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Neeta Raj Sharma *Editors*

Crime Scene Management within Forensic Science

Forensic Techniques for Criminal Investigations

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Forensic DNA Analysis: A Powerful Investigative Tool

1

Lovepreet Kaur and Shiwani Guleria Sharma

1.1 Introduction

The use of deoxyribonucleic acid, i.e. DNA, for the testing in criminal justice explains the term forensic DNA analysis in simple words. It was first introduced in 1981. The term forensis which is a Latin word has given birth to the forensic science where forensic means pertaining to; thus, the term forensic sciences means the use of various applications for the resolution of criminal disputes either criminal or civil. The DNA analysis has become an indispensable part of the modern forensic science; with the use of PCR techniques, it has become a major tool for the analysis of the biological material. The method of DNA analysis used in forensic science is also known by the popular term DNA profiling [1]. The main focus of this science is on the use of the genetic material in case of the criminal justice for solving the cases and answering the concerns related to the cases. It has become a very significant source for establishment and expansion of the databases of DNA collected from the suspected criminals. The technique of DNA analysis involves the use of genetic material in this process; the sample in the form of hair, skin and blood is collected from the suspects that are linked to a particular or various crime scenes [2]. Then the large numbers of isolated DNA sample are interrogated with the DNA databases in order to match the profiles of DNA at the scene of the crime. A DNA database comprises of the profiles of different DNA that are used for the analysis of genetic diseases, genetic fingerprinting or genealogy purposes.

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DNA carries genetic instructions in form of molecules of nucleotides in all living organisms. It is organized into chromosomes in a very structural manner. The nucleotide molecule of DNA comprises of phosphate backbone sugar moieties and nitrogen bases, namely, adenine (A), guanine (G), thymine (T) and cytosine (C). A double-helix structure of the DNA is formed by the attachment of the nucleotides in such a manner that they form two long strands [3]. Each individual DNA contains the specific information related to their heritage. The information gathered from the molecules of the DNA can help to find out certain diseases occurred due to mutations. Only 0.1% of the DNA is unique to each individual which is what the investigator looks for; the rest 99.9% of human DNA sequences are same in every person [4].

Every year, various new technologies are invented to bring out the best approach under the field of DNA profiling. The process of the DNA analysis is not one step; various steps are followed for the analysis of any sample collected. Some of the steps are preparation of sample and extraction of the DNA from the collected sample which is followed by amplifications of the isolated DNA [5]. After amplification, the DNA quantification in which different sizes of DNA present in form of fragments is separated, and at last, the DNA profile matching is done. Various techniques have been updated for the use of the genetic material in investigation. Some of them are restriction fragment length polymorphism (RFLP), polymerase chain reaction, variable number tandem repeat, Y-chromosome analysis, short tandem repeat (STR) analysis, low copy number analysis, single-cell DNA fingerprinting, touch DNA, mitochondrial DNA analysis, etc. [6]. From the past three decades, the development of all these techniques divided the process of analysis under different time periods and frames such as that during first decade only, the method of exploration was used for the analysis in which the utilization of the restriction fragment length polymorphism was done but at that time the analysis through PCR was not specific. Moving further by the second decade, the method of STR was on top for the DNA profiling in criminal cases as these genetic markers provide specific results from the distinct STR loci. Moreover, they have high degree of sensitivity through the PCR amplification. Then the rapid growth of the DNA databases lead to the use of the new Y-STR and use of STR kits on demand in the process of DNA analysis [7]. There are four different types of DNA databases, namely, forensic, genealogical, medical and national; each type of DNA databases has a main role of storing data, but under categories, they are divided. A forensic DNA database comprises of the data that is collected from the crime scene. The collected sample from the sites are sent to the forensic labs where the DNA profiles are generated. These profiles are stored in forensic DNA databases in order to use them for future investigations. They are widely used for the criminal investigations. They are also known as national databases as they are governed by the government. The first national database was developed in the United Kingdom in 1995 [8].

Genealogical DNA databases store the genome sequences which are first submitted by genealogists or geneticist. They are not used for cases that are not registered under police station; they are used more specifically for storage of the genealogical DNA test results. GenBank is one of the most widely used genealogical databases for

the storage of the genome sequences. In GenBank, the data is stored under different categories as per their species specificity. The information related to the genetic variations is stored in medical DNA databases. Such databases comprise of all the medical information about an individual related to their health, disease, variations in genome or other genetic variations. This information stored in medical DNA databases is very helpful in drawing a correlation between various diseases and the environmental factors or a correlation between diseases and the lifestyle of an individual. By this information, the generation of new drugs can be achieved [9]. Medical DNA database is used in forensics in cases where the relation between diseases and the victim is need to be found in order to clarify the case in a better manner. Each database plays a great role in the forensic science as without history of profiles of DNA the comparison of new profiles generated from the suspect would be difficult to analyse to generate end results. Moreover, with the storage of different DNA profiles, we can figure out even if the same culprit or one single person is responsible for two or more crime, and also it will also save time for solving cases. Various techniques have been implemented so that DNA profiling can be used in forensic science effectively. Even the degraded and decomposed samples can be collected, and isolation of the DNA can be done from them. With advancements in science and technology, now the use of nanotechnology has been introduced in combination to forensic science so that new portable devices can be manufactured and the cases can be solved in less time at the crime scene [10]. Nanoparticle such as gold nanoparticle has a quality to attract the charge present on the DNA towards itself so generation of biosensors in combination with the nanoparticle will be a great idea to be utilized in forensic studies to generate portable products. Currently, various techniques of the DNA fingerprinting that are RFLP, VNTR, STR, SNP, low copy number and Y-chromosome analysis are used to solve cases.

1.2 Developments in Forensic DNA Analysis

In early 1990s, the only method used involves the utilization of the blood groups and serum proteins isolated from the crime scenes; in addition to this, the use of various electrophoretic techniques was the main focus for the study in forensic science for solving criminal and rape cases. Various techniques were used under the blood grouping such as ABO typing, MNS system and Rh factor of a person, but due to the high probability of similar blood groups in a population globally, it led to decrease in efficacy of these conventional methods [11]. In addition to this, the markers used in this method of analysis were isoenzyme markers or proteins, but in these techniques, the problem was that the DNA isolation was not successful from that of the highly degraded or decomposed samples collected from the site of the crime. The advancements in DNA profiling increased extensively after 1985; the modern techniques of the forensic science evoke from the first application taken from the work of Alec Jeffrey. In 1985, while working on the myoglobin gene, Sir Alec Jeffrey from the University of Leicester, UK, introduced the new modern technique of the DNA fingerprinting that can be used for solving various cases in a short time

period. He named the repeated sequence of the nucleotides as variable number tandem repeat after his observation that he made in regard to them. He observed the repeated sequences in a specific combination in a nucleotide. Further, he found out that these repeated sequences differ from person to person, thus being helpful to determine clearly about the various DNA sequences and their human link. He termed this method as multilocus testing. He used restriction fragment length polymorphism to solve a rape case. RFLP is used for the analysis of the distinct fragments of the DNA of different sizes. In this method, a restriction endonuclease is used to cut the DNA fragments, and further, with the help of Southern blotting, the location of the repeat sequences is established [12]. Later in 1990, another effective technique for DNA analysis was developed, namely, STR, i.e. short tandem repeat. It is also known as microsatellites and simple sequence repeats (SSRs). They comprise of 2–6 base pairs, short sequences. They are very helpful in representing the discrete alleles that are not identical to each other [13]. But these approaches fail to deal with the problem of the small samples of the DNA as small samples give poor quality of the fingerprints, thus leading to negative results. This issue was resolved by the low copy number analysis which was developed by the UK forensic science service in 1999. Earlier to this, in 1997 at Australian Genome Research Facility, another successful development of the single-cell DNA fingerprinting method was invented by Dr. Lan Findlay. This technique was less time-consuming as compared to the others [14]. Currently, analysis through restriction fragment length polymorphism (RFLP), short tandem repeat (STR), variable number tandem repeat (VNTR), dot blots of allelic sequence information and mitochondrial sequence determination are widely used, and results are acceptable. A broad range of specialists work under the one field that is forensic science; some of them are criminalistics. They use logical and critical thinking for the investigation of cases, digital evidence analysis, expertise for fingerprint, dentistry, odontology, nursing, pathology, and toxicology under which various substances used by criminals to attack on victim are studied and questioned documents are maintained by the investigators to keep a record of every individual (Fig. 1.1).

Currently, introduction of nanotechnology to the forensic science is under process, and various methodologies are adopted in order to save time. Moreover, a single integrated platform for the extraction, amplification and sequencing of the DNA has already been developed with the help of microfabrication of capillary electrophoresis, but validation of such techniques is still under the process in order to utilize them freely in forensic sciences for investigation purposes [15], such as the development of Sci-Fi, a handheld device that can be taken to the crime scene. It is defined as a lab on the chip; the chip would be enough to test the samples at the crime scene in order to generate their sequences of the DNA. This method will provide great advantage as the number of samples can be tested at a single time and place; moreover, it is less time-consuming. Morphological analysis of the skull using three-dimensional computer automated techniques is under study in field of the forensic biology. In addition to this, determination of the colour of skin, hair and eyes with the help of the various techniques of gene sequencing is under the next-generation technologies of the DNA fingerprinting. Use of virtual autopsy, that is, virtopsy, is in

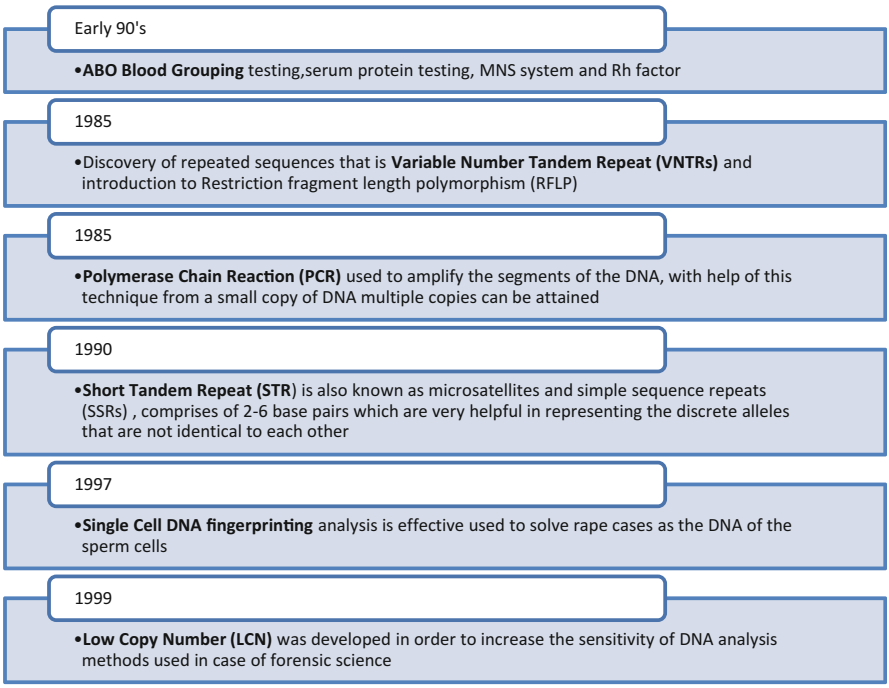

Fig. 1.1 Developments in various techniques of DNA analysis used in forensic science

the near future in coordination to the forensic biology. In this method, the collection of the images will be done [16].

1.3 Steps in Forensic DNA Analysis

The entire process of the DNA analysis is divided into four major parts, namely, serology, which further comprises of collection of sample followed by storage and its characterization. After serology is biology in which the isolation of the DNA is carried out; this involves extraction, quantification and amplification of fragments of the DNA and STR markers. The next step requires the technological aspects in which separation or detection of the DNA is done. After this, interpretation of the data is carried out to determine the characteristic of the isolated DNA from the sample [17]. At last, the role of genetics is there in which the statistical interpretation is done. This helps in the final analysis of the sequence of the DNA isolated from various samples. The genome sequence is compared with that of database DNA or with the suspect's DNA extracted so as to find out the actual culprit behind the crime. The detail process is explained as follows:

1. **Serology**: This is the initial step of any DNA analysis. In this step, firstly, the investigators report the crime site registered by police administration. Under this process, further collection of items, photography of crime scene, storage of samples like semen, hair, skin patches, blood or saliva, is done [18]. Further, after sample collection, the storage of them is mandatory in order to carry out any future investigations if required.
2. **Biology**: The next is based on the biotechnological aspects in which the collected samples are taken transported to forensic labs where the extraction of the DNA is done from both the victim and the suspected samples collected. After extraction, the quantification of the DNA is done in which the average concentration as well as the purity of the DNA are estimated. For the process of the quantification, spectrophotometer is used in which a fluorescent dye is used such as ethidium bromide or SYBR green dye is added to the samples; then, the samples are run on an electrophoretic chamber. The separated bands are visualized on transilluminator or on the gel documentation system [19]. The desired fragments of the DNA are amplified with the help of polymerase chain reaction in which from a small copy of DNA, multiple copies can be attained by using various enzymes such as DNA polymerases. DNA polymerases can be isolated from various organisms such as from bacteria. *Thermus acquaticus* generates Taq enzyme, similarly Pfu enzyme, from *Pyrococcus furiosus* and vent from *Thermococcus litoralis*. This process of amplification of DNA is done under controlled conditions. In the case of forensic science, PCR plays an important role for the identification of the repetitive DNA region [20]. After amplification, with the help of the STR markers, the regions are located, and the final analysis between the two sequences is done.
3. **Technology**: In this process, the separation and the detection of the isolated DNA sequence are done. The human and non-human DNA is separated so that the comparison of the other sequences from database can also be done to find out the person responsible for conducting a crime [21]. With the help of the bioinformatics tools and new technologies, the genome sequences are compared. GenBank is one of the most widely used genealogical databases for the storage of the genome sequences. In GenBank, the data is stored under different categories as per their species specificity. Thus, it helps to distinguish between various sequences of the DNA.
4. **Genetics**: In this step, the statistical interpretation of the collected data is done, and a final report is generated. The report contains all the information starting from the registered date of the case to the final report analysis. The information in a report consists of the photographs from the crime scene taken as evidence, list of samples collected as evidence, reason why the a specific person is taken as suspect and analysis of the DNA genome sequence that shows the matching of the two sequences giving clear information about the accused [22] (Fig. 1.2).

All the above-mentioned steps are carried out in forensic DNA analysis in order to solve the cases related to the criminal, paternity, and mass disaster and rape cases. The three possible outcomes are expected from the results obtained that include

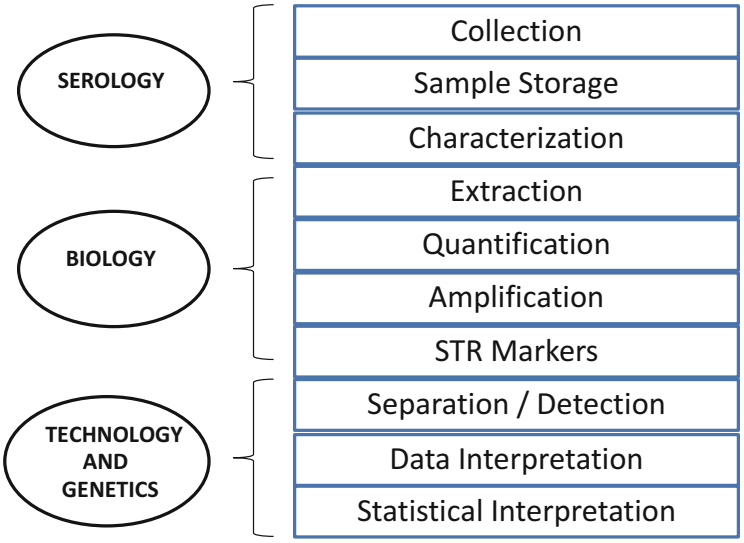

Fig. 1.2 Overview of steps involved in DNA testing

exclusion, inclusion and inconclusive results. Various techniques are discovered for the proper determination of the samples; some of them are low copy number, restriction fragment length polymorphism (RFLP), short tandem repeat (STR), variable number tandem repeat (VNTR) and mitochondrial DNA. Some of them are discussed as follows.

1.4 Various Techniques of DNA Profiling

- Restriction fragment length polymorphism (RFLP)
- Polymerase chain reaction (PCR)
- Short tandem repeats (STR)
- Low copy number (LCN) analysis
- Variable number tandem repeat (VNTR)
- Y-chromosome analysis
- Single-cell DNA fingerprinting
- Mitochondrial DNA (mtDNA) analysis
- Single nucleotide polymorphism

1.4.1 Restriction Fragment Length Polymorphism (RFLP)

This technique is used for the analysis of the different sizes of the DNA that are present in the form of small fragments. These fragments are formed by a digestion enzyme which is restriction endonuclease. It cuts the sequence of the DNA at a

specific site. The fragments obtained after the enzymatic reaction are separated using agarose gel electrophoresis. Furthermore, the fragments are separated by the technique of Southern blotting. Then with the help of multiple probes, the repeated DNA sequences are labelled with radioactive isotopes such as P^{32}. In place of radioactive isotope, a chemiluminescent dye can also be used. The probes can be either multilocus or single-locus probes as per the analysis requirements [23]. The difference in the DNA sequences present in a homologous manner can be detected with the help of RFLP. Most of the RFLP markers are highly specific in nature and work well under codominance conditions. Moreover, the method of development of the RFLP probes is quite simple. This technique was first used by Alec Jeffrey in 1985, when he was approached by the police; they convinced him to help them in the investigation of a rape-homicide case. This technique is effectively used in studying the evolutionary relationships, wildlife migration and breeding pattern in case of animals and for the detection as well as diagnosis of certain diseases [24]. As for the detection of various disease, the researcher can collect the DNA of family members so as to draw a comparison and find out the location of the affected gene and similar patterns of inheritance if occurred.

1.4.1.1 Advantages of RFLP

- A wide range of detection and diagnosis of diseases can be done.
- Specific for locus, thus helping in detecting the gene responsible for a particular disease.
- The evolutionary relationships can be studied.
- Highly reproducible in nature.
- Codominant markers making them species-specific.
- Utilize in genome mapping.
- No prior information on DNA sequence is required.

1.4.1.2 Despite of the Advantages of this Technique, Some of the Demerits Are

- Slow and tedious.
- Requirement of large amount of DNA sample.
- The quality of DNA should be good.
- Expensive technique.
- Radiolabelled probes are required.
- Probes are not available for every species.

1.4.2 Polymerase Chain Reaction (PCR)

This technique was developed in 1985 by Kary Mullis. It is a molecular biology approach that is used to amplify the segments of the DNA. With the help of this technique from a small copy of DNA, multiple copies can be attained by using various enzymes such as DNA polymerases. DNA polymerases can be isolated from various organisms such as from bacteria. *Thermus acquaticus* generates Taq

enzyme, similarly Pfu enzyme, from *Pyrococcus furiosus* and vent from *Thermococcus litoralis*. This process of amplification of DNA is done under controlled conditions [25]. This technique has a wide range of applications in the field of biotechnology; it can be used in genetic engineering, protein cloning methods, forensic DNA fingerprinting and paternity testing. In addition to this, it is effectively used in the analysis of various environmental samples. In the case of forensic science, PCR plays an important role for the identification of the repetitive DNA region [26]. These repetitive DNA are present outside the coding regions of the DNA. They are different from individual to individual. The process of the amplification requires two oligonucleotide primers; these primers are designed in such a manner that they are able to hybridize the opposite strands of the target sequence.

1.4.2.1 Polymerase Chain Reaction Method Is Carried Out as Follows

1. **Initialization:** This is the very first step in which only DNA polymerases are required, as they are needed to get activation by heat. For this purpose, the heating chamber is raised to 94–96 °C. Sometimes, in the case of thermostable polymerases, the temperature is increased to 98 °C for 2–10 min [27].
2. **Denaturation:** In this step, the hydrogen bonds break which are present between the two complementary bases of the DNA molecules, thus leading to melting or denaturation of the DNA. Temperature 90–98 °C is maintained for 10–20 s [27, 28].
3. **Annealing:** In this step, temperature is lowered so as the primers can anneal to the single stranded templates of the DNA. For 20–40 s, the temperature is maintained at 50–65 °C. The temperature should be specifically maintained as it should not be too low or too high; a moderation is required so that hybridization of the primer can occur on the specific target of complementary strand. In this step, the DNA formation begins when polymerase binds to the primer hybrid template [28].
4. **Extension and elongation:** In this step, the addition of the free deoxynucleotide triphosphates (dNTPs) from the reaction mixture; these are complementary to the template in the 5′-to-3′ direction. An optimum temperature is used for the thermostable Taq DNA polymerase enzyme which is 72 °C for 3–15 min for the last cycle of the PCR. After this step, the temperature of the chamber is decreased to 4–15 °C. This stage is termed as final hold; the main purpose of lowering the temperature is to cool down the reaction chamber [27].

This technique is highly preferred for the forensic analysis when the sample is minute or damaged as the amplification of DNA can be achieved by using this method and further analysis can easily be carried out. For instance, a rape case was registered in police under Indian panel code, the victim was brutally gang-raped, the sample was collected to confirm the criminal, and a real-time PCR was performed for the quantification of the DNA samples at State Forensic Science Laborartory. It can also be used for the detection and diagnosis of various diseases in such a manner that we can find out whether the disease has occurred due to mutations or some sort of inheritance [28]. Preimplantation diagnosis is widely used in case of in vitro fertilization approach as this helps in the prevention of defective births of neonatal.

Table 1.1 Various advantages and disadvantages of PCR in DNA analysis

Advantages	Disadvantages
Replication of specific nucleotide sequences from low levels of DNA or degraded DNA	Requirement of special markers that are specific for locus
Creation of large amount of DNA from a very small sample	Lower specificity towards culture or staining
Detection of diseases	Costly protocol
Require small sample for analysis	Chances of contamination
Less time-consuming	Possibility of amplification of unknown flora

Two widely used techniques for the process of DNA profiling under PCR are allele specific oligonucleotide and amplified fragment length polymorphism.

- **Allele specific oligonucleotide (ASO)**: It is short sequence oligonucleotide of 15–21 bases of nucleotides which is synthetic in nature and complementary to the sequence of the variable target DNA. In case of molecular techniques such as Southern blotting or dot blot which are effectively used in forensic science investigations, it acts as a source of probe. It is used for the diagnosis or detection of diseases such as sickle cell anaemia, which is caused by an altered mutation in the codon region [29]. In ASO, a complementary region is prepared to the test region in order to diagnose the disease.
- **Amplified fragment length polymorphism (AFLP)**: It is a technique used to detect various polymorphism among the different genomic regions. It was demonstrated in 1993 by Vos and Zabeau. It is used for the identification of various variations in genetics in same or distinct strains. In a single time frame, AFLP has a great capacity to amplify 50–100 fragments in one go. In addition to this, it is highly preferable technique for the analysis in criminal, paternity testing and generating linkage maps for the process of further quantitative trait analysis [30]. The process of amplified fragment length polymorphism involves the cellular DNA digestion with the help of some restriction enzymes followed by ligation of site specific adapters to those restriction fragments. The next step is the amplification of the fragments by the use of primers which are corresponding to adapters and restriction sites. At last, gel is run over an electrophoretic chamber to obtain bands which can further be visualized [31]. This method of DNA analysis is widely used in the study of various taxa; the main advantage of this is if the genomic makeup is not known, still one can do analysis and study of taxa by using this approach. Some of the demerits include the development of the locus – specific markers for the individual fragments are difficult (Table 1.1).

1.4.3 Short Tandem Repeats (STR)

They are also known as microsatellite and simple sequence repeats (SSRs). Just as variable number tandem repeats, the STR are short sequences of 2–6 base pairs long.

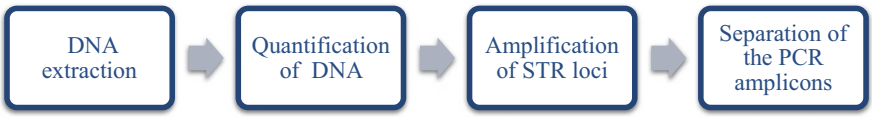

Fig. 1.3 Steps involving in DNA profiling through the process of STR are explained

In 1990, this technique was successfully used in forensic DNA analysis for the investigation purpose as they represent those alleles which are distinguishable from each other. In case of evidence, loci is stable, and even small amount of sample can be used as a short length of fragments is required [32]. This technique of DNA analysis in forensic science requires the use of polymerase chain reaction (PCR) for the process of amplification of short tandem fragments. STR is widely used in genetics for the construction of the linkage maps through linkage maps; diagnosis of genetic disorders can be done. STRs are divided on the basis of the length of the repeats as mono-, di-, tri-, tetra-, penta- and hexanucleotides. Due to the polymorphic nature and loci specific of the STRs, they are considered by the manufacturers to be in kit (330). They vary in size from person to person; such repetitive sequence does not affect the genetic health of the individuals. Mostly, they are found in non-coding regions, but in case of coding regions, they are even less than 10%. Special codes are used for the representation of the STRs, for example, D13S317; in this, D means DNA, 13 is the chromosome number on which the STR is located, and S stands for STR while the unique identifier is 317 (Fig. 1.3).

The very first step is the isolation of DNA by a process called DNA extraction, which is followed by the quantification of the DNA in the sample and at last the separation of the PCR amplicons [33]. The separation of amplicons is done on a genetic analyser by the utilization of bioinformatics tools that help to analyse the resulting data and compare the data from one specimen to databases which has the housing previously generated STR sets, thus helping in the final determination of the criminal among the suspects under study from the crime scene.

For investigation purposes, the samples in the form of bloodstains, semen or some biological traces from the victim's body are collected from the crime scene. This collected sample is investigated by forensic scientist in order to use such evidences for tracing the criminal by comparing the DNA profiling reports with databases of the DNA; after this analysis, the criminal can be found easily if the profile matches [34]. For instance, in Fig. 1.1, the sample from the crime scene was collected and then compared with the two suspects. From the analysis, it has been observed that the suspect 2 DNA profile shows repeat sequences of the STR loci which were identical to the evidence (Fig. 1.4).

The above diagram clearly explains that the repeated sequences were observed in case of sample 2; they are matching with evidence collected. Thus, this is how the process of short tandem repeat helps to solve the forensic science cases in order to trace the main criminal of the scene [36].

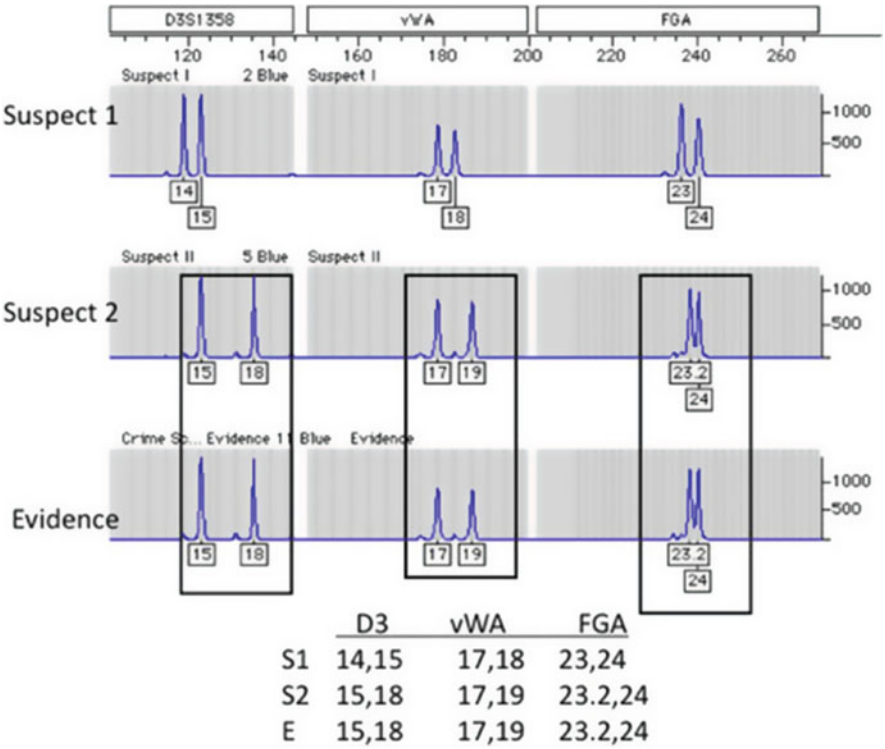

Fig. 1.4 The repeat sequence of Samples collected and the evidence [35]

On the Basis of the Pattern of Repeats, the STR is Divided into Following Categories:

1. **Simple repeats**—they contain similar length and sequence of the units.
2. **Compound repeats**—they are formed by combining two or more simple repeats.
3. **Complex repeats**—multiple repeat blocks of variable length of the units with intervening sequences are present.
4. **Complex hyper variable repeats**—due to allelic nomenclature issues, they are not widely used in forensic studies. The alleles differ in size and sequence, thus making it difficult for genotype reproducibly [37].

Various Applications of Short Tandem Repeats are as Follows:

1. They allow multiplexing due to narrow size of alleles.
2. From degraded DNA samples, the information can also be recovered by using STR method as they only require short repetitive sequence to analyse information.
3. The chances of mutation are low in this method.

4. Use of separate chromosomes in STR markers makes the technique more simple and unique, thus preventing any problem related to linkage between the markers.
5. They are highly preferred in DNA profiling as they can easily amplified by using PCR techniques without any complications.

1.4.4 Low Copy Number (LCN) Analysis

This technique was developed in 1999 by the UK Forensic Science Service. It was developed in order to increase the sensitivity of DNA analysis methods used in the case of forensic science. Low copy number refers to the process of analysis of template DNA which in amount is less than 200 pg in a sample. In case of standard techniques, the PCR cycles are only kept up to 28, but in case of LCN, for the better quality of results, the cycle's number are increased to 38 PCR cycles [38]. In order to increase better sensitivity to results in case of LCN, various approaches are used in which the amendments are made in case of pre- or post-PCR cycles. Other methods that can help in achieving better results via this method include the use of nested PCR, reducing the volume of the PCR, and the use of pure formamide in case of sample preparation that need to be used in capillary electrophoresis.

There are Various Disadvantages of this Technique as:

- The chances of the error are more as compared to other techniques.
- These profiles generated by LCN are not much reproducible.
- Major problem occurs when different profiles mix during LCN typing; then the results are not reliable.
- In case of LCN, the assay is very sensitive so the samples that need to be analysed require effective handling.
- Some reagents and chemicals used may contain extraneous DNA in very low amounts; these can interpret the results.
- High chances of contamination.

This technique is used for the identification of the cases where biological evidences are compromised and other method for DNA analysis cannot be used. An example of this technique is used in DNA analysis where the results were not reliable due to inappropriate measure used by the technicians in forensic laboratory [38, 39]. In 1998, terrorist attack happened at Ireland in which 200 people severely wounded and 29 people died; the police administration suspected an electrician behind the attack. He was 38 year old. Evidence was collected from the site, and that to sample was collected from the suspect and LCN analysis was done, but unfortunately, the results did not show any matching between the suspect and the evidences collected. At the courtroom, the judge declared that due to the ineffective handling, not using of appropriate measures for handling the evidence and not using precaution while doing LCN analysis, the results are inappropriate [39].

1.4.5 Variable Number Tandem Repeat

In 1985, this technique of DNA analysis was discovered by Alec J. Jaffreys. They are comprising of 7–10 base pairs and are also known as minisatellites. In human genome, either one copy of the variable number tandem repeat locus or multiple copies are present. These VNTRs are inherited from parents to offspring. As per their number, they are divided into two categories, i.e. unique loci and multiple loci [40]. An example of the use of VNTR in forensic DNA analysis is as follows:

Dr. Jeffrey conducted DNA analysis on various cases to know the real culprit. He conducted an analysis by taking samples from Leicestershire area crime scene, along with the police. The saliva and blood samples were collected from 4000 men, but none of the sample matched the evidence collected. Later, a person named Colin Pitchfork confessed that he was paid money to give false sample. After this, he was prisoned, and a sample of him was taken, and DNA analysis was done. The results obtained showed matching of the VNTR to the evidence collected from crime scene. Pitchfork was the first person who was sentenced to jail as he convicted the murder. With the help of VNTR technique, various other cases were solved. In 1987, with the help of this technique, a rape case was solved in the United States. The semen sample collected matched to the traces recovered from the body of the victim [41].

1.4.6 Mitochondrial DNA (mtDNA) Analysis

Mitochondrial DNA is isolated from the mitochondria of the cells. Mitochondrion is also known as the power house of the cells. Mitochondrial DNA is present in a circular form. It comprises of 16,569 base pairs. A minute variation is present between the sequences of two individual as this sequence of base pairs is highly conserved but entirely functional in nature. Out of this 16,569, the 1000 base pair long sequence which is known as the control region consists of non-coding D-loop. The non-coding regions contain hyper variable regions which further undergoes variations [42]. The variations are termed as single nucleotide polymorphic (SNP) regions; they are the main regions of this sequence and are more focussed by the forensic investigators. This technique of mitochondrial DNA analysis is highly effective in forensic investigations in cases where nuclear DNA analysis cannot be done. Such possibility occurs in case DNA damage is high due to either burn samples or hair without root. Moreover, this method is highly used in case of lineage studies as mtDNA is inherited only from mother to offspring, so to form an analysis on lineages, this method can effectively be used. Various steps involved in the process of DNA profiling via the use of mtDNA include primary visual analysis, preparation of the sample followed by DNA extraction and then steps of PCR, i.e. amplification and post-amplification. It is important to carry out PCR step carefully as the area of interest is a hypervariable region [43]. After the PCR, the isolated product is quantified, purified and automated; DNA sequencing and data analysis are done. It is advised to handle the samples carefully starting from the collection to analysis in order to prevent any mixing of the samples and to prevent

cross-contamination between samples. This technique is highly used in case of the missing individual. A reference sample is not available or in case of mass disaster [44].

The first case solved in forensic science by using this methodology was related to a 3-year-old child who disappeared from her own residence. After 2 years of this incident, the remains of a human child were found approximately 3 km away from their residence. From that site, the skeletal remains were collected from the desert. Using techniques of DNA profiling, the control regions of mtDNA were recovered. Those recovered regions were matching to that of her mother mitochondrial DNA [44]. It was observed that mtDNA typing can be used in case of missing individual cases as well as in sexual assault cases.

1.4.7 Single-Cell DNA Finger Printing

In 1997, another great technique of DNA fingerprinting was developed by Dr. Lan Findlay; along with few of his colleagues, he developed the method of doing DNA profiling from a single cell. They discovered this new approach at the Australian Genome Research Facility. In this method, a single cell is isolated by using the technique of microscopy prior to final analysis, the cells obtained for identification are collected by swabbing the material, and then, with the help of microscope, initially, the identification of the cells is done [45]. This method of DNA analysis is effectively used to solve rape cases as the DNA of the sperm cells is highly conserved and they are compacted in a protein head. Moreover, this technique is quite fast solving cases in hours, thus making it easy to find out the criminal at the same point. Single cells can be obtained from the fingerprints or marks on pens and car keys, but the only limitation to this method is the requirement of the DNA. More than 1 ng of DNA is required which is equal to 200 cells.

1.4.8 Y-Chromosome Analysis

In this technique of DNA analysis, the more focus is given on the different types of marker, i.e. amelogenin marker. These markers are only present on the sex chromosomes. A specific part of the Y chromosome of the males is used in forensic DNA analysis. This technique is widely used in case of paternity disputes of male child, in case of sexual assault and traces the donors of the missing persons. Various new systems have been developed in order to analyse the short tandem repeats present on the Y chromosome; one such system is applied biosystems [46]. Y-DNA analysis involves the analysis of short tandem repeat segments on Y chromosome. These STRs are first recognised as genetic markers. These repetitions vary from person to person, and STR present on the Y chromosome contains a unique DYS number. In case of this method, the test usually involves the examination and analysis of the 10–100 short tandem repeats that are present on the Y chromosome.

The sample from the crime scene and suspect are isolated and looked for these repeated sequences.

1.4.9 Single Nucleotide Polymorphism

In cases where DNA is badly degraded, the technique of DNA analysis used is Single nucleotide polymorphism (SNPs). Single nucleotide polymorphisms are present in abundance in human genome. They particularly have low rate of mutation, and the size of the amplicon is also small. Basically, SNPs are caused due to point mutations. They are present in the noncoding regions of the genome sequence. For the sequencing under this technique, some basic steps involved include the development and identification of SNP with the use of shotgun sequencing, PCR amplicon targeted sequencing and RNA sequencing. The short fragments can be amplified by using SNP technique for DNA analysis in forensic science; thus, this make easy to solve the cases where DNA sample is degraded or low quantity of DNA template is available. Moreover, due to their low mutation rate, they are regarded as more stable in nature so they are effectively used in the reconstruction of the pedigree and lineage. They can also be used in the identification of the individuals and phenotypic inference studies [47].

In the Case of Forensic DNA Analysis, These SNP are Divided into Four Categories:

1. **Identity testing single nucleotide polymorphism**—in case of individualization where low inbreeding coefficient and high heterozygosity is required. They provide the genetic information in order to distinguish between the different individuals and also help to exclude those suspects or samples that are not part of the putative family member [47].
2. **Lineage informative single nucleotide polymorphism**—in this, the markers are used for the identification of the missing persons through the process of the kinship analysis. Tightly linked SNPs are used that function as haplotype markers (47, 48).
3. **Phenotype informative single nucleotide polymorphism**—they are used to establish the high link of the probability regarding the phenotypic characteristics of an individual such as skin, hair and eye colour as an investigation [48].
4. **Ancestry informative single nucleotide polymorphism**—they are used to establish the high link of the probability regarding the biogeographically characteristics of an individual in link to the phenotypic relationships [48].

Single nucleotide polymorphisms (SNPs) have great advantages in forensic DNA analyses because of the presence of abundant potential markers and amenability to automation. In addition to this, they can be used for the phenotypic identification of the suspect as the physical description of a person can help to portray the individual, thus making it easy for the bureaucrats to solve some cases. Despite of all these advantages, some limitations are that SNPs are biallelic in nature so they are less

informative for the identification testing as compared to the other methods such as STR [49]. Moreover, some ethical and legal concerns arise in the use of single nucleotide polymorphism because the noncoding DNA regions are used and some rules and regulations are set by the higher authorities that need to be followed as the privacy is the main concern in any case of the DNA profiling. Gene-gene interaction acts as a hurdle for solving the cases of phenotypic informative SNPs.

1.5 Challenges in Forensic Science for DNA Profiling

- Various challenges are faced by the investigators at the site or even with the direct contact with victim as in some cases like rape or sexual assault when the victim is not ready to give evidences or even sample for the DNA profiling to match with the suspected person. This could be due to family or social issues that some people do not want to disclose the right information; thus, it's a challenge in such cases to catch the culprit [50].
- The sample collection needs a great focus as in some cases when the requirement is of blood sample but the authorities related to case fail to provide it and as in some cases the law enforcement people give a number of items collected from the crime scene to the forensic laboratory to solve the case while in actual the requirement is not so. It requires a critical thinking to choose the item which should be sent for further investigation [51].
- Chances of error are high in case of handling the sample of DNA. Sometimes the sample is collected from more than two to three individuals. This can even lead to mixing of samples. Mixing of samples led to challenge for analysis for the DNA in order to produce desired results [52].
- Requirement of developed new bioinformatics tools in laboratory for the handling of large number of samples in case of mass disaster. In such cases, there is requirement of handling, managing and analysis of the collected samples in a huge number; thus, the trained technical staff is required. Moreover, the laboratories are not prepared to handle the complex mixtures [50, 51].
- Conflict of interest between the bureaucrats and forensic investigators as they tend to look more in the history but the scientist shows their interest towards the future [51].
- Sometimes, even the bureaucrats do not want to disclose the right criminal due to social conflicts and corrupt officers, so they try to misinterpret with the data in order to hide the truth, and there is a possibility that DNA can be replaced by the non-criminals sample even at the crime scene [50].
- In case of DNA analysis for solving criminal cases, the degradation of the samples is the major issue which occurs due to mishandling of samples and inappropriate labelling issues. DNA degradation starts with contact in sunlight or heat so proper handling by technicians is required [51].
- In order to mislead the investigators, sometimes, the culprits try to use synthetic or fake marks of DNA. They left fake marks of DNA at the site which creates complications to analyse the different sample. One such case was reported in

1922; John Schneeberger who was a Canadian physician uses fake DNA sample. He raped one of his patients and left someone else semen sample, so at the time of investigation, the blood sample collected from John and the semen collected did not match; thus, it led to confusion among the investigators to solve the case [52].

- Trouble with various instruments that are used, old instruments and biological contamination of the tools led to unreliable results.
- Hacking of the DNA databases is the main concern. The DNA databases comprises of all the information regarding DNA profiles; thus, hacking by the past culprits with the use of technological innovations is the main cause and the challenge that the forensic sciences has to face.
- Various ethical norms act as a major hurdle in case of solving sensitive cases like rape or acid attacks as the privacy of the individual with regard to community and religion affects the process of solving cases. Sometimes people are unwilling to disclose what actually happen. Such scenarios act as a challenge for forensic science investigators for solving a case. Moreover, the privacy concern is related to DNA because it contains a lot of information of an individual such as family relationships and diseases related to a person [51].
- Another major challenges faced by the investigators is at the times when the damage to the body is more, in cases related to burn, sometimes, the criminal in order to hide any evidences burns the body of the victim or the surrounding area; this led to great challenge for the extraction of the DNA from such sites [50].
- A large number of cases are solved per day globally using various techniques of DNA profiling, so numerous data is stored regarding profiles of the DNA of either suspect or victim. For the storage of this data, an expansion of the DNA databases will lead to overburden of crime laboratories which is another major challenge that requires scientific experts to maintain the privacy of this data in order to prevent it from hacking [52] (Fig. 1.5).

1.6 Cases Resolved Using Various Techniques of DNA Analysis in Forensic Science

1.6.1 Case 1

A married couple was found dead in the city of Kicevo. Their bodies were corded and hanged. The samples were collected from the crime scene; blood was extracted from the body of the victims, and the nail debris was taken into consideration as a sample. The collected samples were transported to the forensic laboratory for the process of the DNA analysis. Another sample from rope was isolated as suspect to find actual criminal. The method of DNA profiling was continued with extraction of DNA and amplification of it, and with STR typing, the DNA profile was generated which was sent to forensic DNA database of Macedonian. It was observed that the results showed matching of DNA profile to an unknown male to that of sample collected from the legs of male victim. At the same time, another case was reported of burglary in church. During the investigation, they found bloodstains near the

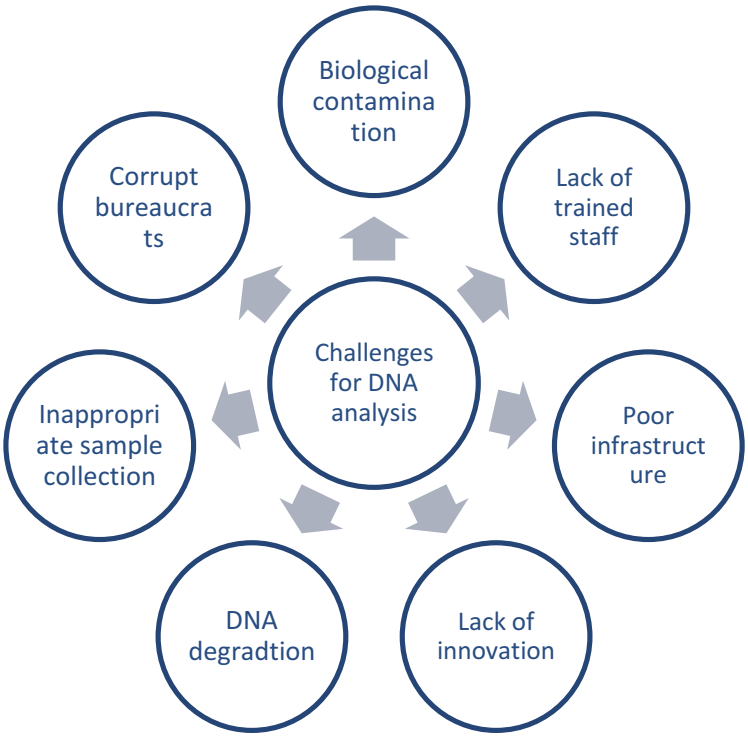

Fig. 1.5 Challenges in forensic DNA analysis

window of the church. The sample was collected from the suspected person, and the DNA analysis was done. The results matched to that of the suspect. The same DNA profile of the culprit was used to run analysis with earlier two profiles of couple murder, and it showed positive results [53]. Thus, the same person was responsible for the murder of married couple and the burglary of the church. This was concluded from the reports analysed by performing DNA analysis.

1.6.2 Case 2

Another case was solved using DNA analysis techniques in forensic sciences. This case was reported regarding the murder of a young man of 35 years old. He was found shot dead in his backyard. Policemen and a team from forensic science reached the crime scene, and as per the witness, several men were observed at the scene. Just after the death, all left. The team started their research and collected three evidences: a handgun, pair of gloves and sleeves of two shirts. The DNA was isolated from the collected evidences, and by using VNTR method, the case was resolved [54]. The results showed matching of profile to an unknown person; this profile was then matched with the DNA data profile stored database of CODIS. The

hit was matched, and shooter was sentenced to in prison for 65 years for murder and robbery.

1.6.3 Case 3

In Bronx, by using low copy number, a case was solved which was related to a gunshot. In 2008, a guy (victim) and his brother had some argument with a group of teenagers. By one of the member of that group, the victim died who was shot with a gun by two bullets. Days after the defendant went to New York, later, he was arrested there as a gun was found in the cavity of the wall in his apartment. From the evidence of the video shoot, an eyewitness to bullet shot case identified that the same man as the shooter. The sample was collected from the person and sent to forensic laboratory to conduct and matched the evidences collected from earlier crime scene. With the technique of low copy number, it was clarified that he was the shooter [53]. He was sentenced to jail for 20 years for the case against weapon charge and manslaughter.

1.6.4 Case 4

A female body was found degraded in an open field during the month of July in 1990. The identification of the body was near to impossible for analysis by individual clothes and fingerprints. The investigators collected fragments of some bones, specifically heel bone and fibula. The samples collected were sent to forensic laboratory for the further DNA isolation and analysis. They tried to amplify the hyper variable regions within HV1 and HV2 present in mitochondrial DNA. On the other side, blood sample was collected from the putative sister of deceased. After analysis, the profile showed matched sequence between the sample collected from decomposed body and her sister. The technique of mtDNA of DNA profiling was used to solve this case as the mtDNA methodology is highly effective to provide reliable results in case of totally decomposed bodies and missing cases [54].

1.6.5 Case 5

In 2012, from the last known place of King Richard III in Leicester, a skeleton was found at Grey Friars. In order to find out if the remains excavated belong to that of King Richard III, the sample was collected in the form of bones, and sequencing was done. The HV1 and HV2, the two hyper variable regions of the mtDNA, were sequenced from the collected sample and collected. This sequence was compared with the sequence obtained from the sample collected from living relatives of King Richard III. The results showed perfect match between the two sequences, and thus, it was confirmed that the remains are of King Richard III. The technique of mtDNA

was widely used for the DNA profiling in cases where mass graves were found in order to determine their lineages to human [53].

1.6.6 Case 6

A 16-year-old girl's body was found in a dense forest on July 6, 2017. She went to school on 4 July and didn't come back, so her parents filed an FIR in nearby police station. Later, her body was found. The case was handed over to Central Bureau of Investigation as numerous marks were found over her body; the team of CBI examined the case and found that the little girl was gang-raped and murdered. The samples from the crime scene were collected which included blood and semen samples; for more evidence, a bottle of liquor and clay were also collected from the crime scene. Later, from approximately 250 people from nearby areas, the blood sample was taken in order to match to the DNA isolated from semen sample, but initially, the results came negative. A percentage and lineage test was conducted by the investigators, and fortunately, they found some match which was with a family from Kangra. Then the samples were collected from that family member to carry out further research and find the true victim. After narrowing down their analysis, they found a person named Anil whose sample was showing matched to that of the DNA sample collected from the crime scene. Anil was arrested by the police, and from the details given by him, the other five suspects were also arrested [53]. By using DNA profiling technique, their samples showed match to that of the semen sample collected from the site. Thus, the case was solved, and culprits were punished.

1.6.7 Case 7

In 1995, a murder case was reported in Utah. A female named Beslanowitch was 17 years old and found dead near Provo River. Her skull was crushed with a hard stone which was later collected from the crime scene for investigation. At that time, the techniques of DNA analysis for forensic science were not fully developed, but the officer investigating this case was curious and dedicated to know what has actually happened. So, later in 2013, when new DNA technologies were introduced, he decided to forward the case to forensic team along with the stone collected from crime scene [54]. By using the technique of touch DNA and with the help of forensic vacuum for the extraction of the DNA, an analysis was made. From the results obtained, the DNA matched to a bus driver who was working in 1995 in a resort near to crime scene. The criminal was arrested and sent to prison.

1.6.8 Case 8

A lady reported a case that she was raped and filed a case against the person to be responsible father of her child. The police started the investigation, and they decided

to carry out the method of DNA fingerprinting to solve this parental issue case. The blood samples were collected from the women, child, and the person he filed a case against. Following the initial step of the isolation, the DNA was extracted by organic extraction method. Further, the quantification of the DNA was done. In order to increase the quantity of the DNA sample, PCR technique was used for the amplification, and at last, the sequence was generated. With the help of the STR technique of the DNA fingerprinting, a 16 loci STR sequence was generated and analysed [53]. The results obtained were quite shocking as the alleles obtained showed matching of the maternal alleles to that of the child, but the suspected person was not showing any matching of alleles, so he was not supposed to be the biological father of the child. Thus, the techniques of DNA profiling in forensic science help to secure the innocent suspects and to punish the real culprits.

1.6.9 Case 9

In woody mountains of the Italy, two corpses covered with very thick vegetation were found. The investigation on them was carried out by the Bresic Forensic Institute; they collected the remains from the area and carried out various investigations on it to determine their age, sex and morphological characteristics from the collected samples. The necroscopic investigators concluded that both were males and their cause of the death was injuries due to gunshot or stabbing. In addition to it, by using mtDNA technology, they were able to analyse the DNA profiles which later showed matched to the three suspects that were arrested for this crime [53].

1.7 Applications of DNA Analysis in Forensic Science

- **Generation of DNA data banks**—Presently, the technique of the DNA fingerprinting is widely used in forensic science for solving various cases, and a lot of DNA is isolated to generate a sequence. Each individual has a specific sequence, and each sequence plays an important role for generating a DNA database. In order to preserve the DNA fingerprints of all the collected samples on daily basis, the Federal Bureau of Investigation (FBI) has created data banks [55]. These data banks are maintained and handled by potential team of expertise, thus providing a number of resources to the people working and maintaining them. Moreover, these databanks are maintained in order to solve the criminal or any other cases by the use of techniques of DNA fingerprinting.
- **Paternity determination**—The technique of the DNA analysis plays an important role to solve the paternity dispute cases of various offsprings. Moreover, it can also be used to identify the dead person. In the cases in which the bodies of the victim are completely burnt, the bones can be used to determine the DNA of the body in order to find out the true identity of the person. Earlier, the ABO blood typing was used, but now with the advancement in science and development of

the new technologies, we can determine the DNA of the offspring and match to their respective parents to solve parental issues [55]. ABO blood typing is not used as the potential technique to solve such cases as the blood is inherited to child on the basis on dominancy in character, so it is preferred to go for DNA fingerprinting to solve such cases. 99% of accuracy is there in DNA typing. The blood samples from the child and parents are collected, and sequencing is done. The matching for the strands of the two samples determine the actual parents of the offspring. A case was solved by using DNA profiling, in which a man was doubting that out of the three of his sons, he is not the biological father of the first one, so he approached the forensic lab and as per their advice he collected hair sample from the comb and sent that to lab. Later on, the results obtained showed the matching of DNA profile of all three to him; thus, his doubt on his wife was wrong [56]. This is how the method of DNA profiling is successfully used to solve paternity cases, and it is widely accepted on legal basis.

- **Identification of rapists**—Various cases have been solved with the help of DNA profiling. Currently, a rape case can easily be solved with no time. Samples can be collected by the technicians either through vaginal swab or through semen found at the site of the crime. Furthermore, the geneticist can separate the sperm cells from that of women cells by various techniques to determine the DNA of the man from the semen sample. Firstly, the purification of the sperms is done with the help of the restriction enzyme. Following it, fingerprints are generated which are further compared to the blood collected from the suspect as well as to that of the evidence collected from the crime scene [57]. For example, a gang-rape case was solved in 2007; a girl was found dead in a dense forest. The samples as evidence were collected which included a bottle of liquor and stains of blood and semen. Later, by using the method of lineage and percentage test, the CBI was able to found out the culprit Anil who was hiding far from the place of the crime. From the sample collected, DNA profiles were made and then were compared to the profiles of the other four suspects along with Anil. The results showed match, and they were punished for their crime [56]. Thus, the techniques of DNA analysis play an important role to identify the rapists easily.
- **Identification of human remains**—It is a very complex process to examine the remains of an individual excavated from a site, but with the advancements in science of the forensics, one can identify the remains of a person. It can be done by collecting the samples such as bones and then isolation of the DNA from it, further analysing the DNA profiles in order to find out their relationship with those alive [58]. A very careful analysis needs to be done with no hope as sometimes the corpse found is not in condition even to isolate anything. Various cases have been solved by using this approach. One such is regarding the remains of the King Richard III of Leicester. His skeleton was found at Grey Friars. In order to find out if the remains excavated belong to that of King Richard III, the sample was collected in form of bones and sequencing was done. The HV1 and HV2, the two hyper variable regions of the mtDNA, were sequenced from the collected sample and collected. This sequence was compared with the sequence obtained from the sample collected from living relatives of King Richard III. The

results showed perfect match between the two sequences, and thus, it was confirmed that the remains are of King Richard III. The technique of mtDNA was widely used for the DNA profiling in cases where mass graves were found in order to determine their lineages to human [57, 58].

- **Identification of murderers**—The science of forensics has a broad spectrum of applications in the field of solving various criminal cases, and the results are accepted by the legal system because the scientific protocols are used to solve the cases. Various steps are involved for the identification of a murderer by DNA profiling starting from the recognition to the evaluation of the evidences collected from the crime sites. The collected samples are sent to the forensic labs for the analysis of the DNA profiles of both the victim and suspect in order to find out the true relationship between them [59]. Various factors are involved for the introduction of the forensic science to the identification of crimes some of them are societal vagaries and obscurity, with all the advancements in science and technology where a number of positive aspects are there but negative impact led to the involvement of forensics of the field of crime solving branch. Globally, a number of cases related to murder have been solved by using the techniques of the DNA analysis; for example, a case in Delhi was solved by CBI in which a housewife was murdered by her own best friend as she was having relationship with her husband. In this case, the women was found lying on the floor flooded with blood, a knife was found in the dustbin of their kitchen, and few scratches of skin were isolated from the body of the victim. Later when sent for investigation from the skin samples, isolated DNA profile was generated. The samples were collected from all the close family and friends. Later the match of profile was observed with her own best friend, and the case was solved [58].
- **Epigenetics**—It is another emerging field of science that plays a great role in the forensic field. This field focuses on the study of the non-heritable changes. Basically, epigenetics involves the alterations of the DNA that does not affect the sequence of the DNA, but the activity of the DNA is affected. The main focus is given to the aging and diseases in epigenetics. In addition to this, the DNA methylation profiling is regarded as one of the most important techniques used for the investigation purposes [60]. It is used for the identification of the tissues and the aging process. Epigenetics is an application of DNA profiling that is widely used in forensic sciences.
- **Analysis of non-human DNA**—In some cases, it has been observed that the bodies are found in forest. The condition of them is worst to conduct a study and analysis on them in order to achieve the target results. In such scenarios, it is important to find out if the DNA isolated from the sample is human or non-human as various microbes are present on decomposed bodies [61]. The type of DNA, either human or non-human, can be determined by using genome profiling technique in which patterns are obtained on electrophoresed gels using multiple DNA samples. But only with the help of a single primer, the diagnosis is done. The advantage of using genome profiling is that in a very short time span, the results can be obtained; moreover, limited technical skills are required**.** In addition to this, real-time PCR technique of the DNA analysis can also be used for the

detection of the nuclear gene target which is specific in nature. A foxhead box (FOXP2) can be used to distinguish between the kinds of DNA. It is more specifically either human or non-human DNA. Use of FOXP2 is taken as a quantification method. The application of the short tandem repeat can easily determine the presence of non-human DNA; in addition to this, single nucleotide polymorphism and the chip technology can be used for the identification of the distinct species in case further analysis is required during the investigation of the samples collected from the scene of the crime [62].

- **Drug sourcing**—In cases related to the narcoterrorism, it is important to figure out the origin of various drugs. The method to determine the source of the various drugs such as opium and heroin is known as drug sourcing. The methods of the DNA fingerprinting such as mtDNA markers and chloroplast DNA are effectively used to identify the geographical origin of the drugs with the help of the mutlilocus system that is used for the prediction [63]. In various countries like Brazil, STR multiplex system was used to create genotype of marijuana samples. A case related to drug transportation and murderer was solved with the help of the DNA profiling method where the drug dealer was arrested later on the basis of the results obtained.

1.8 Nanotechnology in DNA Analysis

Nanotechnology is the branch of the science that deals with the nanoparticles in the field of the research and development. The word 'nano' is a Greek word which means dwarf. The size of a nano is 40,000 times smaller than the width of the human hair; in other words, a virus which is 100 nm in size describes the size of a nano. The use of nanoparticles in forensics helps to solve the cases easily; mainly, it helps to reveal the hidden evidences from the samples collected at the crime scene. Forensics is a wide branch under which the use of the nanoparticles is used for the several investigations such as fingerprinting development, drug identification and manufacture of biosensors [64]. Some of the applications of nanotechnology in forensic DNA analysis are discussed as follows:

- **In-depth diagnosis**—The field of the nanotechnology helps to solve the various cases from the evidence collected; the samples are diagnosed and examined at very nanoscale level. In cases of samples in which the heavy metals and gunshots are there, the DNA fingerprinting can be done with the help of the nanotechnology [65]. By using the approaches of the nanotechnology, the samples can be handled at the nanometre scale, and in-depth analysis of the cases can be done.
- **Amplification of DNA**—The samples for the DNA extracted from the skeletal and body fluids can be amplified in high quality; with the help of the polymerase chain reactions for these, different types of the nanoparticles are used such as magnetic and copper nanoparticles that involve the use of the silica in forensic analysis. For instance, in the process of the PCR amplification, the use of the nanoparticles is introduced due to their ability of uniqueness in different physical

and chemical properties. Gold nanoparticles (AuNPs) are used to increase the specificity of the polymerase chain reaction. In addition to the gold nanoparticles, the carbon nanotubes and the silver nanoparticles are also used to enhance the specificity of the PCR [66].

- **Biosensors based on nanoparticles**—Nanotechnology has a great ability to create and manipulate devices; thus, it is widely used for the production of the kits used in the forensic laboratories for the determination purposes. Various chip-based technologies can be used for the analysis of the DNA in a very short time [66]. A vast variety of applications are there of nanotechnology such as electric engineering, biomedical science and material sciences due to which the nanoparticles can be utilized in every field of the science.
- **Drug detection**—An important role of the nanotechnology is in the identification as well as the examination of the toxic substances isolated from the critical samples such as hair, blood, saliva and fingerprints collected from the crime scene. One such example was quantification of the illegal drug cocaine with the help of the titanium nanoparticle. The cases related to sexual assault, robbery and physical harm are solved via this method. In such cases, the psychotropic substances are given to the victims which can be analysed via nanotechnology [67]. Various biosensors have been developed which are utilized on in field analysis of the detection of drugs. New nanosensors are used to detect the drug clonazepam from the samples of the blood and skeletal with the help of the melamine modified nanoparticles of gold. Detection of a narcotic drug such as a codeine sulphate can be done using a mobile phone; this new way of investigation of various drugs at the crime scene is widely practised.
- **Fingerprint identification**—Nanocomposites are used in combination to the hybrid calcium sulphide quantum dots in order to generate fluorescent in the nature; this fluorescent helps in the better analysis of the fingerprints of the DNA present on a surface, and hence, nanoparticles can be used to detect surface DNA prints. It has been observed that curcumin-based nanoparticles extracted from the turmeric are very selective fluorescent probes used in trinitrotoluene detection even in an aqueous solution [68]. Moreover, in order to enhance the clarity of the fingerprints, the integration of the nanotechnology plays an effective role. The use of the gold nanoparticles along with their binding to the long hydrocarbon chains helps to enhance the quality of the fingerprints; thus, it clearly depicts them for further analysis. The latent fingerprints that are present on the multicoloured can be visualised easily with the help of the nano powder [69].
- **Explosive residue detection**—For the investigation in which the detection of the trace amounts of the explosive components is needed to be done, the nanoparticles can be widely used. The detection of the toxic elements can be done from the tissues, contaminated samples and surfaces with the help of wide varieties of nanoparticles for example; in blasts, the investigation is a challenging process as every leftover remains in the form of the fragments [66]. With the help of nano-based sensors and analysers, the investigators can detect microscopic particles with the help of the powder of the gun in which high resolution scanning microscope is used. Moreover, in cases of use of illegal drugs where an accused

handling explosive substances can be caught as in his fingerprints, the traces of the drugs can be found easily with the help of the nano-engineered powder [67].

- **Extraction of DNA**—The nanoparticles help in the extraction and the amplification of the DNA. Nanoparticles can be used to quantify the DNA samples that are used in the forensic studies such as mitochondrial DNA, which can be quantified in a very short span of time. In case where the samples of the DNA are in small quantities, they can be quantified with gold and zinc nanoparticles [65]. For example, the sample of the urine is source of DNA isolation. With the help of the genomic DNA isolation, methods of filtration and centrifugation along with the help of the nanoparticles of carboxylated magnetic, the DNA can be isolated on a solid phase adsorbent. Further, the intact isolated DNA can be amplified.
- **Ion beam analysis**—It is one of the most widely used techniques used in the investigation in case of the forensic science in order to examine the material collected in the form of the evidence from the crime sites. It is used to detect, identify and analyse the material or residues that are extracted from the samples of the gunshot and soil, thus helping in the determination of the elemental characteristics of the collected material [66]. This technique uses the millielectron volts to determine the thickness, nature as well as the position of the elements. Thus, with the help of the X-ray and gamma rays, in addition to the nanoparticles, it can help to analyse various samples collected from the crime scene as per the requirements of the case.
- **Nano-trackers**—In order to combat the crime, various preventive measures are used in the form of patterns on the products that are kept secret from the suspects or public so as to prevent theft; for this purpose, barcodes are used. In case of the forensic science, the nanoparticles are injected into the body of the prisoners so as to track them via nano-trackers that are injected in their body. In addition to this, cases of burglary can also be investigated by using nanotechnology [67].
- **Estimation of time since death (TSD)**—The estimation of time since death plays a crucial role for solving any criminal case specially related to murders as the time estimation helps to know the accurate time and cause of the death. Earlier, the forensic investigators used to estimate the time via physical examination of the body they used to make an analysis on the basis of the algor mortis, changes in the colour of the eyes, changes in the decomposition of the body and the contents from bowel or urinary bladder. With the advancements in science and technology, now the investigators give more focus towards chip method for the analysis of various amino acids as well as determination of biochemical changes [68]. In the forthcoming years, it is expected that with the help of fluorescent nanoparticles, the estimation of the time of death can be done in which the nanoparticle will be used for the quantification of the various body fluids, thus making it easy and time-consuming for solving criminal cases. The actual time of death is very important to solve critical cases as the more time is given to the culprit, the more delays in the process of case solving will took; thus, in every murder-related case, the first thing the investigators tend to do is the determination of the time since death and use of nanoparticles are making it easy [69] (Fig. 1.6).

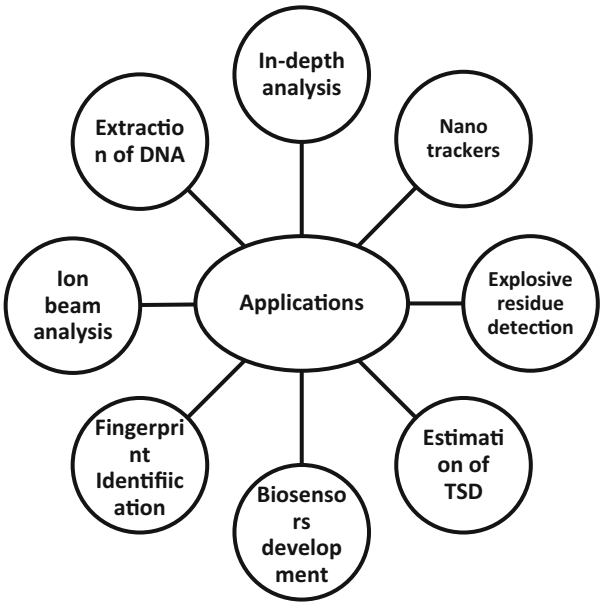

Fig. 1.6 Various applications of nanoparticles in forensic investigation

1.9 Future Advancements of Nanotechnology in the Field of Forensic Science

1. Development of the new advanced media which can be used for the multimodal imaging in solving cases [70].
2. Designing of the nano-structured materials.
3. Conversion of the bioenergy [70].
4. Analysis of the thin films for the biomedical purposes—this will help to do in-depth analysis of the various samples collected from the crime scene [71].
5. Manufacture of the molecularly imprinted polymers in the form of the nanoparticles. These particles can be used for the nanosurface analytical applications [70].
6. Characterization of the functional materials for the investigations on the structure and vibrational properties of the various materials via studying their physico-chemical properties [71].
7. Enhancement in the field of the nano-engineering [70].
8. Introduction of nanotechnology in the field of science for researchers with better tools and equipments in order to increase the number of the skilled workers under this field to make the processes more efficient [71].
9. Awareness for international links with other scientists working on nanotechnology can bring about better advancements for future [70].

1.10 Collection of DNA Samples and Issues Related to Sampling

The first step in investigation of any crime scene by the forensic team is collection of the evidences as much as they can. This step requires analytical and logical skills as the evidences play an important role to solve the case. The samples are collected by the expertise of the team and are preserved for further testing in the forensic laboratory. Samples can be in the form of blood marks and bloodstains, hair, skin, nails, urine stains, scraps from finger nails, teeth and bones in case of burnt cases and different types of touched objects such as cloths, table, vehicle parts, glass, bottles and cosmetic [72]. So, the collection of them should be done separately, and clear labelling is required to distinguish each sample from other. The method of the collection of samples should be clean and precise for example firstly. Identification of the type of sample should be done; then the procedure of collecting it should initiated. But various issues are related with the DNA samples. Sometimes, the sample is minute; due to heat, it may get degraded. Chances of contamination are high. Mixing of the samples and various ethical issues are related [73].

1.11 Methods of Sample Collection

1. **Use of Swabs**—In this method, the first step is creating a defined hole with swab either dry or wet, the co-extraction using multiple swabs is recommended as it enhances the retrieval of the DNA easily. Sodium dodecyl is recommended to be used in case of moist swabs [74]. The yield of the DNA is more from the moisten swabs so after collecting the sample the swab should be immediately frozen to carry out the further processing in the laboratory.
2. **Micro dissection**—This technique of the sampling is used in order to isolate the samples in which the focus is on the specific target cells; with the help of the fluorescence nanoparticles, the labelling of the samples can be done in order to recognize their distinct morphological characters [75].
3. **Laser microdissection**—It helps to distinguish between the cells from the male sample to the female cells, thus making the analysis easy at the first end of the sampling. It is advised to use this method at the time of the sample collection so that the initial labelling between distinct samples can be done [76].

1.12 Issues Related to Sampling

1. The minimum requirement of the DNA sample in order to carry out analysis is 0.1–0.5 ng approximately, but in some cases, the samples are highly regarded, so it is advised to do extraction of the DNA careful in order to prevent any further loss of the sample [77]. The use of organic solvents is recommended while doing the extraction of the DNA.
2. In cases of bomb blast were the number of victims is high and the crime scene is highly scattered, the chances of the mixing of the DNA samples is more due to

poor labelling of different samples collected or due to inappropriate measures used during sampling [78].

3. When the bureaucrats try to collect samples rather than reporting the expertise team in order to save time in such cases they do not use preventive measure and techniques for the collection of the samples, thus resulting in cross-contaminating it with other samples [77]. This makes it difficult for the forensic lab analysts to analyse the sample clearly, so the chance of false results are more. It is advice to report the forensic team at any crime scene in order to collect the samples without any contamination.
4. Degradation of the samples is the main cause to false results or delay in the processing time of any criminal case. The degradation of the DNA occurs at high and humid temperature. It is important to isolate any sample collected from the crime scene in a cool preserving container, and with proper handling, it should be submitted to the forensic lab for further investigation [79]. In addition to this, various environmental issues led to the growth of the bacteria, mosses and lichens on the bodies of the individual are found that led to dilemma at the time of isolation of the DNA whether it is human or non-human DNA.
5. DNA can be transmitted from saliva or when touched by skin directly; thus, it gets contaminated in such cases. Use of the diamond dye chemical is done for sample collection of the evidence, but this process is very cost-effective. In order to prevent such issues, use of cotton or nylon swab is best as nylon swabs or moistened cotton swabs yield high amount of DNA and chance of contamination are less with them [78].
6. The major issues that arises to collect any sample from the crime is the permission from the higher authorities under which the case is reported. It is important to get prior permission from the team to carry out the process of sampling so as to prevent any loss of evidence from the crime site by theft in order to hide the real culprit [77].
7. The storage of samples collected is the main issue as this requires proper security so that not even the family members can access or manipulate anything at the crime scene. Moreover, a large space is required to maintain samples for longer duration [79].

1.13 Forensic Examination Levels

Whenever a case is registered, it is important to recognize the need of various forensic tools for its examination; therefore, the knowledge of tools and services gives an idea to the investigator about different evidences he/she can seize to carry out their investigation. Starting from the physical examination, fingerprint matching, hair and fibre analysis to forensic pathology, anthropology and entomology, various stages of analysis are covered in order to generate the final end report with accurate results [80]. Some of the tools used for examination prior to DNA analysis step in forensic investigation are discussed as follows:

1. **Physical examination—**Different types of evidences can be found during the physical examination of the crime scene that might include marks on tools, footprints indicating a path followed by accused, tire print, torn edges of material like paper, tape or cloth, stains of blood and saliva or some pieces of valuable products that act as a major source of evidence in solving a case. Under physical examination, there are two levels, level one in which the characteristic of the class in relation to the items is taken into consideration such as impressions on tolls, shoe and tire prints. The classification focuses on the model, size and pattern of the various designs, for instance, the size of the foot print any brand if they can determine or pattern [81]. The next, level two, includes the determination of the accidental characteristics such that the investigators look for the items like broken glass and plastic fragments. The fragments of the broken glass can help to determine the direction of a bullet passed, thus making it easy for the investigators in cases of gunshot and murder to establish a link between the origin of shooter and direction travelled by accused.
2. **Fingerprint matching**—This type of examination plays a very important role in forensic science as fingerprints are different from person to person and are representing an unique pattern of lines and ridges that exit on the plantar surface of a person. The identification mark from that of the site of crime can be taken and compared with the database containing patterns of fingerprints stored in it, and matching can be done easily, but now a technique of automated fingerprint identification system is used in which biometric scanning of fingerprints is done electronically [81].
3. **Examination with ballistic approach**—Use of ballistic fingerprinting in forensic helps in examination of the trajectory of the bullet. Trajectory means the path travelled by the bullet from the time it leaves the gun barrel to the end point where gun powder comes to rest. Various patterns of the trajectory are observed by the investigators to draw out the final analysis about the direction of bullet travelled [82].
4. **DNA analysis**—Currently, the laws and regulation set by government are in favour of consideration of the process of DNA fingerprinting as important in forensic analysis because it gives the exact matching of the genome sequencing, thus reducing the chances of error in catching the accused. The process of DNA analysis is very simple, but the collection of the data from the crime sites is important in order to isolate the accurate DNA sequence from the collected sample within a given time frame [82].

1.14 Analysis of Non-human Species Via DNA Profiling

During the analysis of various samples collected from the site of crime, the chances are more of presence of non-human DNA, and distinguishing of a non-human DNA from a human one is very important to determine the results accurately. During forensic investigation, different categories of the biological material is found at the site. It can be in the form of any botanical material; microbial in nature, presence of

dog or cat hair, feathers of birds, saliva, faeces or other animal products can be present. In some cases where bodies are found in damp forest areas, the chances of growth of lichens, bacteria and other microorganisms are more; such organisms generate a challenge for the forensic investigators to isolate any other form of material if present near or on the surface of the body [83]. For instance, in a rape case, if the body of the victim is found in forest, the growth of microbes will interfere with semen or any other sample present near or on surface of the body, as when evidence will be collected the DNA might be of microbe rather than the actual convict; thus, it is important to analyse the sample properly to determine the nature of the DNA isolated. Various methods of genome profiling can be implemented in order to multiply DNA fragments isolated from the sample collected from the site of the crime either by using a random primer or with the help of electrophoresed gel pattern, but these methods sometimes fail to generate reliable results in case of the presence of mixed genomes [84]. For the detection of a human nuclear gene target forehead box P2 (FOXP2), it can encode a transcription factor that accumulates any changes in the amino acids which are involved in human lineage development of the characteristics of language and speech. Thus, FOXP2 can be used to distinguish between a human and non-human DNA. In addition to this, various other methods of DNA analysis can be used such as single nucleotide polymorphism, short tandem repeat, variable number tandem repeat and restriction length polymorphism.

Recently, the technique of the mitochondrial DNA is widely used in the analysis of the non-human DNA due to their salient features such as mitochondria present in almost all eukaryotic cells, and they are termed as powerhouse of the cells as they generate energy that organisms require sustaining livelihood. Mitochondria contain their own genetic information; moreover, they possess remarkable features of the genetic variations such as size, conformation and arrangement of genes [85]. This arrangement of genes is different from person to person or organelle to organelle either in plants, animals or humans. The main advantage of use of mtDNA in forensic DNA analysis lies in their high copy number of the cells. This method is highly used in case of lineage studies as mtDNA is inherited only from mother to offspring so to form an analysis on lineages, this method can effectively be used. Various steps involve in the process of DNA profiling via the use of mtDNA include primary visual analysis, preparation of the sample followed by DNA extraction and then steps of PCR, i.e. amplification and post-amplification. It is important to carry out PCR step carefully as the area of interest is a hypervariable region. After the PCR, the isolated product is quantified, purified and automated. DNA sequencing and data analysis are done [84]. The samples are advised to handle carefully starting from the collection to analysis in order to prevent any mixing of the samples and to prevent cross-contamination between samples. The traceability of food products and endangered species or drugs can be determined with the help of mtDNA technology.

1.15 Future of Forensic DNA Analysis

Various technical developments led to the advancements in the DNA profiling with short time and greater probability of accurate results. The future of the DNA fingerprinting in the field of the forensic will be great with faster, higher and reliable results [85]. With increase in research under this field from past years, focus on the development of more new methodologies has also been increased. The demand of DNA fingerprinting will be high to solve various cases; already with developments, a large amount of DNA can be isolated even from a degraded sample, and much more such advancements are under study.

- A fully automated instrument for the process of DNA profiling has already been introduced. Earlier, the entire process of the DNA sequencing used to take days to solve a case, but now only 90 min are enough for the DNA extraction, amplification via polymerase chain reaction followed by DNA separation and detection, sizing and at last genotyping. With the help of such advancements in short time, the convict can be easily caught as a suspect can be taken in custody for 4–5 h and samples of suspect can be taken, and within a day, the results of DNA sequencing can be achieved [86].
- Sci-Fi—It is a handheld device that can be taken to the crime scene. It is defined as a lab on the chip; the chip would be enough to test the samples at the crime scene in order to generate their sequences of the DNA. This method will provide great advantage as the number of samples can be tested at a single time and place; moreover, it is less time-consuming [87].
- A single integrated platform for the extraction, amplification and sequencing of the DNA has already been developed with the help of microfabrication of capillary electrophoresis, but validation of such techniques is still under the process in order to utilize them freely in forensic sciences for investigation purposes [87].
- Multidisplacement amplification technique can be utilized for the amplification of the whole genome where the amount of the template DNA is quite small [88].
- In case of the identification of body fluid samples, some challenges are faced by the forensic biologists in order to solve this issue. The use of mRNA approach for the identification of the blood, semen and saliva is used in which analysis is made through the specific sequences of mRNA [89].
- With improvements in the PCR assays, more data will be available from various biological samples less than one database; thus, this would provide higher sensitivity towards the information obtained from the sequence analysis of the alleles [90].
- In the coming future, more data will be available in the databases; internationally exchange of data will also benefit to solve old cold cases related to murders and other crime as with exchange of international data more access will be there to more STR loci which will eventually benefit the investigators to solve cases [85].
- The collection of the numerous data from the short tandem repeats (STRs) and single nucleotide polymorphism (SNPs) will serve as a next-generation

sequencing (NGS) approach with the involvement of the parallel sequencing. The information from NGS will require specialized tools of the bioinformatics in order to process the data [85].

- Introduction of phenotypic inferences extracted from a DNA sample so as to compare them to pharmacogenetic information for molecular autopsy. This will also help in determination of the type of tissue and expression analysis [87].
- Some techniques like LCN typing of DNA profiling require new approaches in which the increase in availability of the templates is needed; in addition to this, there is a need of generation of the contamination free reagents that will support LCN analyses with decontamination protocols [86].
- More focus need to be given on the extraction of the DNA as the samples are collected with the help of cotton swabs and from which the DNA is not efficiently removed during extraction, so the new approaches should be developed for efficient extraction of the DNA [85].
- Already various techniques have been developed to isolate the DNA from the degraded samples isolated from the crime scenes, but some PCR inhibitors fail to reproduce the very informative Q profile. For such cases, the smaller PCR amplicons are required. These amplicons can be in the form of the miniSTRs or SNPs [86].
- A better future of the DNA profiling in forensic science can only be seen if the cost for various analyses is reduced. Moreover, technological advancements are needed in laboratories to hold large amount of the data and also to secure the samples for future studies [91].
- The future of any research is dependent on the funding as for the development and research of any new scientific protocols required some sort of funding. Companies or investors only invest in projects if they see any sort of benefits in the forthcoming years so in order to attract the investors to the field of forensic, awareness among people related to various applications and uses of DNA fingerprinting in forensics science is needed [91].
- In the USA, on-site DNA analysis technique has already been introduced; up to some extent, the analysis of various samples can be done on the crime site with the use of various handheld devices [92].
- In future, the use of portable devices for the DNA detection will be more. In comparison to the old PCR techniques, the need is to develop a device that is portable and quantitative and can easily be operated by public. An example of such is a growing demand of the personal glucose meters, i.e. PGMs. These PGMs are used for the quantification of DNA. In addition to this, they can also quantify organic molecules, proteins and metal ions that are linked to the functional molecules of the DNA sensors [90].
- Developments of biosensors that utilize nanoparticles are in demand as nanoparticles such as gold nanoparticles have ability to attract the charge present on the DNA sequence. Thus, various biosensors can be used for the identification of the DNA such as DNA field-effect device (FED); when it is covered by a gold nanoparticle (AuNPs), it attracts the DNA towards itself, thus helping in identification [93].

- An improvement in the statistical analysis of various DNA samples is required in order to attain the reliable results. Moreover, such approaches will lead to improve in selectivity when dealing with the mixture of the DNA samples [85].
- Coupling of the nanotechnology with the microfluidic devices is under research in order to generate high throughput of the DNA profiling [91].
- Morphological analysis of the skull using three-dimensional computer automated techniques is under study in field of the forensic biology. In addition to this, determination of the colour of skin, hair and eyes with the help of the various techniques of gene sequencing is under the next-generation technologies of the DNA fingerprinting [92].
- Use of virtual autopsy that is virtopsy is in the near future in coordination to the forensic biology; in this method, the collection of the images will be done [93].
- Introduction of phenotypic inferences extracted from a DNA sample so as to compare them to pharmacogenetic information for molecular autopsy. This will also help in determination of the type of tissue and expression analysis [93].
- Some techniques like LCN typing of DNA profiling require new approaches in which the increase in availability of the templates is needed; in addition to this, there is a need of generation of the contamination free reagents that will support LCN analyses with decontamination protocols [94].
- More focus is needed to be given on the extraction of the DNA as the samples are collected with the help of cotton swabs and from which the DNA is not efficiently removed during extraction, so the new approaches should be developed for efficient extraction of the DNA [92].

1.16 Summary

The use of deoxyribonucleic acid, i.e. DNA, for the testing in criminal justice explains the term forensic DNA analysis in simple words. It was first introduced in 1981. The term forensis which is a Latin word has given birth to the forensic science where forensic means pertaining to; thus, the term forensic sciences means the use of various applications for the resolution of criminal disputes either criminal or civil. The DNA analysis has become an indispensable part of the modern forensic science; with the use of PCR techniques, it has become a major tool for the analysis of the biological material. Various techniques are discovered for the proper determination of the samples; some of them are low copy number, restriction fragment length polymorphism (RFLP), short tandem repeat (STR), variable number tandem repeat (VNTR) and mitochondrial DNA. The entire process of the DNA analysis is divided into four major parts, namely, serology, which further comprises of collection of sample followed by storage and its characterization. After serology is biology in which the isolation of the DNA is carried out; this involves extraction, quantification, amplification of fragments of the DNA and STR markers. The next step requires the technological aspects in which separation or detection of the DNA is done. After this, interpretation of the data is carried out to determine the characteristic of the isolated DNA from the sample. At last, the role of genetics is there in which

the statistical interpretation is done; this helps in final analysis of the sequence of the DNA isolated from various samples. The genome sequence is compared with that of database DNA or with the suspect DNA extracted so as to find out the actual culprit behind the crime. Various challenges are faced by the investigators at the site or even with the direct contact with victim as in some cases like rape or sexual assault when the victim is not ready to give evidences or even sample for the DNA profiling to match with the suspected person. This could be due to family or social issues that some people do not want to disclose the right information; thus, it's a challenge in such cases to catch the culprit. Currently, the requirement of developed new bioinformatics tools in laboratory for the handling of large number of samples in case of mass disaster is more. In such cases, there is demand for handling, managing and analysis of the collected samples in huge number; thus, the trained technical staff is required. Moreover, the laboratories are not prepared to handle the complex mixtures. Recently, the use of nanoparticles in forensics helps to solve the cases easily; mainly, it helps to reveal the hidden evidences from the samples collected at the crime scene. The nanoparticles help in the extraction and the amplification of the DNA. Nanoparticles can be used to quantify the DNA samples that are used in the forensic studies such that mitochondrial DNA can be quantified in a very short span of time. In case where the samples of the DNA are in small quantities, they can be quantified with gold and zinc nanoparticles. In future, the use of portable devices for the DNA detection will be more in comparison to the old PCR techniques; the need is to develop a device that is portable and quantitative and can easily be operated by public. An example of such is growing demand of the personal glucose meters, i.e. PGMs. These PGMs are used for the quantification of DNA; in addition to this, they can also quantify organic molecules, proteins and metal ions that are linked to the functional molecules of the DNA sensors.

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Death Acre: Tales of Dead Body Land

2

Gestión de la escena del crimen dentro de la ciencia forense: Técnicas forenses para investigaciones criminales

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Abstract

The crime scene can be defined as the place or object that is subject to require a forensic examination. To this classic definition, in 2018, the ISO 21043, also including a body (living or deceased) of a human being or an animal is considered a crime scene as well. That is why each deceased, whether in a hospital or elsewhere, must be treated as a crime scene and therefore follow the protocol detailed below to establish the cause and manner of death. This information and

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documentation are crucial for experts who are involved in the analysis, as well as the legal and health authorities of each country to make the best decisions.

Our working group has especially focused on the handling of corpses during the pandemic, which is why we have included several quotes regarding contaminated bodies. We estimate that the documentation generated at crime scenes in this period will constitute not only a record of legal interest but also epidemiological and historical, especially due to the restrictions that several countries have placed on the practice of autopsies. Faced with this, the external examination and its documentation are enormously valuable, since many times they will be the only records that can be obtained from the body.

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Keywords

Crime scene · Technologies · Cause of death · Manner of death · Postmortem interval · Material evidences · Quality control

2.1 Purpose of the Work on the Crime Scene [1, 2]

Work at crime scenes allows obtaining information regarding methods to commit crimes, places, types of stolen species, among others, and recovering forensic evidence.

Forensic evidence allows:

- Show the kind of crime happened.
- Identify the victim and the offender.
- Link the crime scene to others crimes happened in the past or future.
- Corroborate or discard statements of witnesses, victims or suspects.
- Help to elaborate hypotheses in the case.

2.2 The Processing Methodology at the Crime Scene

The processing methodology at the crime scene (places or bodies) is as follows:

1. Evaluation
2. Observation
3. Documentation:
   (a) Take notes.
   (b) Photograph.
   (c) Sketches, mapping.
   (d) Videos

4. Search.
5. Collection or evidence
6. Processing/analysis and reporting.

These six steps constitute the basic methodology of the crime scene investigation. However, considerations unrelated to the judicial investigation may make some of these stages become indispensable, especially in the pandemic situation that has been declared in the world in 2019.

2.2.1 Stages of Work at the Crime Scene

2.2.1.1 Evaluation

The forensic team leader must determine the universal precautionary measures, that is, he must evaluate the possible elements of risk for the investigative team. One of the most frequent is the biological risk.

It is essential for the research team to bear in mind that blood or other body fluids that can transmit diseases such as HIV, hepatitis, tuberculosis and hantavirus can always be found on the scene. Therefore, the corresponding safeguard measures must always be adopted.

The most frequent routes of infection at a crime scene are:

Through the mouth
- Eat, drink and smoke on site
- Transfer of microorganisms to the mouth using fingers or contaminated utensils (pens, pencils, etc.).

Through the skin
- Accidental inoculation with sharp instruments (weapons, broken glass ...)
- Unsafe handling of weapons, bad techniques to open doors, windows, or objects.

Through the eyes
- Splashes of infectious materials in the eyes.
- Transfer of microorganisms to the eyes by fingers contaminated. Through the airways
- Inhalation of airborne microorganisms To avoid any type of biological risk from the researchers, it must be assumed that the scene ***is always contaminated*** [3] and that is why all the personal protection elements (PPE) must always be used.

The ideal PPE to face contaminated crime scenes or potentially contaminated with any infectious agent are:

– Biohazard suits [4, 5]
– Head cover

- Transparent face shield
- Glasses
- N95 masks
- Latex or nitrile gloves
- Shoe covers
- Plastic bibs

All these elements must be disposable, and after each use (each case), they must be collected in biological waste bags (their color may vary according to the regulations of each country, but they must bear the respective biosecurity logos) and be disposed of as such. Local investigating agencies, hospitals and medical legal services have protocols for the management, collection and elimination of hazardous waste, and investigators at the site of the event must know them and know where they must be collected and finally disposed of as waste.

2.2.1.2 Observation

This process in pandemic case only will help to know what kind of scene you need to process, indoor or outdoor, and what type of elements you will need to do a safe work.

2.2.1.3 Documentation

The documentation of each case will be decisive for all subsequent stages of the judicial investigation. That is why it must be done professionally. The greatest last advances in the study of crime scenes have occurred in the field of detection and documentation of traces, fingerprints and other evidences

This documentation stage consists of four types of records:

- The first is the detailed ***note-taking*** that the forensic team must carry out from both the crime scene and its surroundings; this note-taking must be detailed, narrating antecedents such as date, place, weather conditions, identification of the victim, clothing, place where the victim is, medical records or medications if applicable, among other antecedents that must be recorded.
- The second is the ***photographic record***, which must be carried out using the usual methodology of forensic photography, that is, general, particular and detailed photographs. These photographs must be taken without and then with a metric witness, as well as without and with identifying labels (letters or numbers). The photographs must show and record everything that is related to the deceased and the environment in which it was found, such as, for example, the entire body of the victim without being undressed, possible identifying elements (face, scars, tattoos, moles), injuries visible and fingerprints, among others. To ensure bio-protection, the photographer must permanently clean his photographic equipment, preventing the equipment from being contaminated at the scene, and also possible cross-contamination.
- The body must be photographed as it is, without touching it, to record aspects related to identity, clothing, stains and injuries. If the cause of death is suspected

to be infectious, before undressing, the clothing should be sprayed with technical solution,[1] and wait a few minutes. Clothing and bedding should be considered contaminated.

- The third record is the ***planimetry,*** the application of this process must be evaluated in each case since in some of them it may be unnecessary, for example, if the deceased suffered injuries, was taken to first aid and died in a hospital, where there are no elements of the place where he suffered the assault or accident. If the investigating team requires it, the plan must follow all the rules of forensic planimetry.
- Finally, the fourth documentation process is the ***recording of filming or video***. In this filming, the records must be made with the greatest respect for the victims and without audio narration, the movements of the cameraman must be slow and showing the entire environment of the scene.

  All types of photo documentation must be made with items from the agencies in charge, and not with personal or private elements. Likewise, each of these elements must be cleaned after use, to avoid cross-contamination of the evidence and the operators.

Documentation of findings should always be systematic and complete. Nevertheless, some details should not be overlooked[6]: The technical solution to use:

- Should not interfere with the determinations of DNA and toxic substances that may need to be carried out later
- Must not stain the clothing or affect the areas of different coloration that the corpse may present.
- Its chemical formula must be known and safe for researchers, other people, animals and the environment. Our working group has been using a mixture of hydrogen peroxide and quaternary ammonium in aqueous solution in low concentrations, which meets these requirements, based on the EPA recommendations.

  United States Environmental Protection Agency: www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2.
- ***Verify if the body was moved***, extruded [7], taken off suspension, if there were resuscitation attempts, or any other maneuver, before the arrival of the research team, and record these data, for its correct interpretation in later stages.

[1] The technical solution to use:

- should not interfere with the determinations of DNA and toxic substances that may need to be carried out later
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- Its chemical formula must be known and safe for researchers, other people, animals and the environment. Our working group has been using a mixture of hydrogen peroxide and quaternary ammonium in aqueous solution in low concentrations, which meets these requirements, based on the EPA recommendations.

  United States Environmental Protection Agency: www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2.

- ***Describe and photograph the exact location and position in which the body was found***. If a fall from a place or building at height is suspected, record the distance between the body and the building line.
- ***Work accidents***: it is necessary to find out what risks were in the workplace and if the worker was aware of them; if the worker was wearing their protective equipment: helmet, gloves, safety shoes, among others; if the tie-down straps, knots or scaffolds were in good condition;
- If the body shows ***no external injuries***, it is possible that he may have been ***poisoned***. Check for vomit or stool stains on clothing or bedding; examine the bathroom; check the drawers of the furniture, trash cans in the bathroom, kitchen and outside, to verify the presence of medicine containers, pharmaceutical forms (pills, ampoules), syringes, among others.
- Do not forget the documentation of those evidences that cannot be photographed or sampled, such as ***smells, sounds or weather conditions***.

2.2.1.4 Search: Identification of Traces, Footprints and Other Evidences

At a crime scene, there may be traces, fingerprints and other objects of various kinds. Often it is not possible to know whether or not they are related to the fact that we are investigating, so it is not easy or obvious to decide which ones have "criminal interest" and which ones are not.

Many signs or tracks are not obvious to the naked eye. A fingerprint on an opaque surface, a washed stain, they are difficult to find, photograph and sample. For their search and recognition, forensic investigators have incorporated ***different technological items*****,** such as illumination with light sources outside the visible spectrum, development with chemical products, systems and devices to take samples.

2.2.1.5 Sampling and Lifting of Traces, Footprints, and Other Evidences

In this stage, the evidence found at the scene is collected and kept in the best conditions so that they can be transported to the laboratory where they will be analyzed. Sampling, packaging and transportation are mostly standardized, but techniques and inputs are constantly emerging that contribute to improve their ***quality*****.**

2.2.1.6 Processing/Analysis and Reporting

This is the most difficult stage in crime scene work. No matter how much knowledge forensic investigators have, there is hardly one scene the same as another, and new situations always arise. On the other hand, the experienced researcher is subject to cognitive biases [8] against which he must be alert. Showing what was at a crime scene and explaining what happened to people without much knowledge, sometimes months or years after the event, is a great challenge. The best help is to document everything, even if it initially seems like a minor detail. The second thing is to take into account that, in all cases, the manner of death can be due to natural causes, homicide, suicide and also accident. No possibility should be dismissed at the beginning of an investigation. Only at the end of it, it is possible to decide on the one in which the antecedents have more inferential force.

2.3 Searching Evidences: Forensic Lights [9]

In forensic investigation, it is possible to find patent (visible) or latent traces, fingerprints and evidence. "Latent" are those that, to be detected, need some system of development or enhancement. One of the methods used to reveal latent evidence is the use of light in different colors, intensities and wavelengths, especially that which is outside the visible spectrum.

To do this, flashlights called forensic lights are used that provide illumination within, below and above the visible light spectrum that ranges from 380 to 750 nanometers (nm). These luminaires are designed to emit a beam of light that changes colors by modifying the wavelength [10].

To achieve a successful analysis process from the use of these lights, it is necessary to eliminate the sources of natural light, determine what biological evidence will be sought and choose the type of luminaire that will provide the light with the wavelength that will allow observing what searched, as well as the filter to be used to observe and photograph the objective.

It is essential that in the expertise carried out with forensic lights, the equipment and the wavelength be identified, since it is proven that UV light applied at a determined intensity and time, alters DNA, a quality that is used to sterilize food, and even animals of experimentation [10–12].

2.4 Removal and Lifting of Marks, Traces and Vestiges (Sampling of Material Evidence) [12–15]

Physical evidence can be anything, from huge objects to microscopic particles originated in the commission of a crime and is collected at the crime scene or at related locations.

Considering all the sources of information available in investigations, physical evidence plays a fundamental and valuable role. When physical evidence is properly recognized, manipulated, collected, and preserved, it provides the best perspectives for providing objective and reliable information about the incident under investigation. However, even the value of the most carefully recovered and well-preserved evidence become useless if the chain of custody is not properly kept.

From the beginning to the end of the forensic process, it is essential to be able to demonstrate the "life" of the physical evidence to ensure their traceability from the crime scene to the courtroom. Therefore, locating and identifying physical evidence at the crime scene, as well as, identifying what is missing, is one of the most difficult tasks. Also, the most important evidence may not be the obvious or visible to the naked eye, could be a microscopic one.

Normally, the recognition of physical evidence begins with an observation of the scene. Based on this and considering the context of the case, the possible hypotheses, the nature of the incident, and the characteristics of the surfaces that may keep different types of physical evidence. The searching process implemented will be flexible and methodical. Basic procedures may be necessary to detect physical

evidence, such as using finger powders to reveal fingermarks or using chemicals to visualize traces of blood.

Once the physical evidence is recognized, collecting and packaging methods are used (e.g. taping, tweezers, swabs, bags or boxes, sharps containers, etc.) Each piece of evidence is labeled, sealed, and preserved according with the legal regulations of each country. Evidence collection may need to be prioritized to avoid degradation or loss. Documentation is an integral part of the collection process, including the exact location of evidence prior to collection.

Under difficult conditions, it may be preferable to collect a larger number of evidence and carry out the selection at a later stage of the investigation. Recognizing and collecting evidence requires experience and training.

As part of the evidence collection process, in many cases, collect evidence from the scene and control samples are necessary, for example, when the investigator collected fire debris as a physical evidence from an arson scene. In situations where the evidence is large, representative samples should be collected, for example, when large quantities of drugs are seized. This explains that the sampling process requires experience and training.

2.4.1 Why Is the Evidence Identification and Collection Process Are Important?

- The evidence which is not identified and collected at the crime scene, could be inevitably lost and that can change the course of the investigation.
- Collecting only what is most obvious or attracts the investigator attention, can result in the loss of other relevant evidence.
- The appropriate methods of collection, packaging, and storage make it possible to avoid degradation, contamination, or loss of the evidence.
- The indiscriminate collection of unnecessary evidence can overload the laboratory with objects of no importance and, therefore, hinder the investigation.
- The experience is important because the investigator will understand the case and that will help to collect the right evidence to help solve the crime (Table 2.1).
  The investigative team can take evidences of different crime scene. The type of evidence taken, collected and preserved will depend mainly on the objective of the investigation. All the evidence must be accompanied by its respective chain of custody all the time.

2.4.2 Quality Control in Evidence Collection at the Crime Scene

Whatever the origin of the evidence collected at the crime scene, it is essential to have in mind that forensic evidences are unique and unrepeatable, and investigators must preserve the integrity of the evidence collected and avoid cross-contamination, so the laboratory gets the evidence in ideal conditions to do the analysis and deliver

Table 2.1 Finding and Handling Evidences [15–17]

What evidence can be found at the scene of the crime?		Probative value	Ex. cases in which they are	Recommendations
→ Powders → Liquids → Tablets → Traces of powders invisible to the naked eye → Plants, plant material	Possible illicit drugs	Detection and identification of drugs or precursors, purity, origin and manufacturing methods	• Production, trafficking and misuse of illicit drugs	Safe transport and storage, to avoid their disappearance in seizures Safety measures when taking samples
→ Latent or visible fingerprints	May contain enough DNA for genetic profiling	Personal identification	• All cases in which the offender has touched an object	Very fragile The examiner's gloves avoid leaving their own fingerprints, but do not
			or surface with his bare hands (drug packages, stolen objects or vehicles, homicides)	Prevent the destruction of existing fingerprints Easy contamination and degradation of biological material
→ Visible or latent footwear footprints		Brand and model of the shoe or tire Individual shoe or tire identification Stopping distance calculation Reconstruction of traffic accidents	• Burglar • Homicide	Destruction by environmental factors, outdoors
→ Tire tracks			• Car accident • SS leak	
Visible or latent biological material → saliva → blood/blood stains	They can contain enough material for DNA testing	Identification of the type of biological material Species identification Individual identification	• Violent crimes • Sex crimes • Trafficking in persons • Homicide	Risks inherent of handling biological material Easy sample contamination Easy degradation (shipping and storage precautions)

(continued)

Table 2.1 (continued)

What evidence can be found at the scene of the crime?		Probative value	Ex. cases in which they are	Recommendations
→ semen → hair → epithelial cells				
→ Human remains	Whole or fragmented, recent, decomposing or skeletonized corpses	Body identification cause of death manner of death Postmortem Interval	• Natural death • Accidental death • Homicide • Suicide • Multiple victims • Natural disasters • War crimes • Terrorism	Risks inherent in handling biological material Easy sample contamination Easy degradation (shipping and storage precautions)
→ Bones	May contain enough DNA for profiling	Body identification Species determination		
		Determination of sex and age		Adequate and dignified management of human remains Respect and consideration for the bereaved Debris may contain other evidence: projectiles, fibers, debris Planimetric- written and photographic fixation of the body before lifting Indispensable medical collaboration
→ Teeth	May contain enough DNA for profiling	Identification of the body Age estimation	• Charred, fragmented or decomposing bodies	
→ Bite marks		Identification of species/individual of the biter		Easy contamination and sample degradation

	May contain enough DNA in saliva for profiling		• Homicide • Sex crimes • Child abuse	
→ Dark spots on hands, clothing, injuries	May correspond to shot residues	Shooting distance Gunpowder manufacturer/type identification	• Homicide/injury/other crimes committed with firearms	Sampling as soon as possible Take samples before moving and/or washing the examined items Protect the hands of the deceased before moving
→ Glass shards (any size)		Direction of impact Glass type/ manufacturer/trademark	• Theft in property • Traffic accidents	
→ Textile fibers, threads, fabrics		Type and color of clothing	Violent contact Transportation of corpses (e.g. fibers + stains + hair)	Due to their small size, they go unnoticed, they can be lost
→ Human hair or animal hair		Species/ethnicity/personal identification		The sequence of sampling methods is important to optimize it
→ Paint chips (any size)		Brand and model of a vehicle Restrict search scopes	Vehicle robbery Traffic accidents	
→ Tools → Firearms → Projectiles	Revealing and fixation of searched/deleted and recovered elements.	Brand, model/manufacturer/ country or place of manufacture/ codes, etc. Conventional/modified, handcrafted weapons Serial numbers preserved/erased Single/ multiple projectiles/caliber/ manufacturer/weapon relationship (fields and striations) Elements impacted on the way: paint, glass, fibers, organic remains	Theft in real estate Arms trafficking Organized crime Violent crimes Homicides Vandalism Suicides	Safety measures when picking up a firearm and disarming it Protect gun edges, tool tips, firearms bore scratches, to prevent damage or modification Tools and weapons can have other types of evidence: organic fluids, fingerprints, residues Visualization and recording of searched/deleted and recovered elements
→ Identity documents/ passport	Security elements of official documents	Typewriter, computer, printer, ink Authenticity official document	• Suicide note • Fraud • Testament	Writing suicide notes comparison Documents can have other types of evidence: organic fluids,

(continued)

Table 2.1 (continued)

What evidence can be found at the scene of the crime?		Probative value	Ex. cases in which they are	Recommendations
→ Banknotes → Other official documents → Handwritten/ typed notes		Author of manuscripts and signatures	• Organized crime • Illegal border crossing • Identity substitution	fingerprints, residues, traces of drugs of abuse, footprints, footwear
→ Signed documents			• Trafficking in persons • Illicit drug/arms trafficking • Money forgery/identity documents/invoices	Visualization and fixation of searched/deleted and recovered elements

the results the to the court. The integrity of the evidence can make a huge difference during their analysis and the results the investigators can get [18].

To ensure the quality of the evidence collected, the first thing that the crime scene investigator must do is to avoid cross-contamination during the packaging process and also keep the chain of custody.

Is very important that control samples of the evidence are collected together with the physical evidence at the scene. The evidence and the control sample should be analyzed together. This will not only guarantee the quality of the results but also show that the expert followed the Standard Operating Procedures "SOP" of the forensic laboratory.

2.5 Examination of the Corpse or Remains

2.5.1 Objectives of the Examination of the Corpse at the Crime Scene

2.5.1.1 Processing/Analysis of the Scene (Body)

The medicolegal investigation at the crime scene has four objectives. Some of them can be achieved at the moment; others require autopsy or laboratory tests.

- The first objective is the ***identification of the body.***
- The second is to determine the ***postmortem interval***
- The third is to ***determine the cause of death***[2]
- The fourth is to determine the participation of third parties, that is**,** ***the medicolegal manner of death***[3]

2.5.1.2 Identification

Identification can be carried out by three methods,

(a) ***Presumptive identification***: it can be obtained through identification documents, recognition by relatives, and/or because the victim is the owner or is linked to the property or vehicle where the deceased was found.
(b) ***Positive or scientific identification***: Using any scientific identification method, such as fingerprints, DNA, dental records, medical implants, among others.
(c) ***Circumstantial identification***: It is the identification by means of other characteristic elements of each victim, such as clothing, tattoos, earrings, rings, etc.

2.5.1.3 Postmortem Interval [19–24]

Traditional estimates of the date of death or postmortem interval have been based on a series of observable changes in the body, which occur in a more or less predictable

[2] https://www.ncbi.nlm.nih.gov/books/NBK526015/.
[3] https://www.ncbi.nlm.nih.gov/books/NBK526015/.

and successive way, called ***cadaveric phenomena***. These include livor mortis, algor mortis, rigor mortis and similar changes.

Unless there is a witness to the death or concrete physical evidence to indicate the time it occurred, the postmortem interval should be reported as a time range, since many factors affect the appearance and development of postmortem changes.

Contrary to popular belief, estimating time since death is not an accurate science. [19]. In reality, there is no single precise marker to determine the postmortem interval [25].

The Place Where the Corpse Is Found [13, 26]

The decomposition process depends on a large number of variables such as temperature, relative humidity, the type of vegetation, the pH of the soil, the seasonal season and the circumstances of death.

For decades, it was argued that, at the same temperature, the degree of decomposition of a corpse in the open air, water or buried, has been estimated at 1:2:8 respectively. That is, the degree of putrefaction in a day outdoors would be equivalent to 2 days in water and 8 days buried. This is known as Casper's Rule (1888).

Although today it has been proven that both statements have many exceptions, it should be noted that buried corpses and those submerged in liquids decompose slowest than those lying on the surface.

Forensic Entomology as a Method to Establish the Postmortem Interval [22]

Since the end of the nineteenth century, it is known that corpses are successively invaded by different types of insects, mites, and other arthropods in a predictable way, which is why they are called "death squads". Because of this, they are used to provide an estimate of the postmortem interval.

The study of insects, or forensic entomology is the study and knowledge of insects associated with their arrival at the corpses, directed mainly towards the determination of the postmortem interval (PMI) or date of death. For this reason, it has become an important specialty of forensic sciences, which helps clarify doubts regarding corpses found in particular circumstances [22, 27].

Cadaveric Phenomena or Corpse-Destroying Phenomena [13]

As soon as death occurs, changes begin in the physical nature and/or appearance of the body, which after a few hours become visible. These changes, which are not strictly related to cause of death, have traditionally been used in PMI estimates.

Cadaveric phenomena can be classified according to three criteria:

- According to the ***moment*** in which they occur o Immediate
  Mediates
  Late
- According to its ***nature***
  Physical
  Chemicals
  Biological

– According to its ***influence on the preservation of the corpse***
  Destructive phenomena
  Conservative phenomena **Immediate cadaveric phenomena** are recognizable during the first 3 h after death and consist of the lack of vital signs, without major observable changes in the corpse:

– Lack of pulse
– Lack of blood pressure
– Shortness of breath
– Lack of reflexes
– Corneal opacity (Stenon-Louis sign)

After 3 h, the ***mediate cadaveric phenomena*** begin to be appreciated, which are identifiable until putrefaction begins. The first sign of decomposition is the green abdominal (caecal) spot. Although the color change is generally localized to the right iliac fossa, it may appear elsewhere in the abdomen.

The ***mediate cadaveric phenomena*** are:

– Autolysis (biological phenomenon)
– Cooling (physical phenomenon)
– Dehydration (physical phenomenon)
– Cadaverous lightness, pale (physical phenomenon)
– Cadaveric rigidity (chemical phenomenon)

Autolysis

It is the self-destruction of organic tissues by the hydrolytic enzymes contained in the cells themselves; autolysis is the earliest of the transformative processes in corpses.

Cooling or Algor Mortis

Once death has occurred, the body stops regulating its internal temperature and this begins to approach room temperature. In most cases, this involves cooling the body to room temperature, most often within 18–20 h. There are several formulas for calculating the cooling rate, but none are completely satisfactory. Any estimate of postmortem interval obtained on the basis of cooling should be limited to the early stages of death (24 h or less) and interpreted with caution.

Several factors can influence the body's cooling rate and the rate at which it occurs. The size of the individual is an important factor: the smaller the individual, the faster the cooling, under the same conditions. Exposure to sunlight or heat can also influence the rate of cooling, as can clothing. The temperature most used to calculate the rate of cooling is that of the liver, although rectal temperature can also be used. However, estimates based on these approaches should be presented only in general terms.

One of the most complete methods to determine the date of death is the nomogram proposed by Henssge [28]. In it, he proposes to relate the rectal temperature, the ambient temperature and the weight of the corpse. However, Henssge himself points out that the method is reliable in the early postmortem period, defined as the time it takes for the corpse to equalize its temperature with that of the environment. This period is of great importance since it is the time window in which the temperature is used to estimate the PMI [29, 30].

Dehydration

One of the manifestations of dehydration is the appearance of the ***sclerotic black spot***. (Sommer-Larcher sign). After death, if the eyes remain open, the exposed part of the cornea will dry out, leaving a red-orange to black stain. This is called a "sclerotic black spot" and can be misinterpreted as hemorrhage. Unlike hemorrhage, it will have a distribution corresponding to the position of the eyelids.

Cadaverous Lividities, or Livor Mortis

Also known as lividity or hypostasis, it is a physical process. As long as the individual is alive, the heart is working and the blood circulates. When death occurs, circulation stops and blood begins to settle, by gravity, in the lower portions of the body. This results in a generally purplish coloration of said sloping parts.

Although this process begins immediately, it begins to be visible about 2 h after death at 4–6 h. At this time, the blood is still liquid, and pressing the skin will cause the blood to flow out of the area (pale) and return once the pressure is removed. This situation continues until 9–12 h after death, at which time the pattern will not change, and the lividity is said to be "fixed".

Cadaverous Stiffness, or Rigor Mortis

It is a ***chemical change*** that results from the acidification of the muscles and the degradation of molecules within. Immediately after death, the body becomes flaccid and flexes easily. By reducing cellular pH, blocking chemical bridges are formed between muscle proteins and stiffness occurs. It usually begins 2–6 h after death, in the muscles of the face and neck and spreads to the lower segments until it becomes general at approximately 12 h. It usually lasts 24–48 h, after which the muscles begin to "relax" by autolysis. The onset and duration of rigor mortis are governed by two factors: temperature and the body's metabolic state. Lower ambient temperatures tend to accelerate the onset of rigor and prolong its duration, whereas the opposite occurs in warmer environments.

Late Cadaveric Phenomena

These are those that begin with putrefaction, a fermentation process of bacterial origin, where fungi, insects and mites also intervene.

Insects and other arthropods are the main organisms involved in the major decomposition of the body. They reach the exposed remains shortly after death, often in less than 10 min.

Table 2.2 Decomposition in the Open Environment Evolves in Four Phases

	Period	Approximate duration
1	Fresh	Days
2	Bloat	Weeks
3	Active decay	Weeks/months
4	Advance decay	Months
5	Skeleton, remains, dry	Months/years

Detachment of the Epidermis

The outer layer of skin, called the corneum stratum, is made up of dead cells; however, it plays a vital role in conserving water and protecting the underlying (living) skin. This layer is constantly shed and is replaced by the underlying epidermis. When the person dies, the epidermis begins to separate from the underlying dermis due to the production of hydrolytic enzymes from the cells at the junction between the two layers. The epidermis is then easily detached from the body; This detachment begins with the formation of vesicles and in some cases, the skin of the hands can be separated from the underlying dermis as a complete or relatively complete unit, in the form of a "glove" and can be removed as an intact unit. That detached skin can be used to obtain fingerprints, often with better results than if the skin remained on the hand. Epidermal detachment can be observed after a few hours, in corpses exposed to high temperatures or directly to the sun. It does not occur spontaneously; it requires a tangential force to occur [19] (Table 2.2).

It should be noted that these stages merge with each other and it is impossible to separate them into categorically distinguishable entities. Also, a body rarely breaks down evenly. Part of it may be reduced to a skeleton, while another part continues to have soft tissues [13].

Factors that Retard Decomposition, or Conservative Phenomena of the Corpse

There are three broad categories of factors that retard decomposition: physical barriers, chemical barriers, and climatic factors.

Physical Barriers

Physical barriers to decomposition are those that prevent the body from contacting the environment. A buried body does not decompose as quickly as one exposed on the surface. A body enclosed in a sealed casket or placed in a sealed container will exhibit delayed decomposition.

Chemical Barriers

The embalming process prevents decomposition of the body, replacing natural biological fluids with preservative fluids. The presence of insecticides on, or near the body can also contribute to delaying the onset of insect activity for a period of time.

Climatic Factors
Low temperature delays decomposition. Bacterial growth and insect activity can slow or even stop. At temperatures below 6 °C, most of the insect activity ceases. On the contrary, high temperatures until certain degree, and humidity can contribute to the rapid deterioration of the corpses, altering their appearance and the morphology of the lesions. The fauna present in hot climates also increases the probability of the appearance of artifacts (Pachar Lucio 2013).

Regarding the preservative phenomena of the corpse, the main ones are mummification and saponification or adipocires.

Mummification
In a dry climate, with temperatures alternating between very high and very low, parts of a body or all of it will become dehydrated. The low level of humidity inhibits bacterial action and, in part, the action of insects and other scavengers. Dried tissues and skin will have a parchment appearance and are preserved for a long time with minimal changes. The process can take several weeks.

Saponification (Adipocere) [13]
It is the result of a process of hydrolysis of fatty tissues in wet anaerobic situations, such as immersion or in flooded pits. The tissues take on a waxy appearance and consistency; this process requires a period of several months to complete.

2.5.1.4 Cause of Death

The ***cause of death*** has ***epidemiological importance***, since knowing it is useful to understand the behavior of acute, chronic, congenital, or infectious diseases in a given community. In addition, it has ***legal importance***, to investigate possible third-party responsibilities, insurance coverage and characteristics of the burial, in some cases.

From a medicolegal point of view, the cause of death can be classified into five types:

- Disease
- Asphyxia
- Trauma
- Poisoning or intoxication
- Undetermined.

External Examination of Bodies in Different Causes of Death

Disease [6, 31–33]
A death due to illness can become suspicious of criminality, if it occurs without a previous diagnosis, or precedes a violent event, generally traumatic, such as someone dying of a heart attack while driving and their vehicle crashing later, after losing the address. Also suspected of criminality are deaths that occur in a public place, such as stadiums, shopping malls or workplaces, or in remote and untraveled places.

In the external examination of a corpse, the greatest difficulties are the absence of manifestations of the disease, and the presence of phenomena that can be confused with a violent death. In a corpse without superficial alterations, elements such as medicines or medical documents present in the place can help. In case of doubt, it is necessary to corroborate the diagnosis by autopsy, and make the differential diagnosis with intoxication.

On the other hand, certain diseases produce changes in the skin and mucous membranes, hemorrhages and other externally visible alterations, which can simulate trauma, poisoning or asphyxia, and also, the action of third parties: leukemias, hemorrhagic diseases and immunological or allergic reactions, among others. The definitive cause will be established through an autopsy.

Sudden Infant Death[4, 5] [34]

It is the one that affects a child under one year of age, without apparent cause, despite having carried out:

- Examination of the place of the fact
- Review of your medical history
- Complete autopsy

  During crime scene work, it is necessary:
- Record who was at the time of death
- Write down all circumstances in the child's clinical file: dress, naked, co-sleeping, position, environmental heat

  People with knowledge of the risk factors and with legal authority should visit the scene of the incident as soon as possible and talk with the child's partners.

  The interview should be in a prudent, respectful tone, considering the degree of emotion of the relative, but without losing sight of the possibility of an intentional fact.

  The autopsy must be performed within 24 h of death, and includes examination of the cranial content, histopathological study, imaging, toxicology and metabolic and genetic studies if necessary.

Asphyxia

Asphyxia is a type of death that occurs from lack of oxygen. It can be caused by a decrease in the pressure of O_2 in the ambient air, by obstacles in the respiratory tract at any level, by difficulties at the pulmonary level, the alveolar-capillary barrier, the capacity to transport O_2 by hemoglobin, or by alterations of the enzymes that form the respiratory chain. Many of the agents that cause alterations in hemoglobin or in the cellular respiratory chain are toxic substances: there is an overlap between asphyxia and intoxication.

[4] http://sids.org/.

[5] http://www.nlm.nih.gov/medlineplus/suddeninfantdeathsyndrome.html.

The most common suffocations—those caused by obstacles to the entry of air into the lungs, are called mechanical asphyxia:

If the obstacle consists of the occlusion of the mouth and nose, it is a suffocation by suffocation. If the agent used is the aggressor's hand, finger and nail marks, and even the hand, may remain around the mouth and nose of the deceased. Forensic photography with alternate lights can be helpful in revealing these types of fingerprints[6] [35]

In asphyxia due to suffocation with a soft object, such as a pillow, stains of saliva or mucus may remain on them. Such items must be recorded and photographed with alternate forensic lights [36].

Details such as the content of the nasal and oral cavities, the cervical sulcus, the distance between the feet and the ground in suspended corpses, or the proximity to objects or surfaces against which the corpse may have been hit during the convulsive period, must be documented. and be photographed.

The link or loop used, the presence of ***knots or loops around the neck***, must also be documented and photographed.

A corpse taken from the water may have died from asphyxia by submersion ***or other cause***, and can present injuries by falling or dragging, or by assault. Foamy pompoms, traumatic injuries and their signs of vitality should be described and photographed.

Trauma [35]

Trauma is caused by agents external to the body. Generically, three types of causative agents can be identified (Table 2.3):

Poisoning or Intoxication

Many toxic agents do not produce apparent physical changes: the diagnosis of poisoning is made by identifying the toxic substance and/or its metabolites in the tissues or body fluids of the individual, that is, by means of an autopsy and toxicological examinations.

At the crime scene, toxicological orientation tests can be used, which generally result in a color change or cloudiness. Its positivity must be corroborated by techniques of certainty in the laboratory.

2.5.1.5 Manner of Death

The manner of death, is usually a combination of the cause and the circumstances. Such as, older adult + good hygiene conditions + pneumonia + no external injuries + deceased in bed = natural death. Pneumonia + no external injuries + death in a hospital waiting room could be homicide by omission of medical care. In accordance with the above, it is agreed that the forms of death are:

[6] Photographic camera CANON EOS 800D multispectral.

Table 2.3 Trauma

Type of agent	Type of injury
Mechanical physical (rubbing, hitting, impacting, twisting, crushing)	Blunt – Closed (ecchymosis, hematoma) – Partial breakage of the skin (erosion, excoriation) Wounds – Blunt wounds – Sharp – Stinging – By firearm, shooting
Thermal, radiant or electrical physical	Burns
Chemical	Burns (Local) Poisoning (systemic)
Biological	Bites – Human – Animal Stings

– Homicide
– Suicide
– Accident
– By natural causes
– Undetermined

In different countries, there are different regulations to proceed:

In an inter-agency protocol, all of them must ensure that their procedures are in accordance with the law. In a pandemic situation, it is common for untrained individuals, administrative employees, or funeral home employees to be asked for collaboration. The employees of each organization must be duly informed about the risk involved in each stage of handling the deceased, as well as the individual protection measures and the environment that must be taken. In particular, the risk of transmission of infections and of injuries due to handling must be considered [37–40]

One of the experiences learned in the 2015 due the Ebola epidemic (Africa) is that the handling of corpses was one of the main modes of transmission of the disease. Humanitarian forensics learned from this experience that untrained first responders should not come into contact with human remains of infected people during outbreaks of highly contagious diseases [41]

In a pandemic, several countries have chosen not to perform autopsies or restrict them to the maximum [38]. This is the case of deaths confirmed by COVID-19, or in those in which the exposure data and clinical picture are clear. However, inevitably, there will be cases of people with no medical history, or without identification, or COVID-19 positive, but with violent or suspicious death. In such circumstances, a medicolegal autopsy may be essential [39].

Forensic Photograph of the Corpse [42]

During the examination of a corpse, both external injuries and particular signs, foreign bodies or added substances, must be carefully documented, through descriptions, diagrams and photographs.

The investigator should always take overview photographs, midrange, and close-up without and with a scale, and those that are necessary to illustrate the details.

The scales should be using according the evidence, the scale should avoid any distortion in the picture.

In the case of lesions or spots on the body, whenever possible, some reference point should be included, such as an ear, lip corner, finger, etc., that allows them to be anatomically located.

The expert report with photographs must include some information of the case, the location of the place where it was carried out, the type of camera used, the date it was carried out, name of the expert who took the photographs, description basic of what you want to show in each of them. [43]

Examination of Deceased in Hospitals

In some cases, the forensic team must examine corpses in hospitals. These can be patients who were hospitalized for injuries and died, people who died during their way to the hospital, but whose diagnosis was not made, or cases in which the relatives have made a complaint for alleged negligence of the Health teams.

If there is a possibility that the body is contaminated, it is imperative that the investigating team use personal protective equipment. A useful strategy to prevent the release of microorganisms into the environment when handling the corpse is to place a mask that covers the mouth and nose. Regarding the environment, it is worth remembering the forms of transmission of infections: close contact, through nasal secretions, saliva or mucus, and also by stools, which may not be visible, but are present on bedding, mattresses and objects [44]

Catheters, tubes, syringes, probes or other items that have been connected to the patient should NOT be removed,[7] due both to the risk of spillage or projection of biological fluids, and the possibility of having produced damages related to the cause of death. Ensure that any body fluids leaking from orifices are contained [45, 46].

- Keep both the movement and handling of the body to a minimum.
- If the corpse presents any type of perforation or wound that has been caused by some material connected to its body, cleaning with a technical solution should be carried out and covered with impermeable material, avoiding spillage of body fluids.

[7] https://www.nhft.nhs.uk/download.cfm?doc=docm93jijm4n1413.pdf&ver=23960.

2.6 Clothing Examination [34]

In examining victims, clothing can provide a great deal of information. Often, this valuable source of data is not considered, or it is lost when it is eliminated in the emergency services where injured people go, or after the body is undressed during an autopsy, probably due to ignorance or lack of regulations in this regard.

The richness and variety of traces, footprints and indications that can be found on the clothing is such that they can be considered a real crime scene by themselves [47]

Due to their location, adjacent to the surface of the body, clothing is "injured" before the skin, it retains waste and organic fluids, releases fibers, burns or stains following certain patterns. Most of the people who are victims of accidents, self-inflicted injuries or the action of third parties, wear one or more garments at the time of the events. Just as the brand of the vulnerable agents is more recognizable and typical on the skin and mucosa of the affected person than on the internal organs, due to being more exposed, the clothing constitutes an even more superficial plane, in which blows, cuts, perforations can be identified, residues, stains and other traces, footprints and signs. Such findings may complement the results of the injury examination or autopsy, or even be the only evidence available in a court case.

The characteristics of the clothing allow the forensic investigator to relate its wearer to his daily activities, professions or trades, socioeconomic status, groups of belonging, place of origin, age group and even moods. Clothing represents us, protects us and places us within a gender, age, ethnicity, time, activity and customs. It can also leave marks or imprints on the body. In short, it identifies us, and can be an element of great value in the recognition of people, for example, in case of massive disasters or missing persons [48].

https://www.nhft.nhs.uk/download.cfm?doc=docm93jijm4n1413.pdf&ver=23960

2.6.1 The Clothing: Terminology

A practical method to easily document clothing is the one used by the National Institute of Legal Medicine and Forensic Sciences of Colombia. [49]. It consists of diagrams of the most common garments, on which stains, tears and other findings of interest can be indicated.

Just as we use surface anatomy and anatomical landmarks to describe injuries, there are also landmarks in clothing: cuffs, collar, side seams, buttonhole trim, button trim, waistband, bottom hem, among others, following the same logic of anatomical position. Similarly, buttonholes and buttons are numbered from top to bottom.

Other important descriptive elements are the color, the material, the degree of use, the relationship or not with the context: characteristic work clothes in a work accident, stains that could lead to the trade or profession, thick clothing in high mountains, a party outfit and quirky makeup on a person with a psychiatric illness. Size is a highly variable factor: although it appears on the garment label, there is no standardization of sizes worldwide.

Textile fiber samples, like many other trace evidences, help narrow the circle of individuals to be investigated by the police in a given case, whether they are victims or suspects. Given the smallness and fragility of the samples, the team who collect them must proceed carefully, and avoid contaminating the clothing with their own clothing or hair, using protective equipment.

The closeness between two people and the contact with surfaces, can be demonstrated by the discovery of textile fibers of one in the other, as is the case of armed robberies or some terrorist attacks ([50]). The systematic and protocolized examination, the use of different study techniques and methods, the correlation with other antecedents, and the possibility of documenting the findings and presenting them in court, make the study of clothing a tool of remarkable forensic value.

When undressing a corpse, the cadaverous phenomenon of rigidity is often disturbed. If it is necessary to cut the garments to remove them, the cuts should allow for the subsequent reconstruction of the garment (straight, net). All of the above must be recorded in the crime scene report, to avoid misinterpretation.

2.6.2 The Examination of Clothes in Special Cases

Some situations in which the clothing examination is essential:

- Identity verification
- Assaults and sexual assaults
- Traffic accidents
- Kidnappings
- Clothing as a cause of death

2.6.2.1 Identity Verification

In the face of massive disasters, when it is imperative to implement rapid solutions for the identification of persons, the examination of clothing can play an important role. The collation of premortem information on clothing and personal accessories and data with the remains found, it is faster and easier than other identification methods.

Clothing must be photographed with the same number or code that is given to the body or body segment. As far as possible, garments or fragments of them should not be removed from the body [48].

2.6.2.2 Aggressions, Traffic Accidents, and Sexual Assault

Textile fibers are collected at different times in the forensic investigation, especially at the crime scene and during the autopsy.

The victim's clothing can be examined for bodily fluids such as blood, urine, stool, semen, or saliva. Hairs, pubic hairs, or eyelashes can also be found, which must be compared with samples obtained from the victim. In cases of traffic accident, tire tracks, lubricants, fragments of vehicle paint may remain on clothing, which are not visible on the skin. On the other hand, during extrication maneuvers

(extraction from a vehicle), alterations or artifacts may occur, both in the clothing and in the tissues of the corpse. It is also important to examine the clothing of a deceased in relation to the injuries they present. The coincidence between the number and characteristics of tears and skin lesions must be recorded. It is helpful to photograph tears or cuts with a contrasting color element underneath, to emphasize their shape and size.

2.6.2.3 Abductions

In cases of kidnapping, it is necessary to take comparison samples of the textile fibers of the clothing, as well as the hair and hair of the corpse, to compare them with possible places where the victim could be transported, such as trunks or vehicle seats, or rooms where they could have been held.

2.6.2.4 Clothes and Cause of Death

Clothes such as scarves, ribbons, ties, or parts of T-shirts or sweaters may cause marks on the neck, and occasionally cause death by being caught during a slide, fall, or pull.

It is important to describe and photograph the circumference of the groove (complete or incomplete), the presence of knots, buckles or other accessories, the presence or absence of articles of clothing under the link.

2.6.2.5 Collecting and Conservation of Clothing

Clothing should be kept as far as possible, protected from light and humidity. Each garment must be kept in a separate, clean and dry container. The most recommended material is a paper bags or envelopes (brown paper, wood paper). At the same time, because it is absorbent, it prevents the proliferation of fungi, which usually interfere in the studies of biological evidences.

2.7 Body Management

The handling of the body for its transfer to the place where the autopsy will be performed or it will be delivered to family members must be done in accordance with local regulations, which may be different in different countries. If it is suspected that the death is related to an infectious disease and occurred at home, without diagnosis and without medical attention, the body must be wrapped in the same sheets of the bed where it was, to then be deposited in a body bag ideally 400 microns thick, with a zipper, to then place it in a new bag with the same characteristics and proceed to a safe transfer for those who must handle it.

2.8 Technological Means in Its Right Measure [51]

The information provided by highly complex technological instruments can be great allies, or constitute unnecessary distortion factors. Technological advance by itself encourages people to grant high credibility to their products, without bearing in mind that the principle of its operation is, generally, a "black box" only understandable to those who made the instrument, who are nothing but people. Fallible that contribute their best effort to detect, dimension or clarify certain phenomena that were previously not feasible to identify.

Overconfidence in technological means and lack of knowledge can affect the investigation. Emerging technologies provide greater emphasis to the extent that researchers or system operators manage to obtain more and better information from the data provided by the instrument. However, this does not replace human thought or the flow of its logic. A particle of textile fiber, wood, or a certain metal that is detectable as it was not before, can be gravitating in the investigative process, only if its result is inserted in a time, place and sequence consistent with the rest of the investigation.

2.9 Human Error in Criminal Investigation: How to Avoid It [52–54]

In movies and television shows, a detective solves complex cases using deductive skills, high technology, specialized computer programs, hard work, and luck. In these fictional stories, good wins, evil loses, and justice triumphs. But, in reality, things do not always turn out that way: sometimes the case remains unsolved, the criminal remains at large and justice cannot be done.

Errors in judicial investigation can have serious consequences. Unsolved crimes, unsuccessful trials, innocent convicts, criminals unpunished, and wrongful convictions all contribute to the discredit of the criminal justice system. On the other hand, when you consider the cost of some important research, the loss of effort can be onerous.

Most investigators are competent and dedicated professionals seeking to solve their cases and detain the right people. So, what causes a crime investigation to fail or an innocent person to be blamed? The answer lies in certain subtle traps, which can disrupt the entire process. No one is immune from falling into such traps: some of the best researchers, scientists and judges have been victims of them; However, contributions from Forensic Psychology, Forensic Statistics, Intelligence Analysis, Law and Philosophy of Sciences, have suggested some explanations, which can be classified into three groups that can be combined with each other:

(a) Cognitive biases
(b) Probability errors
(c) Traps in the organization

2.9.1 Cognitive Biases [8]

2.9.1.1 Limitations of Perception and Memory

Human beings often perceive what they expect or believe they recognize what they know, and therefore can come to dangerously premature conclusions. On the other hand, it should be remembered that communication is doubly subjective, since it involves two people. What the speaker wants to say, what he actually says, what the listener hears, and how he interprets the content of the communication, may not be the same. Subjective words, such as "tall", "young", "probably" or "dangerous", have various meanings, depending on the situation and the experiences of the speaker and the listener.

Since people tend to remember the positive and forget the negative, researchers may give more weight to the evidence that supports their hypothesis than to the clues that weaken it. ***Being unbiased and open is the best way to accurately evaluate new or different information.***

2.9.1.2 Intuition

Most cognitive functioning occurs subconsciously, including perception, information processing, memory, and some decision-making methods. Humans use two types of decision making, intuitive and rational. Intuition is situated between the automatic operations of perception and conscious reasoning, it is not a paranormal ability or a form of extrasensory perception and although it functions at a level below consciousness, it is based on normal sensory information.

Different situations require different types of judgment. Faced with doubtful and incomplete data, or in chaotic and uncertain conditions, such as those faced by a policeman on the street or a soldier in battle, intuitive decision-making is preferable. On the other hand, if you have reliable and adequate data, and there is time for a correct analysis, reasoning is what produces the best results. For this reason, complex and rule-governed tasks, such as investigations or court trials, require careful analysis and the use of logic.

2.9.1.3 Heuristics and Biases

Clear and rational thinking is not easy. The brain does not connect easily with uncertainty, and scientific research has shown a poor correlation between confidence and accuracy. Past a certain threshold, increasing the amount of information leads to greater confidence in the analyzes, but not necessarily to greater accuracy. Psychological investigators have identified many heuristics and biases, and some of them can be especially problematic for criminal investigators.

2.9.1.4 Anchorage

The anchor heuristic refers to the strong influence that the first available information has when starting an investigation determine the first approximation, jeopardizing the path to a correct conclusion. Unfortunately, at first glance, many crimes are very different from what they actually appear to be.

2.9.1.5 Tunnel Vision (Satisficing)

It consists of developing a research focused on a limited number of alternatives. The investigator focuses on an individual or incident in such a way that he "removes" other suspects and events from his understanding of the fact under investigation. "Satisficing" involves selecting the first alternative that seems good enough, which may work well for simple tasks, such as buying pliers, but is not suited to conducting a criminal investigation.

2.9.1.6 Availability

It refers to the ease with which examples from previous cases come to mind. The person makes judgments based only on what he remembers, and not on the totality of his knowledge and experiences. It should be noted that it is possible to remember recent events easily, but that it is difficult to find unpleasant events in memory. People use the availability heuristic to determine how common, or probable, a fact is, but in doing so they often consider only limited experience, which can lead to incorrect estimates of probability. The availability heuristic is particularly risky when investigating rare crimes, such as rape with murder of minors.

2.9.1.7 Framing

The presentation of the information influences its interpretation. The framing, or framing, implies that the information is always understood within a context. However, an artificial or inappropriate context can distort the understanding of the true scenario. Dramatic examples of this are observed in the courtroom, when the parties in conflict vary their position regarding the scope of the facts in dispute.

2.9.1.8 Representativeness

People often estimate the probability of an event, recalling a similar incident and assuming that both are identical. The representativeness heuristic is partly motivated by the need to categorize everything. However, similarity in one aspect does not necessarily mean similarity in the others; not all serial killings are for sexual purposes, nor do they affect women. Not all murderers are white, some have below average intelligence, nor do they commit their crimes in the same city where they live.

2.9.1.9 Cause-Effect Biases

Perceptions about cause and effect are susceptible to various mental biases and biases. The linkages of a crime can be affected if the investigator is unable to differentiate between internal (psychological) and external (situational) causes of an offender's conduct, when examining his modus operandi. For example, the level of force used by a rapist may depend on the degree of resistance of the victim and situational difficulties and the place where the attack occurs.

For its part, the phenomenon called "identity fallacy" maintains that major events must have important causes. Conspiracy theories are often rooted in those beliefs. For many people, it is difficult to accept that a spectacular event, or that affects an important person, could have a derisory cause.

2.9.1.10 Biases in the Evaluation of Evidence

Most of the problems that arise with physical evidence are the result of a mistake in the interpretation, and not of defects in the exams to which said evidence is subjected. Bear in mind that, a very important cause of error, is to ignore the context of the events, or of their protagonists, when making the interpretation, sometimes under the pretext of "not being prejudiced".

2.9.1.11 Confirmation or Verification Bias

On the other hand, consists of a type of selective thinking by which investigators notice or seek only evidence that confirms their theory of the case, while ignoring or refusing to seek information that contradicts their own. theory. For that reason, efforts to just verify and not falsify a hypothesis often fail; after all, a single item of contradictory evidence (e.g. DNA Exclusion) can outweigh a large body of evidence against a suspect.

Components of confirmation bias include failure to search for evidence (for example, verifying a suspect's alibi) that would refute the theory, failure to use such information if found, failure to consider alternative hypotheses, and failures in the evaluation of the diagnostic capacity of the evidence.

Sometimes data that appears to support one theory (or suspect) actually has little diagnostic value, as they can also be applied to other theories (or suspects). Hence, trial studies have shown that vivid information has more influence on judges than abstract data. Personal testimonials carry more weight than statistical information, even though this is a compilation of many personal testimonials. For its part, the vehemence of the descriptions of some eyewitnesses has led the authorities to initiate exhaustive investigations about, for example, "victims" of organized satanic cults; eventually many of them turn out to be just people seeking attention.

A fundamental point, and one that is often forgotten in forensic investigations is that what is, as what is missing, can be as important.

2.9.1.12 The Occam's Razor

Finally, it should be noted that "first impressions" often remain even after the initial evidence on which they were based has been discarded. Often called "the curse of knowledge," that fact can lead to wild theories, which people cling to, even though there is no evidence to support them. Such theories violate the principle of "Occam's razor",[8] also known as the "principle of prudence", which indicates that, if there is more than one possible explanation for an event, it is best to choose the simplest one (that is, the one with the fewest assumptions) to avoid the situation becoming complicate more than necessary. Researchers must adopt "Occam's razor", as an

[8] *Occam's razor: (or Ockham's)* is a principle of reasoning formulated at the end of the Middle Ages, and also known as the principle of prudence or simplicity. It is attributed to the Franciscan monk and philosopher *William of Ockham,* although it was known before him. This principle states: A reasoning based on fewer and simpler premises is more plausible. The fewer assumptions, the better.

important principle in science, for the exercise of their profession, always bearing in mind that the more complex theories, the greater the possibility of error.

2.10 Crime Scene Report [1, 55–57]

In the report, it is essential to consider the proper use of language, to avoid slipping biased expressions, subject to double interpretation or simply unnecessary because they do not contribute to the essence of the forensic information process: establishing what is known and envisioning what is needed to know. Therefore, it will be possible to establish those information elements that are established as essential for other researchers to continue the search and interpretation of successive findings.

For all the above, the report must be clear, specifying what has been obtained, this importance, and what was their result. It should not be forgotten try to identify who committed the investigated crime, also if possible establish if that was premeditated, hypotheses and different sequences to understand how the events occurred.

Consequently, only a progressive approach to the logical sequence of the temporality of the events will allow to rule out actors with a casual presence in the place, but at the same time, possible witnesses of what they saw or should have seen when approaching or passing through said site. The analysis constitutes a work element for the investigator, and it does not seem necessary to incorporate it into the report that goes to users, where the presentation of results prevails over details and reflections of the investigative process.

In summary, the report must be brief, directed, and rich in details, because they are the essential elements of forensic information, which will help to establish certainties and generate new investigative hypothesis. It should not be forgotten that a good report is valuable if it meets three conditions: ***short, effective and timely.***

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Small Size, Big Impact: Insects for Cadaver Examination

3

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Abstract

The fields of forensic entomology and entomotoxicology are developing areas of interest in this new era. These two fields are fascinating and are capable of accruing curiosity among researchers. Forensic entomology allows the investigator to estimate the post-mortem interval (PMI), and entomotoxicology enables us to find out if the decedent was intoxicated before death when body tissues are not available for toxicological analysis. The province of forensic toxicology divides toxic substances into special categories according to their chemical and analytical properties. This system of classification helps the investigator in identifying the actual toxic substance present in the biological or entomological samples. The present review is aimed at these two parameters while also focusing on the factors affecting the life cycle of insects feeding on the cadavers.

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3.1 Introduction

Small insects usually cause a big effect in the field of forensic science. Whenever the insects are encountered in any kind of crime scene or involved in legal issues, they are dealt with under forensic entomology, a specialized branch of forensic science. Forensic entomology is a vast field, and both entomology and legal science interact in this particular province. Usually, insects are found inside packaged foods, poisoning cases, termite damage, murder-related cases, and other such medicolegal cases. Cases of negligence in hospitals or due to myiasis might crop up. Pets can also get infected from myiasis due to negligence [1]. Myiasis is an infection caused by fly larvae and is more severe than the other general infections as the maggots are formed in a part of the body and they start feeding on the area. Insects generally come from the open fields and cause such infections.

Many disputes arise when insects and/or insect parts are found in stored products and issues of negligent preservation, packing, etc. crop up. Larvae and flies are commonly found in juice cans or other packaged food products. Insects are sometimes planted in restaurants and other eating places to defame a business house or to blackmail for money/take revenge, etc.

Insects are of great value in criminal cases like violence and murder and also in those cases where the cause and time since death are not known. Medicolegal entomology is concerned with insects which can be used as evidence to solve a crime. It also helps in estimating the minimum post-mortem interval (PMI) [2–7] and any drug intoxication before death [2, 3]. The PMI estimation depends on many factors like temperature, exposure of the body to the external environment, decomposition rate, geographical location, humidity level, and season [8, 9]. Many cases have been reported where the time of death is unknown, and insects of forensic importance have proved to be of great value. When only skeletal remains are found, then maggots can be used for drug analysis [10].

3.2 History of Forensic Entomology

Several species of insects get attracted to a cadaver at different time intervals. These insects feed, reproduce, or live according to the condition and preference of the cadaver [11–22]. The study of forensic entomology was first started and documented in China by the lawyer and investigator of the death of Sung Tzu in the thirteenth century [23, 24]. He described a case of stabbing in an open field of rice with the help of blowflies. After that, many painters, sculpture artists, etc. started observing the decomposition stages of various corpses. Many paintings depicted the maggots eating the body after death. These articles show the insect-based decomposition of the body perfectly [25]. A poem was written by the French poet Charles Baudelaire, titled *Une Charogne*, which depicts the decay of human bodies, and even the sound caused by maggots on cadavers was also mentioned perfectly [26].

In the year 1767, a biologist, Carl Von Linne, stated that as little as three flies can destroy an adult horse with as much rapidity, as an elephant can do [27]. A mass

disaster occurred in France and Germany between the eighteenth and nineteenth centuries, after which, for academic purposes, the graves were exhumed. Investigators found that the dead bodies were being eaten by insects of different species. One of these investigators, Dr. Orfila, observed that the insects play a huge role in the whole decomposition process of the cadaver [28, 29]. A French doctor, Bergeret, gave the first-ever report of forensic entomology in the year 1855 [30]. He estimated the post-mortem interval (PMI) from the life stages of flies from two species, i.e. blowfly pupae and larvae of the moth, feeding on the dead body of a child. There was a mistake in his case study as he supposed that female flies only lay their eggs in the summer season and the larvae will grow into a pupa in spring, which would hatch in summer. Because of this mistake, he thought that two generations of insects were eating the body and that the post-mortem interval was more than a year.

A German doctor named Reinhard gave a systematic report of forensic entomology in the year 1881 [31]. At that time, he was working on exhumed bodies. He found phorid flies, which were identified by a taxonomist Brauer. He concluded in his case study that the bodies in graves were older than 15 years. These phorid flies are now commonly known as coffin flies.

A sub-speciality of forensic entomology, forensic entomotoxicology, is a more advanced and rapidly developing field. This field is concerned with the maggots that feed on the cadaver of a person who has consumed drugs, etc. The first case of entomotoxicology was reported by Beyer and his colleagues in 1980. They used maggots for drug analysis when no tissues were available [32]. This field becomes very important when the body becomes skeletonized, either completely or partially. When maggots feed on such a cadaver, the drugs present inside the body transfer to maggots' gut. These drugs cause an effect on the development rate of insects, creating problems in estimating the post-mortem interval (PMI) [33–36].

3.3 Flies in Direct Relevance with the Corpse

Many different species of flies get attracted to the corpse at different intervals of time. These flies are generally from order Diptera, [32, 37–68], Coleoptera [69, 70], Hymenoptera [70–72], and others [70] (Table 3.1).

Different facts can be revealed while examining the insects scavenging on a cadaver, of which time since death estimation and drug detection are of most forensic value. Different insects have different life cycle durations, and so identification of the insect species needs to be done before any other examination can be undertaken. Blowfly is the most common to be found on a dead body, but other flies and mites can also be found. The job of a forensic entomologist is not an easy one, they have to examine insects that cause diseases like myiasis, and other skin and organ injuries can also occur. Sometimes, the drugs are ingested by maggots, and even the dead maggots can also cause severe problems to the health of the workers.

Table 3.1 Showing different insects that are generally found on the corpse

S. No.	Order	Species/family	Reference
1.	Diptera	*Calliphora vicina*	[33, 44–51]
		Lucilia sericata	[42, 52–59]
		Chrysomya megacephala	[60]
		Chrysomya albiceps	[48]
		Chrysomya putoria	[48]
		Megaselia scalaris	[61]
		Phormia regina	[62]
		Cochliomyia macellaria	[32]
		Calliphora stygia	[63, 64]
		Protophormia terraenovae	[51]
		Chrysomya ruffifacies	[65]
		Piophila casei	[66]
		Calliphora dubia	[67]
		Hermetia illucens	[68]
2.	Coleoptera	*Dermestes maculatus*	[61]
		Dermestes frischi	[35, 55]
		Thanatophilus sinatus	[35, 55]
		Staphylinidae family	[70]
		Scarabaeidae family	[70]
		Carabidae family	[70]
		Histeridae family	[70]
		Silphidae family	[70]
3.	Hymenoptera	Mymaridae family	[72]
		Formicidae family	[71]
		Apidae family	[70]
		Halictidae family	[70]
		Mutilidae family	[70]
		Vespidae family	[70]
4.	Hemiptera	Coreidae family	[70]
		Gelastocoridae family	[70]
5.	Blattodea	Blattidae family	[70]
6.	Dermaptera	Forficulidae family	[70]
7.	Lepidoptera	Hesperidae family	[70]

3.4 Importance of Insects in Criminal Investigation

3.4.1 Estimation of Post-mortem Interval (PMI)

Whenever the remains of a human body are found, the main question that arises is the estimation of the post-mortem interval. Most of the time, the body may be found in a partial or completely skeletonized condition, and the post-mortem interval is

hard to estimate without the help of insects [73–78]. Generally, the insects found on the dead body can be either of 1-day-old infestation or even up to 1 month. The growth rate of the insects always depends upon climatic conditions. This method of estimating the post-mortem interval can only give a rough estimate without much accuracy because the lifecycle of insects is greatly influenced by climatic conditions [79].

The basic protocol to be applied for estimating PMI from insects is to identify the insect and its life stage. The first method generally used to estimate PMI from insects is the life cycle observation, and the second method is to observe the succession pattern of the insects. The species recognition involved, arrival time and the pattern of succession of adult insects on the cadaver, and the information of their rate of development can give an estimation of the death time. Mostly, experimental data obtained from animal cadavers are utilized to estimate the PMI and to apply the findings to real-life investigations concerning human remains [76].

3.4.2 Drug Detection from Insects

The pharmacokinetics of drugs of abuse or prescription drugs in insects depends on the developmental stage and feeding activity of different species. Bioaccumulation has also been observed in parasitoids, predators, and omnivorous species. In investigations related to entomotoxicological samples, species of insects belonging to Coleoptera (beetles) and Diptera (flies) are generally recommended because they come first to attack a corpse and are commonly encountered in crime scenes. Blowflies from order Diptera are forensically important insects because they attack first and deposit their eggs on the corpse within minutes following death. Blowflies identify corpses mainly through the natural odour of decomposing tissues. Other insects like Sarcophagidae (flesh flies) from order Diptera generally reach after the blowflies and houseflies (Fig. 3.1).

The forensic toxicologist generally analyzes the biological and non-biological samples recovered from a crime scene to investigate the cause of death. In day-to-day casework, tissue and body fluids are available as samples. But in cases like unknown dead bodies, which are recovered after a long time following death, no conventional soft tissues and fluids are available. Carrion feeding insects, dipteral, and other arthropods are used as alternate specimens for toxicological investigation [3, 80]. Dayananda R et al. put forward various advantages of knowing these flies and beetle's life cycle, distribution, and ecological behaviour in solving the legal matters [81].

World Drug Report 2020 points out that worldwide, about 0.5 million deaths are attributable to the use of opiates, cocaine, cannabis, amphetamine-type stimulants, and new psychoactive substances (NPS) [82]. In this context, Goff M. L. et al. highlighted the importance of using chemical and instrumental techniques for entomological samples in determining the cause of death in drug overdose cases [83]. Table 3.2 demonstrates several studies that, despite being cumbersome work, entomological evidence is proved as a valuable tool for toxicological analysis of

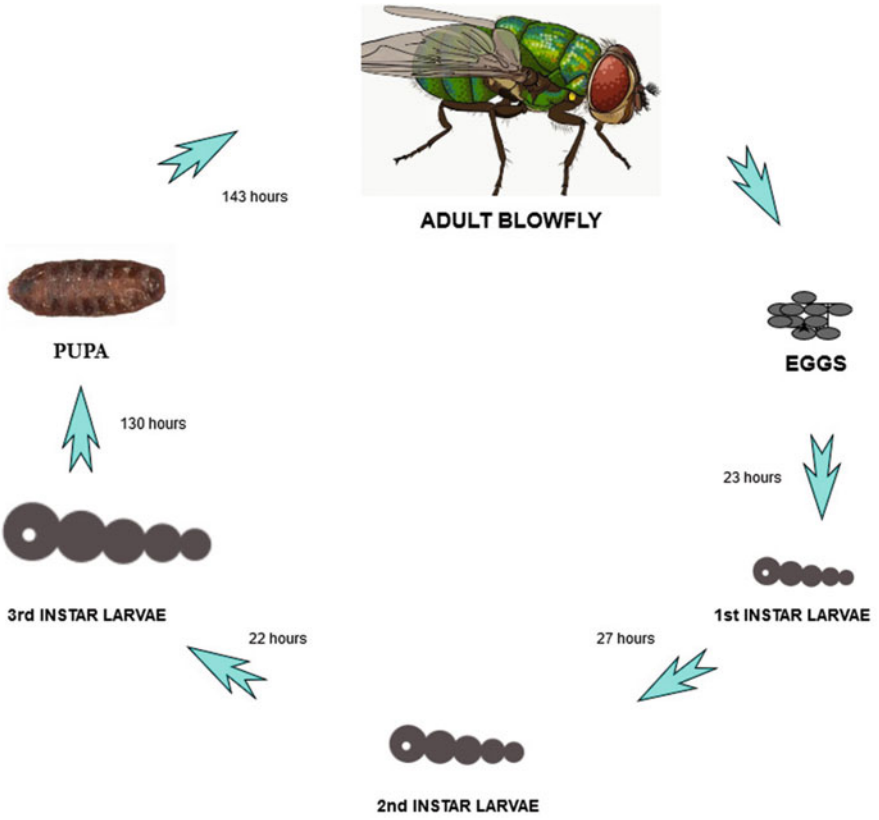

Fig. 3.1 Life cycle of blowfly [75]

numerous drugs of abuse and psychotropic substances overdose cases. Many flies and beetles belonging to Calliphoridae and Sarcophagidae families are used frequently in the determination of drugs and overdose of other prescription drugs and [33, 52, 63, 84–90] pesticides [60, 91] as well as in ante-mortem ethyl alcohol poisoning cases [92, 93] by identifying the ethyl sulfate as a biomarker of ethyl alcohol.

This identification of drugs and pesticides from larvae, pupae, and imago of blowflies and beetles needs an interpretation of chemical and instrumental setup such as TLC, GC, GC-MS, LC-MS, LC-MS-MS, HPLC, and immunoassays [94].

In a case study, the toxicological analysis of human tissues, including blood, and the different fly species /maggots collected from several sites on and in the body showed the presence of drugs in all samples. (See Tables 3.3, 3.4, and 3.5.)

Table 3.2 Studies showing the results of detection of drugs from specimens of blowflies and other arthropods

S. No.	Entomological evidence	Detected toxic substance	Technique used	References
1.	*Calliphora vicina*	Amitriptyline, temazepam, and trazodone + trimipramine	GC-MS	[33]
2.	Lucilia sericata	Barbiturates, opiates, cocaine, clomipramine, amitriptyline, nortriptiline, levomepromazine, and thioridazine	Immunoassay and GC-MS	[52]
3.	*Protophormia terraenovae*, *Calliphora vicina*	Morphine	Radioimmunoassay	[84]
4.	Chrysomya albiceps, *Chrysomya putoria*	Diazepam	GC-MS	[85]
5.	*Calliphora vicina*	Nordiazepam and its metabolite oxazepam	HPLC and LC-MS-MS	[86]
6.	*Chrysomya albiceps*	Amitriptyline, citalopram, and morphine detected. Diazepam—not specified	GC-MS and LC-MS-MS	[87]
7.	*Calliphora vomitaria*	Methamphetamine	GC-MS	[88]
8.	*Calliphora stygia*	Morphine	TLC, immunoassay, and HPLC	[89]
9.	*Lucilia sericata*	Codeine and norcodeine were detected, but morphine was detected only in treated meat	LC-MS	[90]
10.	*Calliphora stygia*	Morphine	Flow injection analysis, chemiluminescence detection coupled with HPLC	[63]
11.	*Calliphora vomitaria*	Endosulfan detected only in meat spiked	GC-MS	[91]
12.	*Chrysomya megacephala*	Malathion	GC	[60]
13.	*Calliphora vicina*	Ethyl sulfate larvae and puparia as promising biomarkers for acute and chronic alcohol abuse	LC-MS-MS	[92]
14.	Calliphoridae and Sarcophagidae families	Amphetamine and alcohol	GC-MS	[93]

Table 3.3 Organic compounds detected in analyzed samples

Compound	Species/family	FL	PFL	Blood	Urine	Liver	Kidney	Reference
Amphetamine	Calliphoridae and Sarcophagidae	+		0.48 mg/L		0.35 µg/g	0.21 µg/g	[93]
Alcohol	Calliphoridae and Sarcophagidae	–		0.45 µg/g		–	–	[93]
Opiate concentrations (µg/mL) in morphine equivalents	*Lucilia sericata*	0.75	0.14	0.72	26.75	2.45	–	[52]
Cocaine concentrations (µg/mL) in benzoylecgonine Equivalents	*Lucilia sericata*	1.99	0.40	1.34	38.75	3.30		[52]

FL Feeding larvae, *PFL* Post-feeding larvae

Table 3.4 Prescription drug concentrations (μg/mL)

Compound	Species/family	FL	PFL	Blood	Urine	Bile	Liver	Reference
Phenobarbital	*Lucilia sericata*	1.70	5.20	15.00	14.90	36.80	32.70	[52]
Levomepromazine	*Lucilia sericata*	0.04	0.06	0.01	0.12	0.53	0.29	[52]
Amitriptyline	*Lucilia sericata*	0.65	0.01	0.40	0.56	13.16	9.42	[52]
Nortriptyline	*Lucilia sericata*	1.44	1.26	1.32	2.14	11.62	120.43	[52]
Thioridazine	*Lucilia sericata*	0.36	0.37	2.06	1.28	1.62	1.26	[52]
Clomipramine	*Lucilia sericata*	5.40	8.73	2.86	0.38	19.70	3.90	[52]

FL Feeding larvae, *PFL* Post-feeding larvae

Table 3.5 Organic compounds identified in arthropod larvae (Medico-Legal Institute of Strasbourg 1988–2002)

Compound	Concentrations (pg/mg)	Reference
Benzodiazepines		
Nordiazepam	21–228	[95]
Oxazepam	44–200	[95]
Lorazepam	155	[95]
Bromazepam	810	[95]
Alprazolam	27	[95]
Triazolam	204	[95]
Barbiturates		
Phenobarbital	500–2250	[95]
Amobarbital	15–0	[95]
Antidepressants		
Amitriptyline	133	[95]
Clomipramine	28	[95]
Dothiepin	280	[95]
Fluoxetine	16	[95]
Venlafaxine	59	[95]
Phenothiazine		
Chlorpromazine	55.116	[95]
Cyamemazine	103–489	[95]
Levomepromazine	45	[95]
Alimemazine	22	[95]
Opiates/opioids		
Morphine	90–182	[95]
Codeine	12–59	[95]
Pholcodine	13	[95]
Propoxyphene	867	[95]
Miscellaneous		
THC-COOH	16–39	[95]
11-Hydroxy-THC	11	[95]
Meprobamate	718–4439	[95]
Digoxin	21	[95]
Nefopam	880	[95]

3.5 Factors Affecting the Growth of Decomposers

There are some factors or parameters which affect the growth of decomposers. These are:

(a) **Temperature**—Temperature is the most critical factor among all the environmental factors affecting the growth rate of decomposers. The arthropods are cold-blooded because the body temperature is directly related to the surrounding environment; consequently, they show variations in the metabolism and growth

rate. The growth of insects is temperature-specific, and it has a low and high point where the development stops, which is termed the threshold temperature level. The species collected for the rearing purpose are kept at room temperature. The rearing temperature varies from species to species and with the different stages of the same species. The species should be kept inside the rearing cages placing the cages in direct sunlight should be avoided. The extreme heat will kill the specimens [96].

The primary environmental factor affecting the growth or metabolic activity of decomposers that are cold-blooded is temperature because at the lower temperature, they develop slowly and at higher temperatures, rapid growth is observed [97]. Rodriquez and Bass performed a study in which four cadavers, of which one was female and the rest of three were male, were exposed to decay in May, June, October, and November. The study concluded that the decomposition rate occurred more rapidly when the temperature was higher and the carrion insects' colonization was great [98].

Numerous studies were conducted to examine the effect of temperature on the growth cycle of insects. And it was observed from those studies that the growth rate was slightly longer at varying temperatures as compared to the mean and constant temperature conditions [99].

(b) **Drug**—Another factor affecting the growth of decomposers is the drug. The toxic substances or the drugs in or the corpse can affect the growth rate of the decomposers or the feeding larvae. For example, heroin and cocaine can drastically increase the growth rate of larvae and simultaneously affect the accuracy of PMI estimation. Similarly, the insects might take a longer time to colonize and decompose a body [97]. According to the in vitro study performed by Monthei on *Phormiaregina*, the low amount of ethanol (0–0.1% w/v) does not affect larval development but can produce some characteristic growth differences such as lower pupal and adult weight, whereas the entomotoxicological effects of oxycodone produced affect the developmental rate of maggots [100]. Ethanol produces significant changes in the maggots' length for the third instar which may further alter the PMI estimation. There is less literature available on the maggots feeding on ethanol-treated flesh [99]. Drugs or toxins can be encountered in the larvae when the absorption rate goes beyond the metabolism rate. But the fact is not yet clearly known that how the metabolism of drugs occurs in larvae and how the larval development gets affected. The changes produced in the growth rate of the necrophagous insects can further produce significant variations or errors in PMI estimation if not considered during the death investigation because most of the drugs delay insect colonization. A rapid growth rate was observed in the larvae of *Calliphora vicina* eating tissues contaminated with PCM (paracetamol) and *Lucilia sericata* larvae feeding on the tissues contaminated with morphine while affecting the size and shape of *Chrysomaalbiceps* feeding upon the tissues contaminated with Diazepam [101].

Drugs in larvae—Numerous experiments were performed to find out the concentration of drugs and controlled substances (for example, opiates, cocaine, antidepressants, derivatives of amphetamines, etc.) to achieve useful

information on the cause of death. The studies have demonstrated that heroin speeds up larval growth and the pupal stage remains for a longer period. So, the total time required for the metabolic growth from the larval stage to adult is of longer duration when the colonies are feeding on the heroin–containing tissues [102].

Drugs in pupae—Miller et al. detected the presence of drugs (drug extracted was amitriptyline) in chitinized insects. The drug concentration was observed higher in pupae as compared to the casts of the beetle's skin (exuviae) [102].

Drugs in beetles (frass and exuviae)—Only a few drugs were extracted by following the method of hair-drug extraction. The concentration of drugs was found higher in fly pupae, whereas, in the faecal materials of the beetles, only a few drugs—cocaine and amitriptyline—were extracted [102].

(c) **Season**—Most of the insects synchronize their cycles according to the seasons. Higher temperature increases the insect activity, while the lower or colder temperature slows it down. The dead bodies exposed to the higher temperature or brighter sunlight will heat up easily and further provide a warmer place to the insects for development, hence reducing the time of growth of the insect colony. In contrast, the humid environment increases the reproduction and growth rate of most insects. On the other hand, high winds will also affect the activity of decomposers by creating a problem to locate and land over the body [98].

As the insects are cold-blooded, they adapt themselves according to the surroundings to survive accordingly within the temperature or seasonal variations. In places with extreme cold weather conditions, there is at least one stage in the growth cycle of insects that is resistant—the stage which survives in low temperatures. Such resistant stage could be any one of all the growth stages, i.e. egg, larva, nymph, pupa, or adult [96].

(d) **Location**—The bodies in damp locations will attract a variety of insects, while those in dry locations will become dehydrated before the insect colonization starts [103].

The moisture needs of insects and mites vary. The location from where the specimens are collected tells about the moisture requirement, as some of the insects in their pupal stage were found to be drought resistant. Apart from it, some require little moisture, while some produce water. Outdoor species have more moisture needs than indoor species. This is because even a minor interruption in the photoperiod can disturb the whole developmental process. In places with extreme cold weather conditions, there is at least one stage in the growth cycle of insects that is resistant, which means the stage which survives in low temperatures. Such resistant stage could be any one of all the growth stages, i.e. egg, larva, nymph, pupa, or adult [96] (Table 3.6).

Table 3.6 Entomotoxicological findings and various drug concentrations in pupae [102]

S. No.	Drugs	Techniques used	Drugs concentrations found
1.	Barbiturates (phenobarbital)	GC/MS	100 μg/g
2.	Opiates (morphine)	Radioimmunoassay, GC/MS	8–1208 ng/g
3.	Bromazepam; levomepromazine	EIA, HPLC	Bromazepam 0.81 μg/g; Levomepromazine 45 ng/g
4.	Cocaine and its metabolite	RIA, GC/MS, GC/NPD	Cocaine 0.49 μg/g Metabolite 0.3 μg/g
5.	MDMA, MDA (metabolite)	HPLC/MS	MDMA 6.6 μg/g MDA 1.8 μg/g

3.6 Conclusion

Forensic entomology is a huge field to discuss due to its relevance with cases where evidence is not even expected. This field is fast developing due to the fascinating behaviour and curiosity of researchers. Numerous researches have been conducted in the past few years, but the relationship between the drug amount in a substrate and the insects reared on it is still doubtful. Different qualitative analyses have been performed with different drugs, but the question arises always for quantitative analysis from entomological samples. The reason for not finding the exact relation between drug concentration of substrate and maggots can be one or many. There is a shortage of literature available for behavioural studies and feeding habits of insects particularly decomposers. Future researches need to be more focused on the ADME of the drug inside the insects as this can help in the quantification of drug from predators. Recent researches finding the DNA material and other crime-related evidence inside maggots gut are of great emphasis in forensic science province because these findings are arcane in their type. The field of forensic entomology and entomotoxicology are accruing and of great value for the judicial system as justice can be delivered to decedents even after a long time.

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Dermatoglyphics and Other Impressions: Deciphering the Potential of a Unique Hominid Niche

4

Shruti Gupta and Tamanna Jaitly

Differentiability of humans from one another taking into account various traits has been the foremost objective of researchers. Uniqueness in everything from cell to soul is a pressing feature in humans. From a walk of a person (gait patterns), the body, the body odor to the shape of the ears, the backside and skull shape and so on, scientists are discovering many astonishing ways of identifying an individual from the other seven billion people in the world.

Individualization of humans is done on the basis of prominent folded skin features present on their palmer side of each hand and on the soles of feet. These features are present in friction ridge skin which leaves behind impressions of its shapes when it comes in contact with an object. These impressions are the most important and unique feature used for recognizable proof of an individual.

4.1 An Overview

Dermatoglyphics is regarded as a study of configurations identified with epidermal ridges on certain body parts like palms, fingers, soles, and toes [1]. Dr. Harold Cummins coined the term "*dermatoglyphics"* in 1926, which is further derived from ancient Greek word "*derma"* meaning skin and "*glyph"* meaning carving [2, 3]. The most fascinating fact associated to dermatoglyphic patterns are they begin to develop in the tenth week of gestation and are completely formed by the 24th week. Basically, dermatoglyphics is the study of ridge patterns of the skin (including palms, toes, fingers, and soles).

The significance of dermatoglyphics has been found in the forensic field of investigation as the ridge patterns of the skin contributes to the undeniable evidence in the court of law. These patterns can be taken into consideration in two forms,

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Fig. 4.1 Basic types of patterns (loop, whorl, and arch)

namely fingerprints and footprints. From the two, fingerprints are regarded as the most reliable evidence in linking a suspect to a specific crime scene. The inner surfaces of the hands and feet incorporate the minute linear markings running down from the tip of the finger to the wrist and from the tip of the big toe to the rear end of the heel, respectively, with furrows between each ridge [1, 2].

The ridges have pores along their entire length that exude perspiration; hence, when an object is picked up, the perspiration runs along the ridges and leaves an exact impression of the ridges, just as an inked rubber stamp leaves its impression on a blank sheet of paper.

Ridges and furrows have evolved on the hands and feet to satisfy three explicit capacities:

1. Exudation of perspiration: emission of a fluid from pores of hand.
2. Tactile facility.
3. Provision of a gripping surface.

Dermatoglyphics is absolutely genetically controlled and thus can be used in detection of congenital malformations [1, 4]. During development of a child, various creases develop in the brain and are consequently reflected on palms and soles as ridges and fingerprints representing different regions of the brain and further being utilized in dermatoglyphics mental intelligence test.

The fine linear markings on the palmer side of the hand, also known as ridges (continuous curved line) along with furrows (depression in between two ridge lines), create patterns on the last joint of the fingers and toes, forming basic type of patterns [5], as shown in Fig. 4.1.

4.2 Fingerprint Patterns

1. Loop
2. Whorls
3. Arch
4. Composites

- **Loop:** The ridges from the one end of the finger digit extends to form a loop by taking a U-turns, forms the head of the loop and takes the exit on the same side. From the other extreme side of the finger digit, ridges flow to the margin of the digit [1]. This side of the loop is "open" side. There are two types of loops (Fig. 4.2):
  - Ulnar loop: The ridges flow in the direction of ulna bone (little finger).
  - Radial loop: The ridges flow in the direction of radial bone (thumb).
- **Whorl:** The ridges make the concentric circle around the center known as "core." The true whorls include two triradii. Whorls are divided into four subgroups (Fig. 4.3) [6]:
  - **Plain whorl:** One complete circuit is observed. The pattern is circular or spiral in shape (Fig. 4.3a).
  - **Central pocket:** Recurves of ridges of plain whorl recurve again over the whorl (Fig. 4.3b).
  - **Double loop:** Two separate loop formations are observed with two distinct deltas (Fig. 4.3c).
  - **Accidental whorl:** Pattern formed from two distinct types of patterns of whorl's subgroups (Fig. 4.3d).

Fig. 4.2 Types of loop patterns of fingerprints of right hand

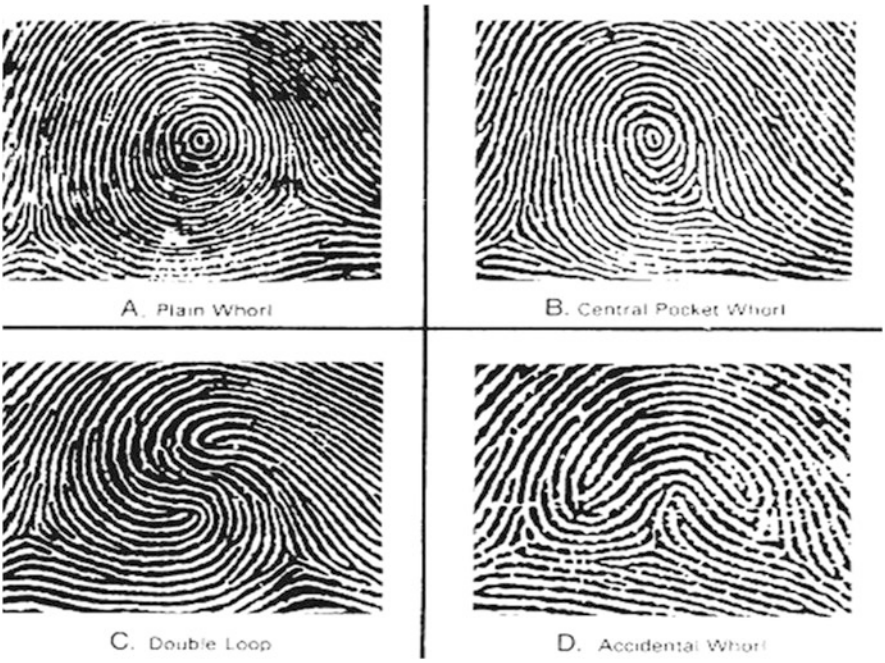

Fig. 4.3 Four types of whorl patterns of fingerprints

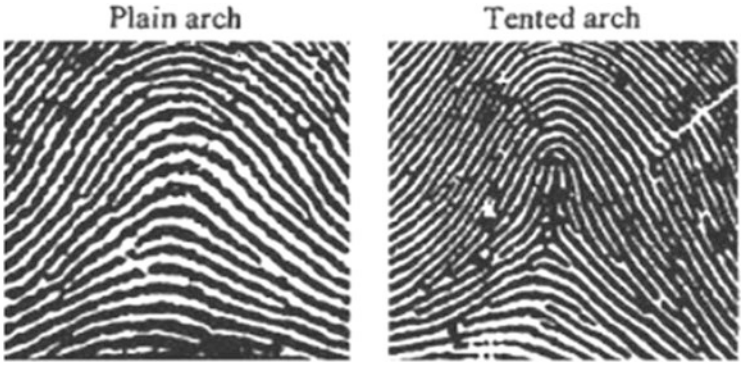

Fig. 4.4 Showing two types of arch patterns found on fingerprints

- **Arch:** The ridges enter from one extremity of the finger digit and exit from the other extremity of the finger digit making a gentle arch in the center. There is no triradius in this pattern. Arches are of two types (Fig. 4.4) [6]:
  - Tented arch: The center raised ridge has a tent-like shape and sharper angle.
  - Plain arch: The center raised ridge has comparatively less sharper angle.

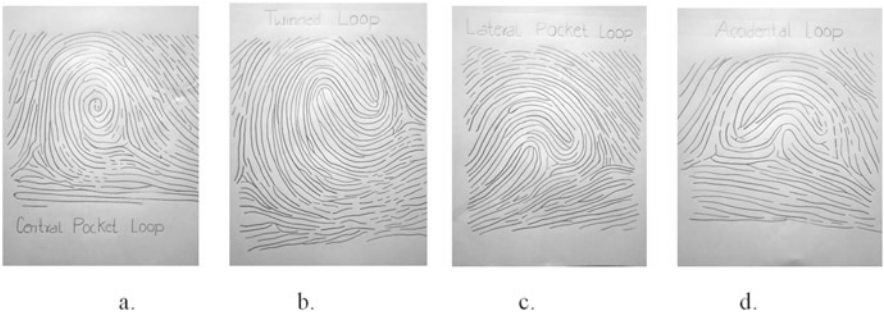

Fig. 4.5 Composite types of patterns of fingerprints

- **Composites:** Composites are patterns found on the fingerprints involving combinations of arches, whorls, and loops. These patterns are subdivided into four categories [1] (Fig. 4.5):
  - **Central pocket loop:** The ridges recurve again within a loop to form a pocket-like structure in a loop. The structure resembles a peacock eye-like pattern (Fig. 4.5a).
  - **Twinned loop:** Two separate loop formations are observed in this type of fingerprint pattern. This pattern is also known as "double loop" pattern (Fig. 4.5b).
  - **Lateral pocket loop:** It is similar to twinned loop except for that the loop curves sharply and bends more on one side making a pocket-like structure. This loop sometimes comes under the category of double loop pattern (Fig. 4.5c).
  - **Accidental loop:** The pattern is a combination of two patterns except for plain arch. The pattern is somewhat distorted in its resemblance (Fig. 4.5d).

4.3 Use of Dermatoglyphics to Identify Left Hand

Dermatoglyphics has been extensively used in determining genetically based abnormalities in individuals. One of the imperative utilizations of dermatoglyphics includes determination of left-handed person. A left-handed individual possesses more radial loops than ulnar loop; more tented arch and a negligible plain arch; more peacock pattern and a minimal double or central pocket loop as contrasted with right-handed person [7].

Identification and production of patterns of fingers and utilizing it in a court of law as a strong evidence is due to the undisputable fact that sequence of ridge details of one person never matches to the ridge detailing in the hands of the other person. These impressions (ridge details along with furrows) from the last joint of every finger are known to be as fingerprints. The pronounced magnificent feature of fingerprint makes it one of the prominent, most preferable and suitable evidence admissible in the court of law. Similarly, the dermal carving are highly

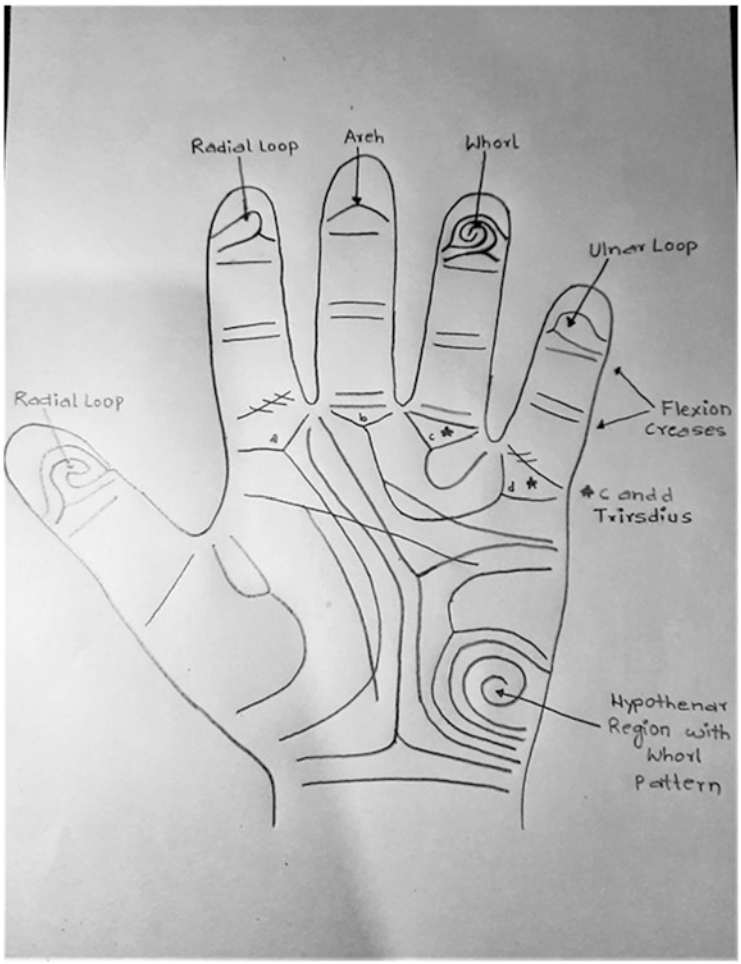

Fig. 4.6 Palm with different types of patterns

individualized means the formation of fingerprints are permanent subjected to no indefinite damage occurs for lifetime [3, 8]. Palm prints along with creases, different types of patterns, and other features are demonstrated in Fig. 4.6.

Fingerprints vary from person to person, including the siblings, father to son, and even the identical twins with identical DNA. The authenticity allows fingerprints to be used in various fields as for biometric security, in banks if signatures do not coordinate in checks due to age factor (finger impression is taken on the posterior of check if tremors are seen in signatures), mass disaster identification, and as a primary evidence in criminal cases.

Fingerprint has been utilized as an extremely predominant tool by law enforcement agencies for distinguishing proof of suspect and solve crimes for more than a

century. Criminal cases involve the entry and exit of victim and suspect at a crime scene. During a crime, the fingerprints are left on different surfaces due to pores of hand and perspiration. As a result, investigators link the evidence found at crime scene with victim as well as with suspect to solve a case by utilizing Locard's Principle of exchange.

Fingerprint identification additionally assists investigators to track a criminal's record, their previous arrests and convictions, to aid in sentencing, probation, parole, and exculpating decisions [1].

In any crime scene, fingerprints are usually present in three forms:

1. Latent prints/invisible fingerprints
2. Patent prints/visible fingerprints
3. Plastic prints/3-dimensional fingerprints

4.4 Latent Prints/Invisible Fingerprints

Fingerprints left at the scene of crime are sometimes not noticeable through naked eye; such prints are known as latent prints. These type of impressions are left behind unknowingly by the perpetrator on various areas at the crime scene. The palmer surface of hand comprising papillary ridges and ridges of friction of foot are subjected to numerous sweat pore openings. The studded sweat pores in hand and feet functions in lubricating the skin through perspiration in the form of sweat so as to keep them moist, warm, and soft. The contact of finger or any palmer region with the external surface (any smooth or polished surface) leaves an invisible print on them. Due to the softness of the surface, the fingerprint becomes hidden and special aid is required to locate and visualize them for assessment and further examination.

At times, chance prints (mostly known as latent fingerprints) are found at the crime scene. These type of prints are left on the objects and evidences by some coincidence and are frequently invisible impressions made by fingerprints.

4.5 Patent Prints/Visible Fingerprint

These prints are easily visible to the naked eye due to any deposition of visible or colored contaminants over the fingers of the culprit. These fingerprints are found on the surfaces touched or contacted by the suspect during his time of committing crime. Visible or colored contaminants can be of any type—ink, mud, blood, paint, etc. Sometimes these prints are not fully developed or only few ridge patterns are seen having minimal identification value subjected to the distinctive characteristics of the friction ridges on the surface of the object/ material contacted. In cases like this, investigators link the half print to the other fingerprints found at different surface areas by photographing directly for the purpose of comparison and maintenance of records in solving the case.

4.6 Plastic Prints/3-D fingerprints

When finger and the palmer surface of the hand comes in contact with any surface with plastic body or semi-solid such as soap, butter, wax, soft putty, tar, grease, and freshly painted surfaces, they leaves a depression and form a mold of fingerprint on the surface. These prints establish three-dimensional impressions and are easily visible.

4.7 Detection and Examination of Footprints

For identification purpose, it has been established that ridge patterns of the fingerprints are unique and helpful. Dermatoglyphic features on the plantar surface of footprints are no less significant for investigation purpose. Detailed observation of a plantar friction ridge gives the conclusive results in exactly the same way as fingerprint identification [5, 9].

Footprints found at the crime scene serves as potential evidence which are left behind as marks or impressions on the contact surface. The dimensions, shape, size, morphology, and overall placing of the footprint help in detecting the entry and exit patterns of the suspect. A bare footprint also reflects papillary ridge details and crease marks from the skin of the plantar surface of foot, and an analyst uses these information to examine the evidence in detail and demonstrate individuality to either establish or exclude associations with the postulated owner of that footprint [9]. Footprint consists of patterns similar to hand (arch, whorl, and loop). A triradius is also present which defines the meeting point of three opposing ridges. An overview of the footprint with basic patterns has been shown in Fig. 4.7 [3].

Ridge density is one of the features of the fingerprint science that has been developed and vastly utilized as an aid in medical and criminal cases. The number of ridges placed in a definite space of a finger, palm, and footprints known to be as ridge density is another trait of human morphology in which sexual dimorphism can be demonstrated. Studies conducted show that the variations seen in fingerprint ridge density pertain to be the ideal tool for determination of sex. The data collected by Acree in 1999 shows that the fingerprints of females have finer epidermal ridge details and significantly persist higher ridge density in a defined space than males [9].

4.8 History

Since earlier times, dermatoglyphics has been used by Chinese as a basis for fortune telling. Observing the ridge details in the hands was a curiosity for a person to know the future predictions and tackling with certain problems and thus healing it. Ancient Indians during that period calls a person as Chakravarti meaning “An Emperor” in case when ten whorls are found in hands [10]. China utilized friction ridge details as

Fig. 4.7 A bare footprint representing different patterns and a triradius

personality determination and as a proof of a person's identity during 300 B.C. in continuation by Japan as early as A.D. 702 and in the United States since 1902 [11].

Chinese invented ridge skin impressions for proof on land contracts, wills, and army rosters back at the time of Tang Dynasty (A.D. 617–907). Followed by China, other nations believed to adopt the practice and used friction ridge skin impressions for individualization purpose [1, 11].

India with reference to the other countries proved it noble to use friction ridge impressions as signatures. In Earlier times (A.D. 1637) joint forces of Shah Jahan and Adil Khan defeated the ruler of Pona (now known as Maharashtra) Shahuji Bhosle who was forced to imprint his impression of hand for acceptance of peace. Utilization of imprints were adapted from Chinese and in contrast Indians reserved the impressions tradition for royality [1].

4.9 Evolution of Fingerprint Science

The detailed study on the friction ridge skin impressions was described in *Philosophical Transactions of the Royal Society of London* paper by Dr. Nehemiah Grew (1684) whereas the uniqueness in the patterns of the fingerprints was recognized in Europe (1788) by J. C. A. Mayer, in his book entitled *Anatomical Copper-plates with Appropriate Explanations.* The detailed drawings of friction ridges and patterns

pave the way to his contribution and became the first to describe that friction ridge skin is unique. Categorizing the fingerprint patterns into nine subcategories and giving names to them was done by Dr. Johannes E. Purkinje (1787–1869) at the University of Breslau in Germany. The results gathered from his significant study provided ample contribution and utilized as the precursor in Henry Classification System [12].

Friction ridges were unique to everyone but their existence was permanent over the lifetime was led by Herman Welcker (1822–1898) of the University of Halle. He printed his own right hand in his study in the year 1856 and the print was seen irreparable or permanent in the year 1897 when he printed it again. He was the first one to study the permanence of friction ridge skin impressions. Welcker, findings was in persistence to the study conducted by Sir William James Herschel (1858). Herschel experimented in the field of friction ridge skin impression in 1858 by having a stamp of right hand of a person named Rajyadhar Konai on the back of the contract made for road binding materials when he visited India. The contract was accepted as valid and as a result spontaneous printing of Konai's hand led to the first official use of friction ridge impression.

Followed by the studies, Henry Faulds mentioned ridge details in his study when he saw ridge details on pottery found on Japanese beach (1880). His independent study includes both the prints from monkeys and people and concluded that ridges were unique and classifiable and alluded to their permanence.

Sir Francis Galton scientifically studied the ridge patterns and details. Most of his studies focused predominantly on hereditary matters which further led him to study anthropometry and the fingerprints. In his first book "*Fingerprints,*" 1892, uniqueness and persistency of friction ridge impressions were discussed. He also added anthropometric measurements and printing of the thumb and all the ten fingers in his book [13].

Following Galton's research, Juan Vucetich experimented with fingerprints. He compiled the fingerprints of criminals and devised his own classification system [14]. Fingerprint science was the first to be utilized by law enforcement personnel for prisoner's individualization using Vucetich's classification system. Vucetich's classification system further came to more practical usage by other countries using a fingerprint system to identify prisoners [14].

Several criminal cases were solved utilizing the fingerprint science. The Rojas murder case of Argentina was the first homicide case solved taking into account fingerprint as an evidence. Rojas's children were murdered and she got a throat wound. She accused a man named Velasquez as she refused to marry him and was in love with another man. Thumbprint was used as an evidence for solving the case, resulting in making Argentina the first country to rely on fingerprints as a method of individualization.

The official use of Henry classification system for identification of prisoners and deciphering criminal cases was done when Sir Edward Richard Henry, Inspector General of Police, collaborated with Galton on a method of classification for fingerprints. Sustaining the help of police officers Khan Bahadur Azizul Haque

and Rai Bahaden Hem Chandra Bose, the Henry classification system was developed [12].

Ongoing research on friction ridge skin impressions paved the way for David Hepburn of the University of Edinburgh, Scotland, to contribute in the research by increasing the level of friction between the ridges and object. Volar pads were evolved found on the palmer side of hand: the hypothenar and thenar as per his paper "*The Papillary Ridges on the Hands and Feet of Monkeys and Men*" [15, 16]. His study was dedicated as the importance and effective use of fingerprints as a means of individualization. Continuing his study and supporting the David's Hepburn's point of view, Harris Hawthorne Wilder, Professor of Zoology at Smith College, compared volar friction ridges of primates with humans. His study suggested the centers of disturbance of primate friction ridge formations actually represented the locations of the volar pads and developed the hypothesis of a relationship between volar pads and primates [15].

By 1900, the Belper Committee in England recommended all the criminal records to be classified by the fingerprint system resulting in the Henry Classification system to be the first one to be utilized as a standard practice for individualization of criminals by means of fingerprints [12]. Contemporary to the fellow members, Alphonse Bertillon employed the fingerprint science for individualization purpose in Paris and France and was given credit to solve first murder case implementing fingerprint as an evidence in Europe [14]. By the end of 1902, systematic use of fingerprints was done by Dr. Henry P. in United States [12].

After many years of utilizing the Henry's classification system for identification, Captain James H. Parke of New York, United States of America, developed the American Classification System. Few years later, the use of the American Classification System became the new trend for analyzing and examining the criminal cases.

In accordance to the advancement in the field, Inspector Ferrier and Major M. W. McClaughry collected fingerprints of all the inmates of federal prison and recorded them for further data analysis and solving criminal cases. By the time, fingerprint recording became the beginning of the U.S. Government's fingerprint collection.

A criminal case was solved by Inspector Charles S. Collins of Scotland who found the suspect's fingerprint on a cash box. Lifting and comparing the fingerprint with the records collected proves the guilt of perpetrator. Earlier fingerprint comparison in the court of law was performed by the forensic expert until the case of Emperor Abdul Hamid where the Indian court decided the individualization of prints could be done solely by the participants and no expert was needed. In continuation of the decision made by Indian court, other high and district courts agreed on the decision that no expertise is required to individualize fingerprints (2001). Contribution of Dr. Edmond Locard "*The Legal Evidence by the Fingerprints*" was proved to be one more example of law enforcement personnel conducting research into fingerprint science. He conducted the study of pores (poroscopy) and applied the results as an aid to fingerprint comparison by lending supporting data [1].

4.10 Fingerprint Development Techniques

Fingerprint development is the first step of the investigation right after the fingerprints are found at the crime scene. Investigators secure the crime scene to avoid tampering of the evidences found. Several development techniques are used depending on the type of the fingerprint (latent, patent, plastic) and the surface (porous, nonporous, and semi-porous) respectively.

4.10.1 Surface Characteristics

- Temperature of the surface (sebaceous material adheres better to a surface that is cooler than the human body).
- Structure of the surface (the rougher the surface, the greater are the adhesive forces).
- Electrostatic forces on the print-receptive surface.

Properties of an unknown surface have to be taken into consideration prior to the development of fingerprints. Surfaces bearing fingerprints can be porous, nonporous, and semi-porous.

4.10.2 Optical Detection Techniques

One of the nondestructive methods with respect to the latent fingerprints is optical detection. It can be applied on both porous and nonporous surfaces. These techniques exclude the application of other conventional fingerprint development procedures. The fingerprint is observed directly under the white light and photographed without any further treatment. Fingerprints contaminated with a colored material such as blood can be significantly enhanced using selective absorption techniques. Commencement of detection of fingerprints by using different illumination techniques should be the primary step in accordance with the surface type and fingerprint contamination.

4.10.3 Detection Techniques for Porous Surfaces

1. **Ninhydrin Test:** The most commonly used method to detect fingerprints is ninhydrin test. The test involves binding of terminal amines of lysine residues present in fingerprint with ninhydrin to form a deep blue or purple color known as Ruhemann's purple.
2. **Secondary Metal Salt Treatment:** Fingermarks developed with ninhydrin can be further enhanced by treatment with a metal salt solution of zinc (Zn) or cadmium (Cd). The treatment results in a color change, orange for zinc and red

for cadmium, due to the formation of a 1:1 coordination complex between the Ruhemann's purple.

3. **Ninhydrin Analogs: Ninhydrin analogs also known as** 1,8-Diazafluoren-9-one (DFO) is used to make latent fingerprints visible. The technique is used to make latent fingerprints visible by reacting with the amino acids present in fingerprints.
4. **Physical Developer (PD):** The technique was developed in 1970s for developing the fingerprints found on the porous surfaces by Atomic Weapons Research Establishment. The technique is effective even when the surface is wet due to its sensitivity to water insoluble components of latent prints. The fingerprints developed from the technique appears dark gray to black in color due to the deposition of silver metal on the sides of the print ridges.
5. **Multi-metal Deposition (MMD):** The two-step chemical process including gold and silver for the detection of latent fingerprints. The technique involves immersing the fingerprint in aqueous gold solution and enhancing the print using physical developer. Porous and nonporous, wet or dry all can be treated by the technique.
6. **Various Powders:** Fine powders are used for dusting over the surface bearing fingerprints at the scene of crime. Particles are adhered to the residues left by ridges on fingers, palm, or feet. This method is also known as powder dusting.
7. **Iodine Fuming:** The method can be utilized for both porous and nonporous surfaces. Fingerprints are developed by the long-used method of adsorption of vapors onto the oily substance formed by fingerprints. Iodine vapors are formed through the process of sublimation. Iodine fuming can be used on fabric and rough surfaces.
8. **Silver Nitrate and its analogous:** Silver nitrate is another technique used for developing fingerprints on paper. The method involves spraying of silver nitrate onto the fingerprint on the paper. The particles react with chlorides to give insoluble salts of silver chloride.
9. **Phase Transfer Catalyst:** Fingerprints are developed on the sticky side of adhesive tapes using the Phase transfer catalyst (PTC) and a dye. Without the catalyst, the process is so slow that by the time the complex begins to deposit on the ridge.

4.10.4 Detection Techniques for Nonporous Surfaces

1. **Fingerprint Powders:** Simple powder, fluorescent powders, and magnetic powders are used for development of fingerprints.
2. **Small-Particle reagent:** It has been widely used method of developing latent fingerprints. SPR base on zinc carbonate hydroxide monohydrate has been formulated. Crystal violet dye and a liquid detergent are also utilized. Clear, sharp, and detailed fingerprint is formed after immersing the compositions into clean and dirty water for variable period of time.
3. **Cyanoacrylate Fuming:** The method involves the vapors of super glue to develop the fingerprint. Fuming is performed in a chamber using cyanoacrylate

polymerization reaction in the presence of water. The vapors formed adhere to the fingerprint, thus making it visible.

4. **Vacuum Cyanoacrylate Fuming:** Development is achieved by placing the evidential objects in a large metal chamber. The fingerprint is developed due to deposition of polymerized cyanoacrylate ester on residues of latent fingerprint. Fingerprints developed in this technique are clear, stable, and white in color.
5. **Vacuum Metal Deposition (VMD):** The process involves coating the evidence with thin layer of metal film under high vacuum. Several metals like gold, silver, aluminum, zinc, tin, and some alloys are used for this method. As a result, reversed developed fingerprint is obtained.
6. **Aqueous Electrolyte:** Aqueous electrolytes on metallic surfaces can be of great use in developing the fingerprints.
7. **Phase Transfer Catalyst.**

4.10.5 Detection Techniques for Semi-porous Surfaces

Fingerprint development techniques are much easier on porous and nonporous surfaces in regard to semi-porous surfaces. Semi-porous surfaces include glossy paper, waxed paper, matt polished surface, and rubber or latex gloves. Particularly for semi-porous surfaces, Iodine/Benzoflavone spray and multi-metal deposition (MMD) are utilized for developing fingerprints. MMDII was particularly effective on surfaces such as latex and nitrile gloves, expanded polystyrene, and waxed paper.

4.10.6 Use of Dermatoglyphics in Diagnosis of Various Pathologies

- **Dentistry:**
  Dermatoglyphics plays a great role in the field of dentistry. Several dental and oral pathologies such as sub-mucous fibrosis, oral caries, dental clefts were pre-diagnosed as per the fingerprint pattern possessed by the individual. Person with attaining a whorl pattern is more likely to have dental caries than a person with loop pattern [17].
- **Diabetes Mellitus Type II:**
  Diabetes mellitus is basically a genetic disease and is passed on within a family. Since dermatoglyphics has its significant role in genetics, the disease can be diagnosed taken into account the dermatoglyphics factor. Patients with Type I disease are more likely to possess whorls and arches and reduced number of loop patterns. Patients with Type II disease holds increased whorls and minimal ulnar loops in both hands irrespective of sex [18].
- **Down's Syndrome:**
  The genetic disorder with a presence of third copy of chromosome 21. The abnormality is distinguished by broad head, wide face with facial disabilities, and decreased intellectual capacities. Individuals with Down's syndrome are

characterized to have high frequency of creases, bilateral, radial loops, and ulnar loops [4, 19].

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Postmortem Toxicology: A Facet of Drugs to Expedite Crimes

5

Shipra Rohatgi, Pooja Yadav, and Dina Shokry

Abstract

Postmortem toxicology is a branch of forensic toxicology that helps in establishing the impact or role of poisonous substances in the death of an individual, whereas clinical toxicology deals with the diagnosis and treatment of adverse effects due to exposure to harmful agents. Conducting toxicology on specimens that recover from the dead is difficult because the redistribution of drugs takes place which alters the concentration of drugs inside the body of the victim; that's why it requires careful consideration when it is examined by toxicologists to interpret their significance. This chapter focuses on qualitative and quantitative analysis (including extraction, identification, and concentration detection) of toxic substances in corpses such as drugs, household poisons, pesticides, and alcohol that are commonly used in criminal activities (homicide, suicide, sexual assaults, cattle poisoning). Most of the medicolegal cases encountered in hospitals (for autopsies) and forensic laboratories are homicidal because ideal homicidal poisons are colorless, odorless, tasteless, and cheap with high availability, and cases of suspected homicide require much more thorough screening than the cases of natural and accidental death. The examination of specimens such as viscera or body fluids is very important to know the nature of poisoning that leads to death for the medicolegal determinations.

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Keywords

Toxicology · Preservatives · Forensic · Quantitative analysis

5.1 Introduction

This chapter provides knowledge of postmortem toxicology focuses on the medico-legal aspects of poisoning cases to find the cause and manner of death in suspected drug-related deaths or poisoning. Nowadays, cases of death due to drug intoxication continue to increase, which means drugs are responsible for expedite crimes. This section of the book explores the morphological and anatomical examination of the victim's body, type of samples collected, proper preservation, extraction methods from matrices, and their analysis.

Toxicology—In ancient times, arrowheads were dipped into a poisonous substance called "toxicon," and "logy" means the study of something, so it is defined as "the science of poisons" which means the study of etiologic agents with reference to their sources, properties, mode of action, recognition, identification, treatment, and their autopsy findings.

Paracelsus (Phillippus Aureolus Theophrastus Bombastus von Hohenheim) is considered the father of ancient toxicology. He states that all "substances are poisons; the right dose differentiates a poison from a remedy." He also proposed that all-natural substances show two types of influences—a harmful one and a beneficial one which were separated by means of alchemy (effective that seems like magical).

A Spanish physician named **Mathieu Orfila (1813)** is known as the founder of toxicology, and he demonstrated the effects of poisons on organs and their associated tissue damage.

Toxicology is selective and is an ever-evolving medical science. Insecticide like pyrethrin is taken as an example; it is lethal for insects because it targets the nervous system of insects but is relatively low in toxicity to animals.

5.2 Branches of Toxicology

1. **Clinical toxicology** (medical toxicology)—It focuses on adverse effects caused by acute and chronic exposure to a large concentration of substances and their diagnosis and treatment that occur in humans.
2. **Applied toxicology**—It is a science which uses modern technologies for the early detection of toxicants in a practice area or a field setting.
3. **Analytical toxicology**—Its concern is the detection, identification, and measurement of xenobiotics and their metabolites in samples recovered from a victim's body.

4. **Forensic toxicology**—It is the discipline of forensic science that uses the principle of analytical chemistry and toxicology for medicolegal investigation of ill effect or in case of death, and the result is used for administrative purposes.
5. **Postmortem toxicology**—It is a branch of sciences that helps in establishing the impact of toxic substances on the death of an individual.
6. **Forensic entomotoxicology**—It is the application of forensic science in investigations of death on alternative specimen like insects in case of poisoning.
7. **Environment toxicology**—It is the study of the surrounding (soil, water, air) of living organisms that is concerned with the assessment of pollutants.
8. **Nanotoxicology**—It is a branch of bionanoscience which deals with the study of the toxicity of nanomaterials.
9. **Occupational toxicology**—It is the application of principles and methodology of toxicology towards chemical and biological hazards encountered at work.

5.3 Types of Cases Encountered in Forensic Toxicology

1. Identification in acute and chronic drug intoxication.
2. To determine breach of policies about the use of drugs in the workplace.
3. Alcohol intoxication: whether this poisoning was homicidal or intentional.
4. Roadside vehicular accidents: suspicion of alcohol and drugs in driver or victim.
5. Child abuse under influence of drugs.
6. Mass toxic tort.
7. Cases related to chemical exposure: identification and nature of exposure.
8. Qualitative and quantitative pesticide exposure leads to death.
9. Water contamination cases.
10. Drug doping used in sports to enhance performance.
11. Cases related to death due to burn.
12. Postmortem redistribution.
13. Toxicological risk assessment of toxic exposure.

5.4 Poison

Poison is any substance that is capable of acting deleterious effect on living organisms when administered eternally or paternally. The dose and exposure of substances make them poison; even medicine in a toxic dose is poison, and poisonous substance in a small dose may be medicine.

5.4.1 Classification

Poison can be classified on various bases:

Table 5.1 Classification of poisons on the basis of mode of action

Poison category	Features	Subcategory	Examples
Corrosive	Erodes and destroys the surface when contact	Strong acids Strong bases Metallic salts	H2SO4, HNO3, HCL NAOH, KOH Zinc chloride, silver nitrate, potassium cyanide
Irritants	It causes irritation, pain, and excessive vomiting	**Inorganic** Nonmetallic Metallic **Organic** Vegetable Animal **Mechanical**	Phosphorus, chlorine Arsenic, lead Calotropis, Arbus Castor, kaner Snake venom Cantharides Hair, diamond dust
Systemic	Directly affect the targeted organ	**CNS poison** **Cerebral** stimulants Depressants **Spinal** Peripheral nerve **CVS poison** **Asphyxiants**	Caffeine, cocaine Alcohol, sedatives Nux vomica Curare, conium Aconite, oleander Carbon Carbon dioxide

Table 5.2 Classification of poisons on the basis of nature

Poison	Feature	Subcategory	Example
Gaseous	It alters the competence of blood as a carrier of oxygen and damage tissue of air passage		CO, CO_2, H_2S, SO_2, and tear gas (N_2O)
Volatile	Slow inhalation of vapors leads to intoxication	Inorganic Organic	Arsine, phosgene Ethanol, formaldehyde, chloroform, chloral hydrates
Nonvolatile		Nonvolatile inorganic anion Cation Organic neutrals Organic alkaline Acidic Basic	Nitrites, cyanide Mercury, lead, copper Organophosphate, organochloride, carbamate Barbiturates, salicylates Morphine, benzodiazepine

- On the basis of mode of action (symptoms)
- On the basis of nature
- On the basis of use (Tables 5.1, 5.2, and 5.3).

Table 5.3 Classification on the basis of poison being used

S. no.	Poison	Characteristics	Example
1.	**Household**	Used for domestic purpose	Disinfectants, baby powder (boric acid), crayons (Pb, Cu)
2.	**Aphrodisiacs**	Increase sexual desires	Cannabis, cantharides
3.	**Arrow poison**	For hunting and kill enemies	Strychnine, snake poison
4.	**Cattle poison**	Used to kill cattle of enemies	Calotropis, Abrus precatorius
5.	**Ordeal poison**	Used to prove and disprove guilty of a suspect; if a person died after administration, he was guilty otherwise not	Croton, odollam
6.	**Abortifacients**	To cause abortion due to irritation of the uterus	Ergot, croton, oleander
7.	**Stupefying**	Used to stupefy, i.e., travelers and women	Datura, chloral hydrates

5.5 Psychoactive and Narcotic Drugs

Psychoactive and narcotic drugs are a broad category of drugs that show their effects by adjusting levels of neurotransmitters, primarily act upon the CNS of a user's body and change the person's perception, mood, consciousness, and behavior, and produce euphoria and analgesics effects. Therefore, they help in treat various medical conditions and also cause dangerous side effects if not taken in a prescribed manner or in case of drug abuse [1].

5.5.1 Classification of Drugs

CNS depressants are drugs that slow down the activity in the brain which leads to a lower level of awareness in a person.

- Alcohol
- Opioids
- Sedative and hypnotics
- Tranquilizers
- Barbiturates

CNS stimulants are a class of drugs that speed up the activity of the brain that temporarily makes a person more alert.

- Amphetamines
- Caffeine
- Nicotine
- Khat
- Cocaine

Hallucinogens are the drug that distorts the CNS activity in user

- Cannabis
- Magical mushrooms (psilocybin and psilocin)
- Lysergic acid diethylamide (LSD)
- Mescaline
- Phencyclidine (angel dust)

5.6 Postmortem Toxicology: Autopsies in Drug-Facilitated Crimes

Nowadays, elevated rates of violent crimes increase due to the proximal and distal effects of drugs that may lead to circumstances of violence [2]. In other words, drugs are a prime cause of expediting violent crimes such as homicide, accident cases under intoxication, manslaughters, sexual offenses like rape, and abortion, which are known as drug-facilitated crimes (DFC). DFC are criminal acts that are carried out by means of administering a psychotropic substance to a person without his/her knowledge with the intention of modifying awareness, perception, impairing behavior, degree of consciousness, judgement, decision-making capacity, and anterograde memory because of their potent central nervous system depressants and mimic severe alcohol intoxication or sedation [3].

Most commonly seen substances and illicit drugs like alcohol, opium, marijuana, cocaine, and sexual assault facilitated drugs (alcohol, GHB, rohypnol) [4]. These substances are not recognized by a victim because of their properties such as colorless, odorless, and solubility (readily soluble).

Ethanol is the most commonly identified substance that has possible relationship to criminal behavior or that had been voluntarily ingested prior to criminal activities like drug-facilitated sexual assaults, homicide, and robbery. It has been followed by cannabis, cocaine, and amphetamines based on their cost, use, and differential pattern of control [5].

These days, drug-facilitated crimes like rape, sexual assault, robbery, and sedation of elderly persons are encountered. In these conditions, the victim is incapacitated through spiking the drink or beverages with a hypnotic drug. Drugs involved can be benzodiazepines (flunitrazepam, lorazepam), hypnotics (zopiclone), sedatives, anesthetics (GHB, ketamine), and opioids. Synthetic opioids are mainly used because of the pleasure and enjoyment they experienced after taking them, and addictive character of these substances leads to an alarming threat for public health and results in violent crimes committed in society [6].

5.7 Death: Toxicological Investigation

In cases that are mentioned above, specific data are retrieved from autopsy, toxicological analysis of specimen, and police reports to determine the nature of poisoning, cause of death, manner of death, and time since death. Analysis of the cause of death reveals the role of substitution drugs in the death process.

The investigation is carried out in violent, unnatural, or unexpected deaths. To investigate the death, all available information includes the terminal events, scene investigation, police reports, and social and medical history. The success rate of arriving at the correct conclusion depends on the combined efforts of the investigator, the pathologist, and the toxicologist [7] (Fig. 5.1).

Death investigation begins with scene investigation by investigating officers who collect samples from the crime scene for further investigation, to know the events actually happened on the scene. After collection of evidence from the scene of the crime like container, vomit, blood, and hair [8], the body of the victim is taken to the hospital for autopsy which is performed by a pathologist. During hospital, pathologists examine the body properly in daylight (never an autopsy performed at night). Injuries, color of skin and wound, syringe marks, smell from internal body and mouth, and ocular changes are noted by him. After observing, collect viscera samples and biological fluids from the victim's body (Table 5.4).

5.7.1 Physical Examination

It is important to get an idea about the type of substance used.

- Pigmentation/dermal clues
- Body temperature
- Hypothermia
- Hyperthermia
- Olfactory clues

5.7.2 Pigmentation/Dermal Clues

1. Cherry pink color—carbon monoxide
2. Brick red color—cyanide
3. Flash pink—cocaine
4. Dry and hot skin—*Datura*, Atropine
5. Blister and bullae—CO, barbiturates, imipramine, methadone
6. Petechiae and purpuric spots—warfarin
7. Diaphoresis—cocaine, LSD, salicylate, pilocarpine, mercuric chloride
8. Erythema—boric acid, cyanide, mercury
9. Flushing—clonidine, ergot, niacin, theophylline
10. Formication—cocaine, arsenic

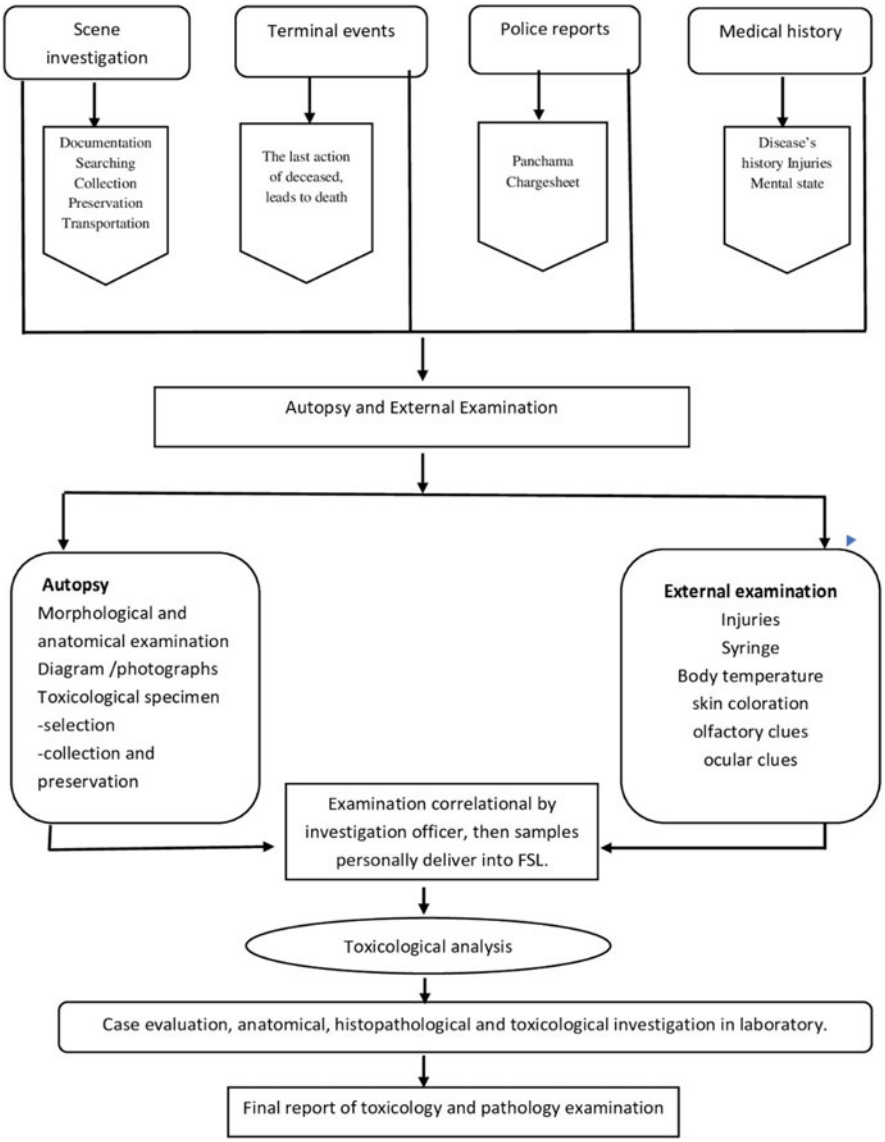

Fig. 5.1 Framework of death investigation

11. Acne brown color—chronic poisoning of phenytoin, bromides, iodides, coaltar products
12. Hyperkeratosis and raindrop pigmentation—chronic arsenic poisoning
13. Cyanosis and methemoglobinemia—nitrates, nitrites, ergotamine, phenazopyridine, aniline dyes

Table 5.4 Artifacts preserved during autopsy for examinations

Artifacts preserved	Sample quantity	Poisoning cases	Preservative used
Amniotic fluid		Criminal abortion	Nil
Bile		Alkaloid, barbiturates	Nil
Blood	30 mL	In all cases especially in alcohol, cyanide	10 mL of blood + 100 mg sodium fluoride (enzyme inhibitor) + potassium oxalate
Brain	500 g	Strychnine, volatile poisons, opiates	10% of formalin
Bone	10 cm of shaft of long bones	Heavy metal, radioactive poisons, pesticides	Saturated solution of common salt
CSF	1–5 mL	Alcohol	Sodium fluoride
Fat	2–5 g	Agriculture poisons especially organochlorine	Nil
Fingernail and hair	1 hair to 200 mg (cut as close to the scalp at vertex posterior)	Metal poisoning	Aluminum foil, envelope, or a plastic tube without any preservative. No need for preservatives
Heart	Full heart Pericardial fluids	Cardiac poisons, aconite, digitalis	Saturated solution of NaCl or rectified spirit
Insects	20 g of maggots collect from inside and below the cadaver	Cocaine, benzodiazepines	Common salt used as a preservative
Intestine and content		In all cases	Rectified spirit
Kidney	Half of each kidney	In all cases	Saturated sol. of sodium chloride
Liver	200–300 g	In all cases	Saturated solution of NaCl
Meconium		Neonatal drugs addiction	Nil
Nasal swab		Inhalant drugs, cocaine	Nil
Placenta		Abortifacients	Nil
Spinal cord		Strychnine, gelsemine	Nil
Spleen		Asphyxiants	Saturated sol. of NaCl
Stomach content	20 mL	In all cases	Nil
Sweat		Drug of abuse	Directly stored in glass bottles
Synovial fluid	10–15 mL	Alcohol	Nil

(continued)

Table 5.4 (continued)

Artifacts preserved	Sample quantity	Poisoning cases	Preservative used
Urine	30 mL	In all cases	1 g of sodium benzoate Fine grains of thymol
Uterus		Criminal abortion	
Vitreous humor	1–2 mL	Volatile poisons, embalmed bodies	Sodium fluoride (10 mg/mL)

5.7.3 Olfactory Clues

1. Fruity smell—ethanol, chloroform
2. Pungent smell—choral hydride
3. Rotten egg—mercaptan, hydrogen sulfide
4. Burnt grapes—cannabis
5. Bitter almond—cyanide
6. Kerosene—organophosphate

5.8 Toxicological Examination

After physical examination, during the autopsy, collect viscera and biological fluids from the deceased body and preserve properly to avoid contamination or decomposition. After collection and preservation, these samples send to forensic science labs personally by investigating officer with maintaining chain of custody.

5.8.1 General Steps in Toxicological Analysis

1. Define the purpose of method.
2. Select an appropriate sample matrix.
3. Sample preparation.
4. Select an extraction method like solvent extraction, total alcoholic method, solid-phase extraction, and accelerated solvent extraction.
5. Choose internal standards.
6. Choose the appropriate detection method such as chemical examination and instrumental investigation.
7. Prepare an accurate report of analysis.

When the toxicology report is prepared by the experts at FSL, it is collected by the investigating officer and brought to the doctor, who studies the report and then gives the final conclusion regarding the cause of death, manner of death, and time scene death.

5.9 Case Study: Drug-Facilitated Crimes (DFC)

A drug-facilitated offense is not a new phenomenon. In the month of June 1982, there was a case reported in Poison Control Center of Marseille, France. In this case, young girls involved as a victim of sexual assault, who had experienced amnesia and found by the roadside. One of them reported having flashbacks; she described that a vehicle stopped and the driver offered her a can of soda which she partially drank and then threw into the street. As per the information gained by police from the victim, the police find a can by the roadside. This can transport to a forensic laboratory for examination. The toxicological examination of the content revealed the presence of triazolam. After the police arrested a suspected person, he confessed that he would pierce a hole in the bottom of the can, then introduce a crushed triazolam tablet, seal it with a patch, and offer an undamaged can to his victim. He took the victim to a deserted place and commit sexual assault with them.

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Forensic Biology: A Passport for Biological Evidence

6

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Abstract

Though acts of crime committed today may have evolved to be part of the cyberspace, most criminal activities originate from the space that all living creatures live in: Earth. From harming fellow human beings to causing disarray in the environment, mankind has been the cause of and victim of criminal activity. From the very first crime to have ever occurred on planet Earth, to the petty theft that occurred in the neighbourhood store, biological evidence has always been a beacon in the run to serve justice.

As time evolved (and so did the means of committing crime), the means of testing biological evidence found has evolved too. Perpetrators who may have gotten away with, say murder, prior to DNA testing are being held accountable now. Forensic biology, in all its glory, is a frontrunner in proving the theory that "no crime can be perfect".

This chapter delves into the field of forensic biology and how it serves as not only a passport for biological evidence but also a seemingly one-way ticket to crime solving and justice.

6.1 Introduction

The first crime to have occurred on Earth (according to most discussions) is that of the murder of Abel by his brother Cain. As detailed in the Bible, Cain struck Abel's head with a rock, smashing his skull and leaving him bleeding to death. Even in this rendition of a crime that occurred when perhaps the concept of crime wasn't even

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born will you find *biological evidence*: entities of biological origin that prove or disprove a theory. The rock smeared with blood and blood on the ground show how and where the crime happened. An injured Abel proves who the victim is, and his blood on Cain's hands tells us in which direction we ought to be looking while searching for a perpetrator. Of course, there are many other questions to be answered, but to a layperson, it is as clear as day—Cain killed Abel.

This was a story of centuries ago, when the world first seemingly came into existence. Today, with studies specific to forensic science and biology, we now have more questions that we can ask (and seek the answers to) and more equipment and scientific method to reach a conclusion.

The biosphere consists of biotic and abiotic components, of which the biotic content far surpasses the abiotic one. Hence, it should come as no surprise that the scope of forensic biology is unfathomable. Every living entity that crosses paths with a crime can be tested as evidence. Forensic biology is one of the strong pillars on which the foundation of the justice system is set. This chapter discusses the evolution of forensic biology, what constitutes a biological evidence, how different types of biological evidence are tested and analysed, and what are some of the issues faced in the field of forensic biology.

6.2 History of Forensic Biology

While mummification, a process Egyptians used to preserve mortal remains of the dead, is the earliest possible record of autopsy studies (say around the year 400 BC) [1], it was in 1853 when Ludwik K Teichmann discovered that the haemin protein found in blood can be crystallised. This discovery let to the description of the Teichmann test, one of two microcrystalline assays run to confirm the presence of blood in a particular sample [2]. In 1903, the popular colour test for blood—the Kastle-Meyer test—was described. Joseph H Kastle made the rough draft for the test in 1901, and Erich Meyer modified it in 1903 and wrote down the three-step procedure, as followed today [3]. The Adler/Benzidine colour test for blood was then introduced in 1904, by Adler and Adler [4]. Eight years later in 1912, Takayama described the procedure to confirm the presence of blood in a sample, by the formation of ferroprotoporphyrin crystals of the haemin protein [5]. In 1930, Hans Fischer received the Nobel Prize in chemistry for artificially synthesising the haemin protein [6]. These are all ground-breaking discoveries and inventions related to confirming or negating the presence of blood.

The most popular method to identify the morphology or "group" of red blood cells in the human body is the ABO blood grouping system, which was prescribed by Karl Landsteiner in 1901 in the now Medical University of Vienna. He formulated the system after finding out that red blood cells would agglutinate if the source varied, as in, if the blood of two persons was mixed. This is the first recorded proof that the make-up of blood was not constant throughout the entire human population.

While all the available tests for blood in the twentieth century were helpful, there were still hurdles that needed to be crossed. For instance, there was no provision to

check for the presence of blood that had already been wiped out from a surface. In a parallel setting in 1928, H O Albrecht realised that the presence blood increased the luminance of luminol in the presence of an alkaline medium. It was only nearly a decade later, in 1936, that Karl Gleu and Karl Pfaanstiel attributed this increased shine to haematin, a component of blood. These findings are what crime scene investigators use to perform the luminol test in crime scenes, today, where the liquid is sprayed over an area suspected to have housed blood and viewed under ultraviolet (UV) light [7, 8].

Towards the end of the twentieth century, specifically in the 1980s, there was a lot of scientific research done in order to make use of DNA as an individualising mark. In 1983, Sir Alec Jeffrys, in collaboration with the Forensic Science Services (FSS) of the United Kingdom, invented DNA profiling, or DNA fingerprinting, wherein short tandem repeats (STRs) in a DNA sequence are used to determine individual characteristics in a person. This was first used in 1983 and 1986 to bring about convictions in the rape and murder of two teenagers in Leicestershire, England [9]. In 1983, Kary Mullis invented the polymerase chain reaction (PCR) technique to make multiple copies of the same sequence of DNA, a step which is now very pivotal in DNA profiling.

In present time, the twenty-first century, we have reached an era where entire genomes have been mapped and the data interpreted and saved (The Human Genome Project 2003) and where all the textbook steps of analysing blood are fast-forwarded to DNA analysis. As of today, there has been research on new software that gives the result of DNA testing based on statistics and algorithmic actions of a computer, that aid in providing the weightage, or percentage of contributions in a mixed or degraded DNA samples.

6.3 Sources and Types of Biological Evidence

There are many situations in a forensic setting in which evidence originates from a biological/natural source, the most crucial and common of which is when a crime is committed against another living being—human or otherwise. In cases of violent acts of crime like murder and rape/sexual assault, it is very imperative to identify and collect samples of body fluids, the most common of which is blood. Other seemingly transparent or non-coloured body fluids like saliva, sweat, and genital discharge may take a keen eye to locate. All body fluids are the store house for DNA samples, the proper testing and identification of which can lead a forensic investigator to identify victims and culprits. Blood and sweat may also be found at the scene of burglaries, where the criminal may get scathed against a broken glass or may wipe sweat on a surface without meaning to do so.

Another common biological evidence found in a violent crime is that of skin and residue under fingernails. In cases of struggle—commonly seen in assaults—the victim may try to break free from the perpetrator, often scratching them enough to pull skin, and (in some cases) draw blood. Skin cells being nucleated also house genetic material, which can lead to making an identification.

Any sample that can provide DNA—toothbrushes, hair brushes with strands of hair, and cigarette butts with traces of saliva—are commonplace in cases of determining blood relations. These articles became most useful in identifying human remains of the September 11 attacks in 2001, after the biggest fragment of human remains measured barely a few inches, as a piece of bone. For maternity disputes, it is considered more helpful to submit samples of hair, as hair strands contain mitochondrial DNA (mtDNA) which is always passed down the maternal line of a family. For paternity identification cases, usually blood or a swab of inner cheek cells is taken as samples.

As with the definition of biological evidence, the origin isn't necessarily limited to human beings. Diatoms play a huge role in determining the cause of death in suspected cases of drowning. The location of the diatoms in the body determines whether drowning occurred anti or post-mortem, and the types and morphology help one assess in what water body drowning occurred. Many types of diatoms can be indicative of the dead individual drifting across water bodies with the flow of water currents.

Most animal parts are crucial evidence in wildlife-related crimes. Claws, fur, hide, teeth/fangs/tusks, horns, antlers, and hooves prove useful in handling cases of poaching, hunting, and illegal trafficking of animals. Certain samples of caviar can help a wildlife forensic practitioner determine the species of sturgeon they were derived from.

Plant parts too are considered evidence of biological origin. The poppy flowers and leaves of *cannabis* are evidence samples in cases of illicit drug production and trafficking. Destruction of growing endangered species of plants is considered a crime, and hence, these plant parts can be used as evidence. There have been many cases of smuggling of sandalwood from the forests of India, the most common targets being in the states of Kerala and Karnataka.

The possibilities for finding evidence that is also of biological origin are endless.

6.4 Biological Evidence as Evidence for Other Forensic Fields

This, however, does not mean that all evidence that originated from a biological source shall be tested as such. Since forensic science is a multidisciplinary field, it is very common to have multiple connections among the various subfields within the study of forensic science. It is often through collaborative effort and teamwork that evidence is analysed and cases are solved.

The examination of human remains post-mortem—an autopsy—is considered to be a study under *forensic pathology*, which also focuses on microbial activity and stages of degradation of human/animal remains at different time intervals.

All body fluids become what is known as "serological evidence" under the subfield of *forensic serology*. It is under forensic serology that all colour and microcrystalline tests for blood are conducted. Other fluids like genital discharge (vaginal fluids and semen), saliva, sweat, and even vomit are tested to confirm their presence or absence in a sample in question.

Organ remains like stomach, section of intestine, brain, and liver are tested in the sub-discipline of *forensic toxicology*, where concentration levels of certain toxins in the viscera are determined through various chemical-related tests.

Bone specimens are studied carefully under the field of *forensic anthropology*, and the marrow of such bone may be extracted for DNA testing and identification. It was mainly through anthropological studies that the remains of the last Russian Romanov family were identified and the causes of their deaths in 1918 was ascertained. Teeth samples fall under the sub-specialty of *forensic odontology*. While the pulp (if available) is extracted for DNA testing, the morphology and positioning of teeth provide information of age, time since death, and presence of dental disease (if any), among other details. Upon examination, bones can relay information like age, height, race, presence of fractures, and cause of death (a crack in the skull can be indicative of a blow to the head).

Often it happens that fine particles participate in cross-transfer of evidence that help provide the link between the scene of crime, the victim, and the perpetrator of the crime in question. A common example of such fine particles, apart from sand, is pollen. Due to its microscopic size and somewhat sticky exterior, pollen is quick to stick on any surface it comes in contact with. While Mother Nature has made the provision for pollen to stick to the stigma of a female flower, pollen is also known to stick to clothing, shoes, skin, and other surfaces that may help establish a link to an outdoor crime scene or an outdoor secondary crime scene. This study of the microscopic morphology of pollen is called *forensic palynology*.

Forensic botany deals with the legal implication of plant parts as evidence of a crime. The most common cases of this instance are when plant parts are used to manufacture illicit drugs, like the *datura* flower and various parts of *cannabis* that are used to make different drug products. Plants also become evidence when endangered species are smuggled or destroyed, or even illegally grown, as in the case of when endangered wood species are used to make guitars and other musical instruments.

Any type of evidence that is found in the wild naturally is analysed by the subdivision of *wildlife forensics*. In case of dead animals, the rules of *forensic veterinary pathology* are nearly the same as that for human remains.

6.5 Testing and Assessment of Biological Evidence

Like all articles that are considered evidence, proper collection, packaging, transportation, and testing are crucial for them to be presentable in a court of law. These practices when done properly, followed by maintaining a proper *chain of custody* (a chronological record of the journey of evidence, from its discovery at the crime scene to the court where it is presented) solidifies the authenticity of what is being presented before the magistrate. This convinces the judge and/or the jury to rely on the evidence put forth in order to reach a decision without hesitation and worry of error.

Wherever possible, samples are tested following the following path in the exact order:

- Presumptive tests—those tests that are highly sensitive, but not specific to one type of substance, that serve as an indicator for what the sample *may* be
- Confirmatory tests—a highly specific test that gives a positive result for one particular substance only, thereby *confirming* the contents of the sample
- Individualising tests—tests that help pinpoint the source of the sample, as in the case of DNA testing

6.5.1 Analysis of Major Body Fluids Analysis of Blood

The most common of all serological evidence is blood, a specialised connective tissue that, by virtue of circulation, indirectly connects all parts of the body to each other via the heart. It consists of three types of cells, or *corpuscles*—the *erythrocytes* (or red blood corpuscles, RBCs) that are responsible for oxygen transport by the use of haemoglobin, the *leucocytes* (or white blood corpuscles, WBCs) that serve the immune system and fights infection, and the *thrombocytes* (or blood platelets) that help clot blood and prevent haemorrhage. All these cells are suspended in what is known as *plasma*—a straw coloured medium that is made up of 90% water and 10% of miscellaneous substances like proteins, urea, fats, and hormones, among others [10].

It is the erythrocytes and leucocytes that have the most forensic relevance in the testing of blood. RBCs have haemoglobin, which houses the haemin—a porphyrin that contains iron and chlorine. This haeme (the complex that helps bind haemoglobin to oxygen) group is what causes a colour change, luminescence, and microcrystal formation in testing for the presence of blood. However, since RBCs are enucleated (lack a nucleus), they cannot be used for DNA testing. This is why WBCs become the source of genetic testing with respect to blood.

Presumptive tests for blood include all colour-based tests and chemiluminescent tests. In the former, a change in colour due to oxidation is a presumptive positive, and in the latter, the emission of a light of certain wavelength is an indication of haemoglobin presence [10].

The most reliable and sensitive of all colour tests is the *Kastle-Meyer Test* or the phenolphthalein test and is considered so because it can detect blood from a sample that is diluted down to a part in ten million and also because old stains of blood can be detected by use of this test [10].

To perform the test, a cotton swab (Q tip) is dipped in distilled water and rubbed over the surface or stain that is suspected to house blood. A drop of phenolphthalein is added, followed by a drop of 3% hydrogen peroxide. The swab goes from colourless to pink when the peroxide is added, if blood is supposedly present. This is because phenolphthalin (colourless)—a reduced form of phenolphthalein (pink) that is formed so by boiling the substance with zinc in an alkaline medium—becomes oxidised in the presence of hydrogen peroxide to form the pink coloured

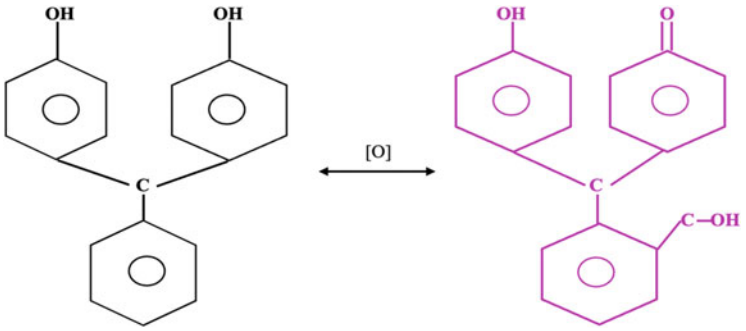

Fig. 6.1 The Kastle Meyer test: Phenolphthalin (reduced, colourless) reacts with hydrogen peroxide to form phenolphthalein (oxidised, pink)

phenolphthalein [3]. The colour change must be observed rapidly, as the pink colouration forms naturally as time progresses. If no colour change is observed, it can be inferred that the given sample either does not contain blood or does not have blood in the minimum quantity required for a positive result (Fig. 6.1).

The Kastle Meyer test gives a positive result for substances like fruits, vegetables, and food stuffs made from these (like tomato ketchup) as these contain peroxides as well.

Other reagents used in the colour testing for blood is *tetramethyl benzedine (TMB)* and *leucomalachite green*. These reagents are used in the same three-step process as the Kastle Meyer test, but the colour change varies. While TMB changes a colourless swab to a blue—green colour, leucomalachite green gives a greenish-yellow tint upon oxidation. Not only are these two reagents less sensitive to blood, but they also are not very cost effective and are known to be carcinogens.

Most crimes where blood is found to be an evidence are violent in nature, and finding just a few drops of blood is rare. Blood is often found covering a large area of the surface it rests on, and while cleaning may be a time-staking process, blood pattern analysts require that the blood pattern remain undisturbed in order to properly study spatter [10]. Hence, in these cases, rapid colour tests are not feasible, and so chemiluminescent tests are used to determine the possible presence of blood on a given surface. Once sprayed, the emitted light is observed under an alternate light source (ALS), as the output may be faint [10]. Such practice, however, is not encouraged if the blood stain is visible, and a sample of it can be collected (Robert Spaulding 2002) [10]. The common reagents for chemiluminescent tests are *luminol* and *fluorescein*.

Luminol (3-aminophthalhydrazide) works the same way as does phenolphthalein in the Kastle-Meyer test; only this time, the colour of light emitted varies from a blue-white to yellow-green. A solution of luminol, an oxidiser, and water is made and sprayed upon the surface. The resultant light emission is viewed in darkness and in the presence of a light source. This reagent is extremely sensitive to haemoglobin and can detect blood diluted up to a part in five hundred thousand. The pattern must be photographed immediately, as the light is best emitted for half a minute only,

following which additional treatment must be done to increase duration of emittance. This, unfortunately, comes at a cost as minute details are lost on multiple treatments.

Fluorescein is also used in the same way, with the additional thickening substance in the preparation, which is used in order to increase the staying capacity of the solution on vertical surfaces, thereby preventing its running off [10]. The solution, however, emits fluorescence and must be lit up with the help of ALS with a wavelength of 450 nm.

While both reagents are not carcinogenic, they cause severe irritation if brought in direct contact with skin, and so, proper care must be exercised while handling them. Studies about whether or not a chemiluminescent test destroys the chances of a DNA test being done vary.

Confirmatory tests for blood include microcrystalline assays and the Vibert's fluid test. In these tests, the microscopic nature of complexes formed with haemoglobin are studied. These tests are specific to blood and can hence confirm its presence in a given sample.

The *Takayama Test*/haemochromogen test is one of two confirmatory tests for blood that checks for the formation of crystals of a haeme complex. In this test, a drop of sample containing the stain presumed to be of blood is placed on a glass slide and gently hated after having a coverslip on top. A drop of pyridine (in an alkaline medium, in the presence of a reducing sugar) is placed over the sample drop [10]. The formation of light-pink coloured crystals in the shape of needles confirm the presence of blood. These crystals are called pyridine ferroprotoporphyrin.

The Takayama test is said to be very sensitive and can give a positive result from very old bloodstains as well. However, proper care should be taken while heating the sample, as improper heating may lead to a false negative result.

Prior to the Takayama test, the *Teichmann test* was widely used as microcrystalline test. While the principle of both tests are the same, the reagents and crystal morphology vary. For the Teichmann test, a potassium halide is dissolved in glacial acetic acid, a drop of which is placed over the sample drop on a slide and warmed. Microscopic inspection will yield brown-coloured rhombic crystals, which are of ferriporphyrin halide.

Another way of confirming the presence of blood is by observing the sample under a microscope, after it has been treated with *Vibert's fluid*, a solution containing sodium chloride and mercury chloride in distilled water. This procedure extracts the red blood corpuscles and can be viewed as red dots/blobs under the microscope [11].

In real-life situations, it is very rare to find laboratories perform presumptive and confirmatory tests as mentioned above. This is because when a stain is suspected to be of blood, it is directly sent in for DNA analysis, where a DNA profile is generated. The DNA profile will not only confirm the presence of blood but also provide information related to species and the source of the blood themselves. Bypassing the traditional analytical steps not only saves on time and money, but the end result will be more accurate, given the nature of DNA testing. This, unfortunately, puts a lot of strain on the DNA laboratories and creates a tremendous backlog of testing and report filing.

6.6 Semen Analysis

Semen is a viscous, white fluid that is secreted by males who have crossed puberty, via the urinogenital tract, upon sexual stimulation. It contains the male gamete, spermatozoa/sperm, along with a complex mixture of sugars, amino acids, and salts to ensure the viability of sperm once it leaves the male body (and preferably enters the female reproductive tract, where fertilisation is due to take place). Each ejaculate from a healthy male is known to hold around 125 million sperm cells, all of which have genetic material of the male source. Hence, finding and testing semen traces are crucial in solving cases of sexual assault.

For **presumptive analysis**, the key component of forensic relevance is *acid phosphatase*, an enzyme secreted by the prostate gland, which is tested by use of Brentamine Fast Blue B. For the test, a buffer of anhydrous sodium acetate is prepared, in which alpha-naphthyl phosphate is dissolved. Another solution of the buffer with the Fast Blue B reagent is also prepared. Next, a portion of the sample stain is cut, and both the reagents are placed on it, one after the other, a minute apart from each other. A rapid purple colouration is indicative of a presumptive presence of semen on the stain. Older stains may not stain purple, due to decreased acid phosphatase activity, [10] but that does not mean that semen is absent from the stain. Another downside to the test is the fact that other body fluids, like vaginal secretions, may test positive as these contain acid phosphatase too.

Semen stains can also be views under alternate light sources at 450 nm, under amber goggles, and give a blue-white fluorescence [10]. This method of identification can be used on light and dark surfaces and covers a large area. However, fluids like saliva and urine will also fluoresce, so care must be taken to look for the exact colour of emitted light. It is noteworthy that an absence of fluorescence does not imply absence of semen.

A long-time popular method of **confirming the presence of semen** is through staining the spermatozoa and viewing under microscope. This is due to the abundance of these cells in semen (an exception would obviously be the semen from men who are aspermic/unable to produce sperm in semen). The *Christmas tree stain* does exactly as the name suggests: light a sperm cell up, like a Christmas tree: the tip of the head stains pink, the bottom becomes dark-red, the idle portion becomes blue, and the tail becomes yellow-green. The reagents involve nuclear fast red (a solution of ammonium sulfate and nuclear fast red in deionised water), and picro indigo carmine (picric acid and indigo carmine in water) [10]. After a drop of the extract is placed on a slide, the nuclear fast red solution is added and allowed to sit for ten minutes. After the excess is washed off with water, picro indigo carmine is added and let to sit for half a minute, following which it is washed off with absolute ethanol. Once complete, the slide is viewed under the microscope to view the stainings [10].

Using the enzyme-linked immunosorbent assay (ELISA) test, a *prostate specific antigen* by the name of p30 is detected to confirm the presence of semen. ELISA is based on interaction between antigens and antibody, and when the reagent is added

to the sample swab, an intense purple colour is observed. Deeper the colouration, more is the quantity of p30 in the sample [10].

The *time since intercourse* not only helps draw a time sheet of events but also helps locate the position of sperm cells in the case of rape (where protection is not used). Motile sperm survives for the shortest period in the vagina (3 h, due to the surrounding acidity), followed by the mouth (6 h) and the longest in the rectum (6–65 h, or until defecation). P30 levels are used to estimate the time since intercourse, as most p30 content becomes obsolete a day after the act [10].

As with the case of blood, most semen samples are sent straight for DNA testing, apart from the p30 test for determining time since intercourse.

6.7 Saliva Analysis

Analysing saliva is often tricky, mainly because it is rarely found as an evidence to begin with. In the cases of bite marks during violent crimes, movement of the victim may cause accidental wiping off of any saliva residue. Items that come in contact with the mouth—licked articles (as an adhesive), cigarette butts, beverage cups, and cans—all produce trace amounts of saliva. Spitting may yield scope for DNA testing, due to its quantity.

Common **presumptive tests** for saliva check for amylase activity (such as the one with starch water and iodine: a blue-black coloration if amylase is absent, no colour change if amylase is present). However, these are not selective at all, as amylase is a component in other body fluids too. Hence, a presumptive positive for saliva via these tests is unreliable.

The best shot at getting a confirmation of the presence of saliva is through DNA analysis. This is possible due to the fact that saliva often contains epithelial cells of the cheek and, in case of oral injury, blood too.

6.8 Urine Analysis

Urine can be presumptively tested by checking the presence of urea (through urease enzyme), by its foul odour on heating or by testing for creatinine with picric acid (yellow-orange colouration). Though DNA typing for urine is rare, it may be possible if the sample is very concentrated [10].

6.8.1 Bloodstain Pattern Analysis (BPA)

Bloodstain pattern analysis (BPA) is the study and interpretation of patterns formed by bloodstains that form when a violent crime has occurred. The practice uses biology, fluid dynamics, and mathematics (and logic) in order to *reconstruct* the crime and piece together the chain of events that are assumed to have occurred during the commission of the crime in question.

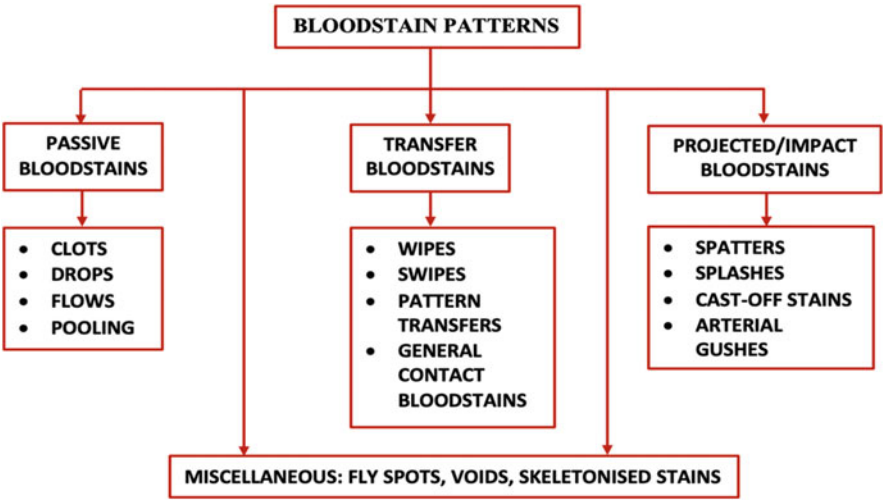

Fig. 6.2 Categories and types of bloodstain patterns [10]

BPA can be defined as "analysis and interpretation of the dispersion, shape characteristics, volume, pattern, number, and relationship of bloodstains at a crime scene to reconstruct a process of events" *(Bloodstain Pattern Analysis by Bevel and Gardener 2002)*. A great amount of work and experience is required to be a bloodstain pattern analyst. Even then, this procedure is not entirely reliable, and many accused individuals convicted by this form of evidence have been acquitted in subsequent trials due to the information not being convincing enough. Hence, BPA is mostly treated as a corroborative evidence, instead of direct.

There are many types of bloodstain patterns an analyst will come across, often from the same scene of crime. Rather than being one big picture, bloodstain patterns are considered to be many minute details that make up a whole, larger pattern. The classification of bloodstain patterns is as follows (Fig. 6.2):

Passive bloodstains form when blood flows due to its viscosity, as well as due to gravitational pull (if blood falls on a vertical surface, like a wall). As a result, passive bloodstains can be observed as a *clot* (when blood becomes jelly-like after being left unabsorbed), *drops* (solitary blood drops that aren't connected to any other pattern part), *flows* (blood dripping due to gravity), and *pooling* (an area filled with blood).

Transfer bloodstains are created when an object comes in contact with blood, thereby creating a pattern by disturbance, and include *wipes* (when an unstained object in moved through blood; like a shoe being pressed in blood, leaving a pattern) and *swipes* (when an object stained with blood is rubbed across a clean surface; like a blood-coated hand leaving a mark on a door frame).

When a pattern is made after an object is struck against blood, a *projected pattern* or *impact pattern* is formed. The types of this include *spatters* (that can be *forward*—when blood droplets move in the direction opposite to the object causing it—or *backward*—when blood flows in the same direction of the spatter causing object),

splashes (when an object is thrown in a pool of blood, resulting in a splash of blood droplets), *cast-off stains* (when an object that bears blood is repeatedly shaken so as to get rid of the blood on the surface, like a hammer being shaken back-and forth), and *arterial gushes/expirations* (when blood sprays out of a cut artery or vein). Expirations can also occur when a person with a lung injury coughs blood out.

Whilst these are the main categories of bloodstain patterns, there are many bloodstain patterns that do not fit in any of these brackets. Such patterns include *fly spots* (that are created by activity of flies, including flying, sitting on, and eating and defecating blood), *voids* (empty spaces in bloodstain patterns that are caused due to an object blocking the area from the blood projectiles, and are in the outline of the obstructing object), and *skeletonised stains* (old stains with cracking edges as the blood dries) among others.

Mathematics—especially geometry—plays a major role in bloodstain pattern analysis, as it is used to determine what is known as *point of origin*, the area where blood first emanated from before hitting the target surface. In order to determine the point of origin, the basic measurements must first be understood. The *angle of impact* is the acute angle formed in between the intercept of the target and the vector of the bloodstain [10], while the *direction angle* is what comes in between the long axis of the blood stain and a zero-degree vertical that is taken as the reference point [10].

To find the point of origin in a three-dimensional space, analysts first find the *area of convergence*, which is the area on the surface where blood first struck before dispersing into smaller patterns. This area is found by unfurling thread along the long axis of many bloodstains, such that they appear to converge at a similar area [10]. The point, or area of origin, is then assumed by drawing strings in the z-axis and may help determine the probable position of the victim when first injured.

Many tools—from basic strings, pencils, and geometric apparatus to the most sophisticated software—are used in aid of visualising the pattern better. The process is also made easier by documentation, which when done properly can help reach a solution better. Photography is a must in order to re-assess the bloodstain pattern again in the future when needed. It is imperative to first click photographs relative to a stationary point, in order to establish where the pattern was found in the crime scene. Individual photographs of fragile patterns must be taken first, and all photographs must be accompanied with a scale to measure. Photographs must also be taken in close-up and far ranges. These photographs can then be analysed as individual parts of a grid or as a whole [10].

Case Study: Sam Sheppard—The Wrong Man [12]

On the morning of 4th of July, 1954, Marilyn Sheppard was brutally murdered in the bedroom of her home in Ohio, USA, having been bludgeoned by an object. As a result, there was blood all over the room, and some drops of blood were found on the floors all over the house. When her husband, Sam Sheppard (a neurosurgeon), was questioned, he told the police that he fell asleep on the couch in the living room the previous night, during a movie watch with their guests. Marilyn bid the guests farewell and went to her room to retire for the night. Sometime later, Sam was

awoken by the cries from his wife, and when he ran upstairs to the bedroom, he saw a "figure" before being knocked unconscious. By the time he regained his bearings, the assailant was downstairs, so he chased the person to the beach further down where they scuffled and he was knocked out again. This inconsistent story, backed with a lack of murder weapon, immediately made Sam Sheppard a suspect. In his first trial, the prosecution argued that Sam killed his wife as a means to legitimise an extra marital affair with a nurse in his clinic. The prosecution also led the jury to believe that the weapon in question was a surgical scalpel, based on a blood print made by a supposed scalpel on the pillow on which Marilyn's head was placed. The defence was denied access to any physical evidence, and hence could not make any assertions with respect to injuries, blood patterns, even the surgical scalpel blood print in question. The trial ended on December 21, 1954, where Sam Sheppard was found guilty of second-degree murder and was awarded a life sentence. After serving 10 years of his sentence, he was released in 1964 and began the process of re-trial, which began in 1966. It was here that the jury realised that the prosecution back at the first trial put forward statements with no concrete evidence whatsoever and went with the flow based on mere assumptions. It was during this time that renowned criminologist Paul Kirk presented the bloodstain pattern analysis report based on analysis of the spatter in the bedroom, which showed that the killer was left-handed, when Sam was right-handed. This evidence, coupled with the now baseless argument of the prosecution, resulted in Sam Sheppard to be exonerated and proven not guilty. The experience, however, took a toll on his mental health, and Sam Sheppard died of alcoholism-related complications four years later.

6.8.2 DNA Fingerprinting

The method of DNA fingerprinting/profiling that has become popular today was first put forth by Sir Alec Jeffrys in 1985, when he discovered that certain parts in a sequence of DNA kept repeating over and over at close proximity to each other and also that these repeating sequences vary from person to person. These repeated sequences came to be known as *variable number of tandem repeats (VNTRs)*, and the process was termed *restriction fragment length polymorphism (RFLP)* as it used restriction enzymes to cut the sequence that held the VNTR. A smaller form of the VNTRs, the *short tandem repeats (STRs),* is commonly tested in most cases. Since its inception, the profiling process has become almost indispensable, as almost every situation in life—ranging from paternity testing to identifying victims of a mass disaster—requires a DNA profile to be made.

When a sample is first found at the scene of crime, it is collected and packaged carefully to prevent damage that could lead to incorrect results. Once at the laboratory, it undergoes three phases of processing:

In the *biology* phase, the sample is processed so as to extract the genetic material, lysing the cells that hold it. It is then measured to check for the amount of DNA recovered. Once all this is done, the DNA strands are cut at the STR points by the use

of restriction enzymes, and the STRs so formed are amplified by the use of the PCR process [13].

Next comes the *technology* phase, where the products of PCR are separated by means of electrophoresis, and the STRs are detected in order to characterise them. The strands are then fluoresced in order to measure them, and these are later assessed to determine the sequence of the tandem repeats, in a process called sample genotyping. The DNA profile bands are then prepared at the discretion of the practice that varies among laboratories [13].

Finally, in the *genetics* phase, the DNA is profile is matched to the profiles of other samples, including that of the reference sample. Should there be no match found, it is inferred that the reference and questioned samples have originated from different sources. A no match is called exclusion. In the eventuality of a match, or an inclusion, the profile is matched with a database bearing profile information of samples of the same demographics. At the end, a test report is generated which includes the probability that a random match may have occurred (as in, a chance that a random person from a demographic can have an STR profile identical to the markers used for the sample in question) [13] (Fig. 6.3).

Case Study: The First Case Solved by DNA Profiling [13]

In November 1983, Lynda Mann—a 15-year-old schoolgirl in Leicestershire, England—was found raped and strangled to death after being missing for an entire day. She was found in a deserted area, which was part of the shortcut between her school and home. With the forensic technique prevalent back then, the police found a semen stain and found it to match a person with blood type A, along with an enzyme profile that was common in only 10% of males. The case ran cold soon after, due to lack of evidence and further leads.

In July 1986, another girl, Dawn Ashwood (also 15 years of age) was found in the same way as Leslie Mann—beaten, raped, and strangled to death in a deserted area. She was found two days after she went missing, having never returned home after going to visit a friend. The modus operandi, along with the genetic details of the semen sample found, were the exact same as the case three years prior. Richard Buckland, a 17-year-old boy with learning difficulties, confessed to the crime against Dawn Ashwood but denied having anything to do with the murder of Leslie Mann.

By now, it had been a year since the DNA fingerprinting technique was formulated. Dr Jeffrys compared the semen samples found in both cases to each other, and they matched. However, the DNA type did not match Richard Buckland's, proving that he had lied. So began a countywide hunt across three villages, where samples of 4000 men were collected and compared to the profile of the semen sample found. It was like finding a needle in very big haystack, and the police were beginning to lose hope at the lack of a breakthrough.

And then, the breakthrough came. Not as a match, but as an eyewitness account, where a person testified to having seen and heard a person brag about how he "provided a sample on behalf of Mr. Pitchfork". The impersonator, Ian Kelly, informed the police that his colleague, Colin Pitchfork, had told him that he had already impersonated his friend and given a sample in the friend's name, who was

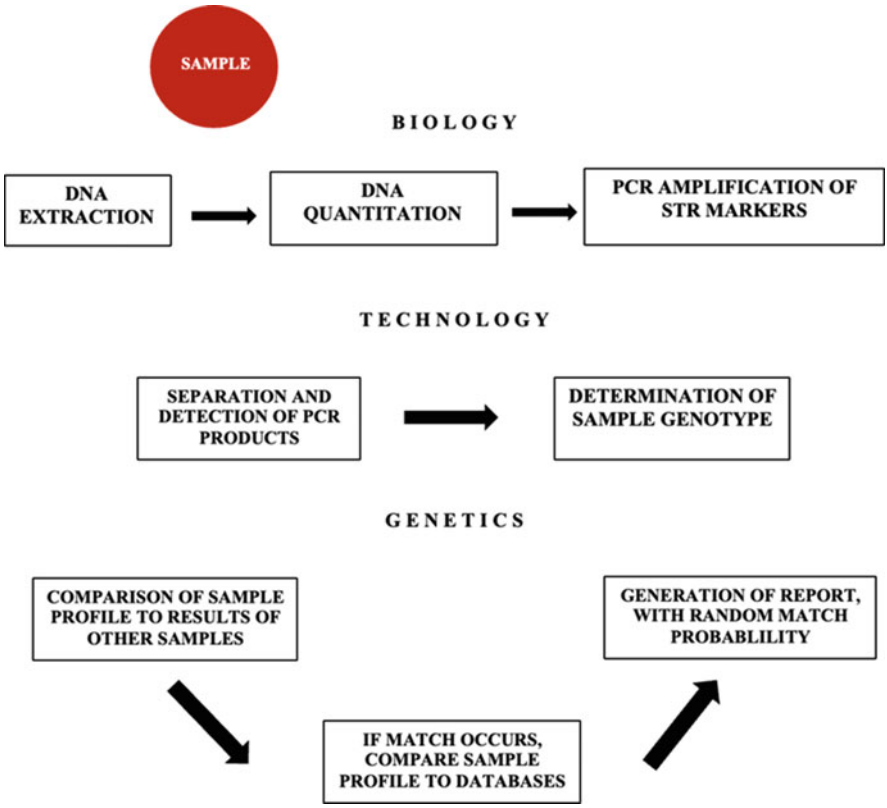

Fig. 6.3 The DNA Fingerprinting Process [13]

being harassed over a sexual assault case. As a result, Colin could no longer give his own sample under his name, so he asked Ian Kelly to do so.

Colin Pitchfork was caught in 1987, and his blood was drawn for testing, where his DNA matched that found in the semen stains on both crime scenes. He pleaded guilty to both rapes and murders, as well as another case of sexual assault, and was sentenced to life imprisonment.

It is only in an ideal, textbook situation where one would find evidence with enough genetic material to be tested that is not only fresh but also free from contamination. While this is true when a reference sample is collected from a person for paternity testing, this is not the case for material collected from the scene of crime. Even if, supposing, a good sample is collected, the time delay from sample collection to sample testing (sometimes even years, in an ill-equipped laboratory) ensures that a mostly degraded sample is available for testing. Perpetrators, too, refuse to cooperate and hand over a reference sample for testing immediately, being

protected by the requirement of a warrant being issued first, which may not be granted if there is insufficient evidence that a warrant is required to begin with.

Thankfully, all these problems become miniscule in the eyes of the *polymerase chain reaction (PCR)* technique, which was developed by Kary Mullis in 1985. The PCR technique makes multiple (in the order of millions) copies of the specified sequence of DNA, which has made it possible to easily genetic material from a given sample, no matter the size. This invention won Mullis the Nobel Prize in Chemistry in 1993.

In the process of polymerase chain reaction, an enzyme is used to replicate a specific region of the DNA strand, by repeated heating and cooling at every cycle (usually, 30 cycles make up the entire process). As a cycle is completed, the target DNA sequence is replicated at every molecule where the said sequence exists. The end result, known as an *amplicon*, is then used for testing by various methods, as it contains enough genetic material to be detected by the equipment used in the testing process [13].

Most handbooks for PCR mandate that the sample be anywhere in the 20–50 μL volume range. This is because an optimal weight is required to get a good quality of amplicon: too small a sample may be lost to evaporation from high temperatures, and a heavier sample may cause an issue with thermal equilibrium; it takes more time for a temperature change on the outside to be transmitted on the inside of a sample with large volume [13].

As the years have passed since the inception of PCR, it has become more easy to perform the technique. Most forensic laboratories add a template DNA sequence to a pre-made PCR kit that contains all the components needed for the process to be carried out. These kits simply require the user to add in a small portion of the template DNA to the sample DNA that needs to be amplified and is best used when the volume of template DNA added roughly matches that of the concentration range for which the kit was designed [13] (Fig. 6.4).

The most important components of PCR include the primers that anneal at the 3' end of the sequence and is what precede the sequence that has to be multiplied, the DNA template itself (along with some information about the actual sequence, so that appropriate primers are selected), building blocks that contain nucleotides, and a polymerase that will help the nucleotides attach in the correct sequence. The most commonly used polymerase is the *Taq*, which is developed from the *Thermus aquaticus* bacteria found in hot springs, as these are most thermostable at the high temperatures at which the PCR process is carried out.

The sample is first incubated at 95 °C for 11 min, and the cycle count on the machine is set to 28–30. In the main process, the DNA strands are *denatured* or undone from its helix, at a high temperature of 94 °C for about a minute. Next, the primers (oligonucleotides) are *annealed* to the 3′ end of each strand at 59 °C for a minute. Finally, the strands are *extended* by the use of the Taq polymerase that will help the nucleotide blocks to get attached to each other in the correct sequence (complimentary to the template strand). This process occurs at 72 °C for a minute. In the final extension, the machine is run for 45 min at a lower temperature of 60 °C,

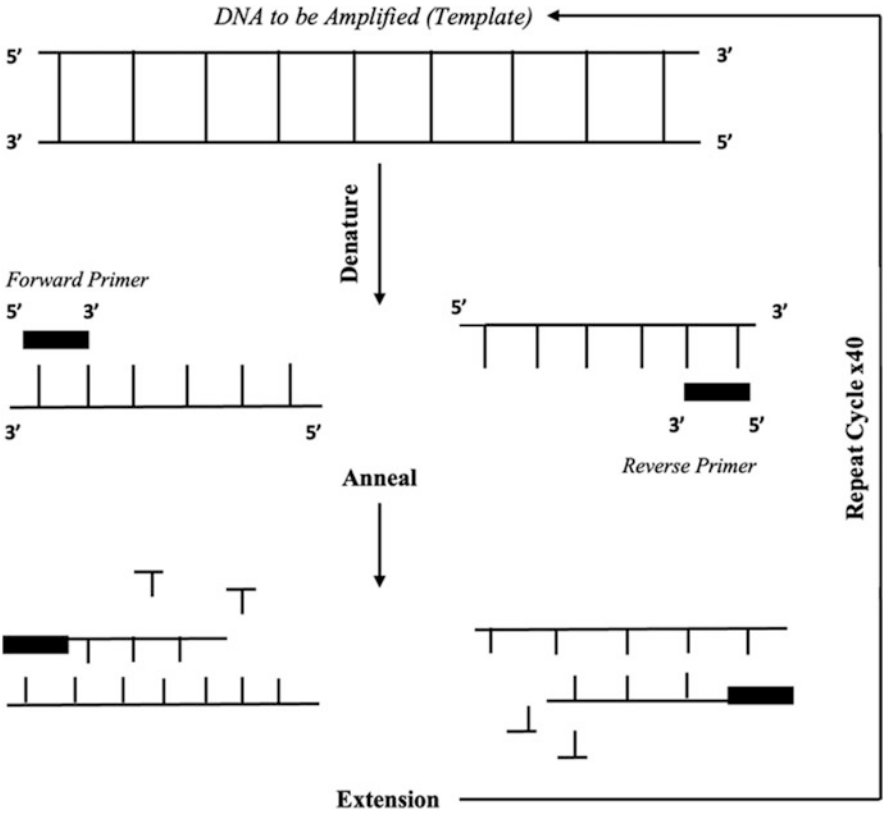

Fig. 6.4 The polymerase chain reaction (PCR) [13]

and the final soak requires that the temperatures be reduced to 25 °C until the samples are removed [13].

The polymerase chain reaction can be conducted in such a way that many copies of multiple regions within the same strand may be made, as long as more than one primer pair is added to the mix. When two or more regions of DNA are replicated simultaneously, the process is called *multiplex PCR*. For this to work without a hitch, the primers used must be compatible with each other, the annealing temperatures must be within the same range, and the excessive complementarity between primers must be minimised; else the primers will anneal to each other, and not the DNA template. The optimisation process, however, will be much more complicated here as compared to when a single region is copied [13] (Fig. 6.5)

Another popular PCR method that came about with new instruments is the *real-time PCR*, which analyses the changes that occur per cycle from the amplification of the template DNA sequence, by monitoring the change in fluorescence signals emitted. The most common of the approaches studied is the use of *5′ nuclease*

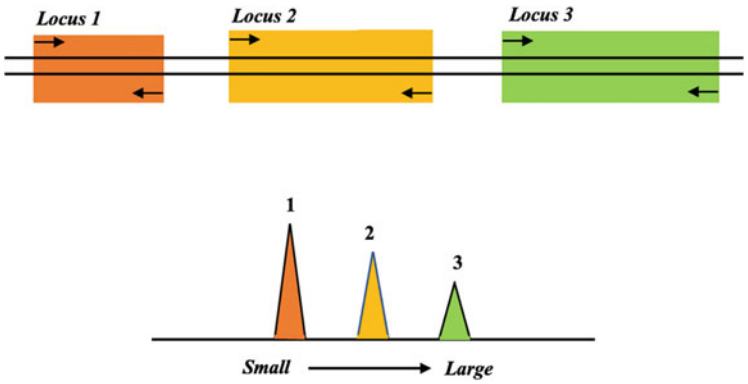

Fig. 6.5 Multiplex PCR with three loci. Primer size varied per loci, such that amplicons could be separated by size [13]

assay (TAQMAN) or by using an intercalating dye like *SYBR Green*, which is highly specific for a double-stranded molecule of DNA.

The TaqMan probes are labelled with two fluorescent dyes—the *reporter (R)* dye at the 5′ end and the *quencher (Q)* dye at the 3′ end—each emitting a different wavelength, which is made to hybridise specifically in the region of sequence between the two primers. The probe usually has a higher annealing temperature as compared to the primers so that the detection starts as soon as the annealing process begins. When the probe is intact and the R and Q dyes are close to each other, there is barely (if any) fluorescence that is emitted due to energy transfer between the two. When polymerisation (extension) begins, the strand being extended pushes aside any TaqMan probes that have hybridised to the target sequence. 5′ exonuclease activity in the Taq polymerase will eat out any probes that have annealed to the sequence. This causes the R dye to be released from the probe, and now that two dyes have separated, fluorescence signals begin emission. The signals increase if the TaqMan probe and the template sequence complement each other [13].

Each PCR process is characterised by three separate phases: *geometric/exponential amplification*, *linear amplification*, and the *plateau region (Bloch 1991)*. These are observed in a plot of fluorescence signals versus PCR cycle count [13].

When the exponential amplification is on-going, there is immense precision in which the amplicons are formed. When the process takes place at near perfect efficiency, the number of amplicons formed double with each complete cycle. A plot of cycle count versus the log scale shows a linear relationship during this phase. This is the optimal place to check for the relation between fluorescence and cycle count, since this is where there will be a consistency between input and output DNA [13].

The linear phase of amplification follows the exponential phase, as amplification efficiency slows down to an arithmetic increase instead of a geometric one, due to components falling below the critical concentration. Given that some components

like the primers may be used up at varied rates in the course of the reaction, the linear phase is not useful for comparison as this phase varies among samples [13].

Finally, the plateau region forms when the accumulation of PCR products becomes stagnant as multiple of the components reach their efficiency limit [13].

During the polymerase chain reaction of short tandem repeats (STR), there are many artefacts formed that can interfere with the process of interpreting and genotyping the alleles present in the template DNA. These artefacts are as follows:

Stutter products are characterised as small peaks near the STR allele peak, which are several base pairs smaller, and are formed when STR loci are copied by a DNA polymerase. These are also known as *shadow band* or *DNA slippage product* and have been found ever since STRs were first described. Analysis of stutter products from a locus has shown that these are usually one repeat unit short than an allelic peak. Stutter products that are a repeat unit bigger than an allelic peak are very rarely observed in commonly used tetranucleotide repeat STR loci. The most common way stutter products are formed is when a region of the primer-template complex becomes unpaired during the extension phase of PCR, thereby allowing a slippage of either primer or template strand such that one repeat unit falls short and forms a non-base-pair loop [13].

Stutter products are roughly the same size as PCR products of alleles, and so it is often challenging to determine if a small peak is actually a stutter product, or an allelic peak formed from a minor contributor in a mixed DNA sample. Laboratories often quantify the percentage of stutter products, as the ratio between stutter peak height and corresponding allele peak height. On studying the alleles from the standard thirteen loci in the CODIS, it was found that while each locus has a different amount of stutter product formation, the longer the allele for an STR is, the greater is the degree of stutter formed. Finally, it was also noted that stutter percentage for tetranucleotide repeats is less than 15% for standard conditions of amplification [13].

Stutter formation may be reduced by using STR markers that have longer repeat units (which have variations on the common repeating strand), with alleles that have imperfect repeat units, and by using polymerases that process faster, as a faster polymerase can copy both the strands of DNA before they could come apart and re-anneal out of turn during extension [13].

Non-template additions form PCR products that are one base pair longer than the actual template strand. This happens when the DNA polymerases add an extra nucleotide to the 3′ end of the amplicon as the template is being copied. For example, in the case of Taq polymerase, an extra adenosine is added in what is called *adenylation*. In partial adenylation, the amplicon does not have an extra adenine at the start of the sequence. These differences contribute to a peak broadening if the resolution capacity of the system is poor. Furthermore, varying adenylation across sample can cause an inaccurate sizing and genotyping of potential microvariants. For these reasons, it is preferred to have all amplicons either with or without an extra adenine, and not a combination of both [13].

Microvariants are those alleles which have a sequence variation of any kind, as compared to alleles that are commonly observed. These may differ at DNA markers by one or more base pairs and are called so because they only slightly vary from full

repeat alleles. Since microvariants do not size the same way as alleles present in the reference allelic ladder, they are also called ***off-ladder*** alleles [13].

Suspected microvariants are easy to spot: while observing a heterozygous sample, one allele will line up with the respective fragment size on the allelic ladder, and one will not. The relative size difference between the questioned sample and an allelic ladder marker run in the exact same conditions (reference) will then confirm if the allele is actually a microvariant or not [13].

When a DNA template exists for a particular allele but fails to replicate in the amplicon during PCR, a ***null allele*** or ***allele dropout*** occurs. Sequence changes are known to occur either within the repeat sequence, in the region that flanks the repeat sequence, or where the primer binds. If a base pair change occurs in the template while the PCR process is on-going, primer hybridisation can be hindered and amplification fails. This causes a failure to detect and replicate an allele in the template DNA. This, however, is extremely rare as the flanking sequence surrounding the STR repeat is known to be quite stable [13].

6.9 Y-Chromosome Testing

The evidentiary value of the Y-chromosome lies in the fact that it is found only in males, as maleness is determined by the *SRY (sex-determining region in Y chromosome) gene*. Given that most of the reported cases of sexual assaults involve men as perpetrators, Y-STR testing becomes handy in testing DNA samples in cases wherein autosomal DNA testing has limited use. This can be seen in cases where the perpetrators are aspermic or have had a vasectomy (thereby nulling sperm content) or in the cases of gang rapes, where the number of perpetrators (and thereby the number of contributors in a sample of mixed DNA) are more than two. Primers specific to the Y chromosome when used in PCR improve the chances of detecting trace amounts of the criminal's DNA and can also be used to verify men deficient in the amelogenin gene [13].

As much as it is a boon in forensic science, the degree of maleness is also a bane for the field. This is because the Y chromosome (the bulk of it, at least) is directly passed down the male line in generations, without variation or recombination. The only source of a change comes from mutations, which occur rarely. Thus, while the Y chromosome can match a suspect to the scene of a crime, it can only point towards blood-related men, and not just one individual. This means that the sample that originated from the suspect will also match his father, brothers, cousins, uncles, and so on. This is why while the Y-STR testing can be a useful tool, autosomal tests for DNA typing always take first priority. And while there is always a sense of uncertainty with regard to finding one person involved in a crime, many relatives having the same Y chromosomes increases the reference sample pool when it comes to identifying a victim of a mass disaster. It also helps when a paternity test needs to be carried out, but the father in question is missing or deceased [13].

There are two main categories of DNA markers that are adopted in use of looking for diversity/variation in Y chromosomes. The first is of *bi-allelic markers* that

include single nucleotide polymorphisms (Y-SNPs) and an *Alu element insertion* (a short strand of DNA that is characterised by the action of *Arthrobacter luteus*), of which the latter was discovered first. These are also called *unique event polymorphisms* since they have a very low mutation rate [13].

The second category is of the *multi-allelic markers* which consist of two minisatellites and over 200 Y-STR markers. Since they have a high mutation rate, these are used to differentiate Y-chromosome haplotypes with a higher resolution. Some Y-STR loci occur more than once on the chromosome, due to some regions being palindromic. When these loci are amplified during PCR, the primers end up producing more than one type of product, which may lead to confusing while determining the number of loci present. This may be mistankenfor the presence of two loci types on the Y chromosomes, when in fact there is just one [13].

Forensic scientists rely mainly on the presence of kits that are commercially available, in order to conduct tests. This is one of the reasons why most forensic science laboratories were apprehensive of testing Y-STRs during the PCR process. Kits available today amplifies not only the loci but also the amelogenin marker. This is essential as the presence of amelogenin confirms that the test has not failed on DNA samples from females since it will produce just one amplicon of the X chromosome. Added to this is the possibility of finding the concentration levels of male and female DNA from a mixed sample, which is sought from studying the amelogenin X and Y peak ratios. However, a quick consumption of PCR components occur when there is a high amount of female DNA, as the amelogenin primers will now have extra sites to attach to [13].

As with all DNA testing, a database is essential, as it serves the purpose of drawing an estimation at how rare the Y-STR profile in question really is. A lack of recombination between the Y-chromosome markers implies that the profile must be combined into a *haplotype* (a set of markers on a single chromosome) in order to search a database and determining rarity [13].

A Y-STR profile can be interpreted in three ways: there can be an *exclusion* as the Y-STRs do not match, and therefore do not have the same origin, an *inconclusive result*, because the data is insufficient to reach a conclusion, or it the results are not clear, or an *inclusion*, where the results from the questioned and reference sample have ample similarities to be considered to have originated from the same source.

Case Study: The Boston Strangler's Guilt Proved Half a Century Later [14]
It was the 1960s, and women across the capital of Massachusetts, Boston, became targets for a serial killer and rapist. By the time the spree was complete, eleven women were raped and strangled with the nylon stockings they wore during the attacks. The perpetrator was not identified for a long time and was soon nicknamed the Boston Strangler. The crimes had started five District Attorney's offices investigating the cases—such was how the locations of all eleven crimes were spread across the city.

In October of 1964, a woman was sexually assaulted by a person posing as a detective. The description given by this victim to the police led them to her assailant, a man by the name of Albert DeSalvo, and when his sketch was published, many

women came forward, claiming he attacked them as well. By this point, the police had yet to make a connection of the serial crimes to the assaulter.

While incarcerated, DeSalvo confessed to being the Boston Strangler to his fellow inmate, and once he was charged with rape, he gave detailed descriptions of the eleven crimes, reducing the mistaken count from 13. The police believed him, as though some parts of his confessions were inconsistent; he had divulged details of the crimes that had been kept away from the public. However, no physical evidence ever proved that he indeed was the Boston Strangler, and so he was given a life sentence for multiple rapes and thefts in 1967. After escaping from his hold at a hospital for the criminally insane, he was caught and placed in a high-security prison, where he was found stabbed to death in 1973. His killer/s were never found.

The last victim of the Boston Strangler, 19-year-old Mary Sullivan, was found brutally raped and then strangled in her apartment in 1964. Among the evidence was a maroon blanket her body was wrapped in, which bore traces of seminal fluid. This remains to be the only DNA evidence in this case of serial crime—six samples that were preserved by the Boston Police Crime Laboratory's lead forensic scientist, in hopes that a future scientific advancement could enable DNA to be matched to a suspect.

In 2013, members of the Boston Police tailed DeSalvo's nephew, Tim, to his workplace at a construction site, where they collected a water bottle used by him and left aside. Once the DNA sample was extracted from the water bottle, a Y-STR profile was generated and matched to the profile of the Y-STR from the seminal fluid found at the scene of Mary Sullivan's murder fifty years prior. The two profiles matched, and that was considered evidence enough to get a warrant to exhume Albert DeSalvo's remains from his resting place, in order to conduct proper autosomal testing. Since he was confirmed to have raped and killed Mary Sullivan, it is now widely assumed that this is evidence that DeSalvo killed the other women as well and is most likely the Boston Strangler everyone wanted to see the face of.

6.10 Mitochondrial DNA (mtDNA) Testing

Mitochondrial DNA (mtDNA) is the DNA that is found in the mitochondrion, an organelle where chemical energy from food is broken down into adenosine triphosphates. The human mtDNA was first sequenced by Fredrick Sanger in England in 1981. This sequence known as the *Cambridge Reference Sequence (CRS)* or *Anderson sequence* gave rise to the revised CRS version, which is now used as a standard reference to which all mtDNA profile reports are compared to [13].

For forensic relevance, human mtDNA is said to be passed down generations strictly from the maternal side of a family. This is because during fertilisation, the cellular components of the zygote are contributed to only by the egg, which is much larger than the sperm cell, that passes on just its nucleus. In the eventuality that sperm mitochondria is passed to the zygote, it is destroyed by the ubiquitin tag added

during spermatogenesis, which highlights sperm mitochondrion for destruction by the zygote's cellular machinery. Thus, a mother passes her mtDNA to her children and is therefore common in siblings and maternal relatives. This however, is not unique to an individual [13].

Like in the case of Y-chromosomal testing, mtDNA is helpful in solving cases of missing persons and identifying victims of mass disaster and can also help in crime solving, when autosomal DNA samples cannot be recovered.

An advantage of mtDNA typing over nuclear DNA typing is the fact that mtDNA has a high copy number per cell, which in turn increases its sensitivity. This proves important in cases where the nuclear DNA cannot be quantified due to its minute quantity, or is severely degraded [13].

When an mtDNA sample is extracted, care must be taken to perform the extraction in a clean environment, as this DNA type is extremely sensitive to contaminations. mtDNA is often extracted from material with less DNA to begin with, like hair, bones, and teeth. Since bones also undergo anthropological study, it is essential that the bone sample is taken in such a way that it does not hamper the main structure of the entire bone to be studied. For example, an analyst may cut the bone in the middle, instead of all the way through, in order to maintain the length of the bone. In the same way, physical examination of the hair strand sampled must be done prior to extraction of mtDNA, as this will destroy the hair strand in its entirety. Comparisons showed that hair from the head gives best results, as compared to that from pubic and axillary hair regions. [13]

Once extraction is complete, the HV1 and HV2 regions of mtDNA (hypervariable regions) are amplified by use of PCR and are the amplicons are then sequenced. The profile report is then compared to the revised Cambridge Reference Sequence, where dissimilarities are noted. The same procedure is performed for a reference sample, following which the dissimilarities of the samples with the revised CRS are compared to each other. Upon comparison, the results can be either of an inclusion, exclusion, or inconclusive (Fig. 6.6).

Case Study: The State of Tennessee Versus Paul Ware—Where mtDNA Was First Used as Evidence

In 1996, 27-year-old Paul Ware was suspected to have murdered a four-year-old child after having raped her. While all circumstantial evidence pointed to him, the defence counsel argued that the babysitter, another man in the house, committed the crime in drunkenness. He was found sleeping next to the body of the victim.

The girl's blood was not found anywhere on the suspect, nor was any seminal secretion found on her. But a small strand of red hair was found in the girl's throat during the autopsy, similar strands of which were also found on the bed at the scene of crime.

Mitochondrial DNA was sequenced and profiled from four sources—the hair strand found in the victim's throat, the strands found in the bedroom, saliva swabs taken from Paul Ware, and the victim's blood. Comparisons showed that both hair samples not only matched each other, but their mtDNA was found to be the same as Paul Ware's. No match was found between the hair strands and the girl's blood.

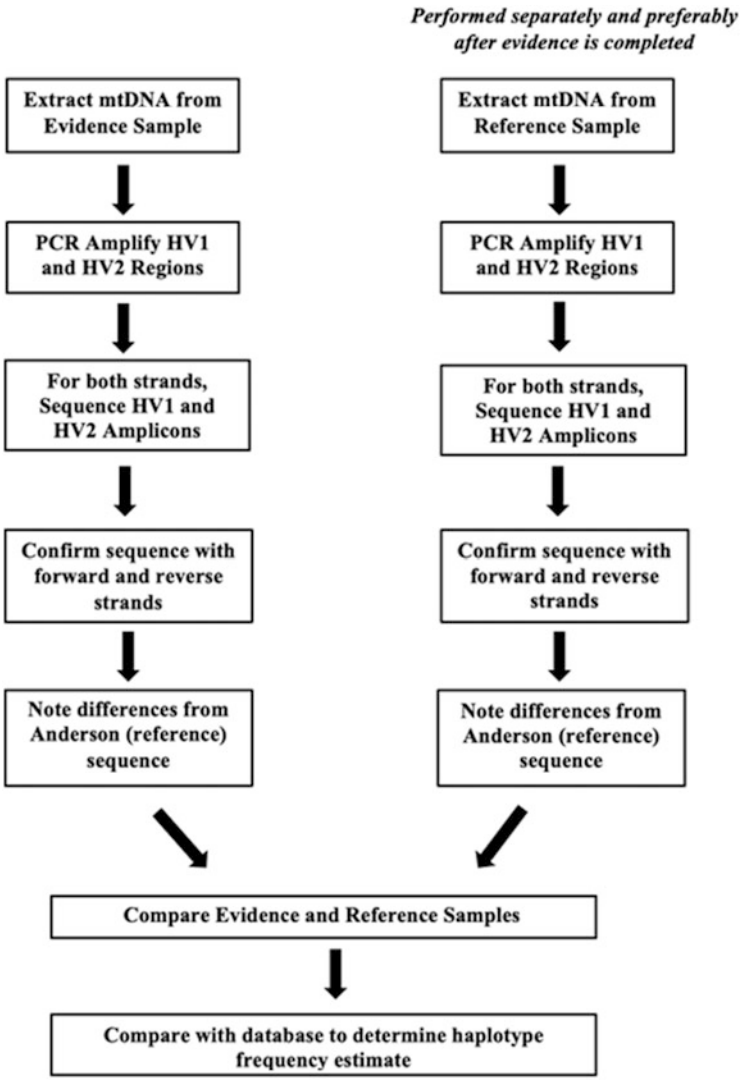

Fig. 6.6 Evaluation a sample of Mitochondrial DNA [13]

Currently, the FBI database has mtDNA sequences from 742 individuals. The sequence obtained from Paul Ware and his hair strands had never been seen before.

6.10.1 Non-Human DNA Testing

While most cases requiring a DNA analysis report is human-based, there are times at which DNA samples taken from non-human sources prove to be the most crucial in

an investigation. Pet animals are almost always near humans, and trace evidence transfer is inevitable. Plant DNA can link a deceased to a crime scene or a living person to a drug syndicate. In more serious situations, the now prevalent threat of bioterrorism needs DNA analysts to be vigilant over microbial DNA from time-to-time.

Animal DNA evidence can be found in such situations where the animal is a victim, where the animal is a perpetrator, or when the animal is a witness [13]. When a pet animal is abducted or abused, the victim's DNA sample can help locate where the pet is being held hostage. In cases of animal bites, saliva traces on the bite mark may prove sufficient to pinpoint which animal bit the human [13].

Case Study: How the Family Cat Helped Incriminate a Killer [15]
In 1994, Shirley Duguay went missing. She supposedly left her husband and three children and ran away. Nearly a year later, she was found dead in a shallow grave near her home in Prince Edward Island, Canada. Along with her remains was a jacket with her blood and many strands of feline (cat) hair scattered across the jacket. While interviewing her husband, Douglas Beamish (whom she had separated from), the investigators found out that the victim lived alone with her cat, Snowball, who had white fur. On a hunch, they confiscated Snowball and drew blood from it in order to have its DNA type identified. The investigators faced an issue, though: no crime lab ever tested animal DNA before. So, they got in touch with the Laboratory of Genomic Diversity, a laboratory that specialised in genetic diseases, and were able to devise a method to test the cat's DNA. They further solidified the testing by putting the same method to use on twenty random stray cats found on the island. This was done in order to rule out the chances of a common ancestor or relative of Snowball having deposited its fur on the jacket and to check if all cats on the island had the same ancestor. Eventually, the DNA of the fur on the jacket matched Snowball's DNA. Douglas Beamish was eventually convicted of the murder and sentenced to life imprisonment. This case was the very first to have tested for animal DNA to solve a crime against a human being.

When it comes to plants, they prove to be important when a cross transfer during a crime may have occurred or when illicit drugs need to be tested for. In the first scenario, if the plant part/grass is very rare, pinpointing the crime scene becomes crucial but was an easy task. This was the case in 1993, when two seedpods from the Arizonian Palo Verde tree was found in the back of a pickup truck and used to place a murder suspect at the scene of crime [13]. Upon DNA testing of the seedpods, it was found that out of twelve trees tested in the area, only one tree's DNA provided a match: the one under which the victim's body was found. Thus, this evidence was heavily used against the accused to get a conviction.

A sensitive test for DNA of *Cannabis sativa* (marijuana) was developed by the Connecticut State Forensic Science Laboratory [13], given that marijuana is the most commonly abused drug in the USA. This test can help link an individual to a sample, which in turn can help nab illegal cultivators and bring down a supply network. Unfortunately, this has limitations too, as if the marijuana is cultivated by means of

"cloning" (wherein a plant part is rooted directly in soil to propagate faster) instead of by seed, many samples will have identical DNA profiles [13].

Marijuana DNA is tested either by *randomly amplified polymorphic DNA markers (RAPDs)*, *amplified fragment length polymorphisms (AFLPs)*, or *short tandem repeats (STRs)*.

RAPD analysis uses short PCR primers which have random sequences that are roughly 8–15 nucleotides. These primers anneal too many regions in the given sample genome, thereby creating complex products of PCR. This prevents interpretation of mixtures that kill the reproducibility quality of this method of marijuana DNA testing. It also is tedious to perform, as the amplification conditions require thermal cycler ramp speeds [13].

In comparison, patterns formed from AFLP analysis can reproduce faster. AFLPs are generated by first cutting the double helix with more than one restriction enzyme, following which specific adapter sequences are ligated to the cut sites. PCR amplifiers that recognise the adapters are used to amplify the fragments of DNA that are variably sized, which are then sifted through by electrophoresis. As a result, one can observe a series of peaks in the 50–400 base pair range that can be scored and compared to other sample results by help of computer software. An advantage to this method is that even highly in-bred plants can be distinguished through their AFLP patterns [13].

Just like human STR markers, marijuana STR markers are also highly polymorphic, specific to unique sites on the genome, and can undo mixtures. There are many STR markers developed for marijuana analysis *(Gilmore et al. 2003)*. In 108 samples tested, a hexanucleotide repeat marker showed repeat units, and amplifying primers did not produce ant cross-reactive amplicons in 20 other samples tested *(Hsieh et al. 2003)* [13].

While all these methods do what is seemingly impossible, there is a lack of concrete databases to relate these findings to. These would sufficiently help law enforcement in making better comparisons, and delivering justice faster.

6.10.2 Microbial Forensics and Bioterrorism

With the onset of bioterrorism, a type of terrorist activity of hazardous substances of biological origin (mainly infectious microbes), there is a new-found urgency to equip DNA and forensic science laboratories to battle with a new era of crime solving. It is crucial to have a method of testing that is highly sensitive and specific that can measure components fast, that has portable apparatus, and that, obviously, give reliable results [13].

The challenges faced are humongous: one has to first identify the organism/agent, gather evidence, and trace the source of the agent, all the while being at a risk to contact the disease while doing the aforementioned tasks, while striving to maintain the chain of custody without contaminating the evidence and environment [13]. There also is a need for well-equipped databases that house information of the species studied and their virulent strains, along with reference material needed to

draw a comparison. Validation testing is required to rule out false positives and negatives as well [13].

Comparative genome sequencing looks to be a great tool for investigating outbreaks as was used in the whole genome testing of anthrax and will most likely be the case in the latest COVID-19 outbreak as well.

Case Study: The 2001 Anthrax Attack [13]
Merely a month after the horrifying September 11 Attacks in the USA, the country was faced with another threat. In October 2001, various news agencies and government offices received anonymous letters with the deadly Anthrax spores laced on the front side of the envelopes. These letters came mainly from the centralised postal service. The end result included 22 positive cases of Anthrax, five deaths from the disease, and a nation full of fear to do a seemingly mundane task: open their mail. In the years that followed, more than 125,000 samples were collected and processed by the FBI in an effort to get to the perpetrators. Yet, no charges were ever pressed, and in the ensuing chaos of nursing to health a post-9/11 America, the Anthrax attack case ran cold.

As is the case with most novel means of evidence testing, most courts do not readily accept evidence from non-human DNA testing. This is coupled with the issues of checking the validity of the scientific theory, validity of statistics used, along with the approval of the relevant scientific community prove that a long distance is yet to be covered in the practice. It also is a problem when finding a reference sample to match findings with take time, and a means to make a match may not even be readily available at the time of investigation. Finding appropriate experts to verify and cross-check the application may also be a big hurdle to cross [13].

6.11 Issues with Forensic DNA Evidence

A forensic science laboratory always has to deal with evidence that are less than ideal—body fluids that are contaminated, samples with degraded DNA, and so on. In most cases, a small taskforce ensures that cases are opened months or even years, after the samples were first collected. Improper collection of evidence often results in wet surfaces getting mouldy, thereby destroying the chances of proper DNA testing of the body fluid in question. Mostly, improper evidence results in problems for DNA sampling and analysis. There are also issues related to features of the testing instrument that may inhibit proper results. Some of the issues faced while handling a DNA based-evidence involves the following:

6.12 Degraded DNA

When left in the environment, DNA rapidly degrades to smaller fragments. While moisture causes decomposition to occur faster, presence of nuclease enzymes will cause natural chewing down of DNA strands. Older DNA profiling methods were not able to detect small fragments of DNA properly, as the molecules must have high molecular weight to detect, say VNTRs by use of the RFLP technique. While high-quality DNA typically has a molecular weight of 20,000 base pairs, degraded fragments of DNA appear as a mere smear, that clearly weigh much, much less. This is why PCR (especially multiplex PCR) is absolutely essential for the DNA profiling process, as very minute fragments can be amplified and brought to the required molecular weight. However, for the process to occur smoothly, the DNA strand surrounding the STR region must be entirely intact, so as to facilitate proper annealing of the primers. Should there be a break in the middle of the strand, the extension process will also end at that particular beak [13].

It is, therefore, best to use STR markers in PCR as it can easily be amplified with small size of product, along with the fact that there is a higher chance that the primers will find an intact strand for multiplication. Furthermore, a limited range of STR alleles are beneficial for degraded DNA samples because both alleles in a pair are identical in size, thereby preventing preferential amplification to occur. [13]

Another way to solve the issue of degraded DNA sampling is by the use of mini STRs. According to *Wiegand and Keliber (2001)*, highly degraded/low amount of DNA can be successfully multiplied by using STR primers that are very close in space to the repeated region, such that smaller but more concise products may be generated. While this is beneficial in its own right, there are many disadvantages as well, the major of which being that multiplex PCR may not proceed at optimal capacity, since small primers limit the loci that can be simultaneously amplified. Also, since different primers are used with the mini STRs, there is a chance of allele dropout from the primer binding site. Yet again, in rare occurrences, a point mutation may occur outside the region flanked by the primer, causing an undetectable change in the PCR products. Regardless, with the help of proper study, experimenting, and care, the use of mini STRs will be very beneficial in forensic science laboratories in the near future. [13]

6.13 PCR Inhibition

Another obstacle faced in processing DNA evidence is the fact that PCR amplification may be hindered by certain inhibitors present in the samples. Often it happens that a sample of blood or semen is found in soil, or on wood, or on wilted leaves if the scene of crime is outdoors. These contaminants may also get extracted along with the genetic material in the evidence. Non-DNA-containing items, like dyes, leather, fibres, and so on may, contain DNA polymerase inhibitors.

These inhibitors can either interfere with the cell lysis process that is essential for DNA extraction, interfere with degrading the nucleic acid present in the sample, or

hamper polymerase activity that in turn will hamper enzyme-based amplification of the DNA sequence. As a result, either some alleles from the loci are lost, or the entire loci may fail in replication altogether, which in turn causes production of partial DNA profiles which look as though they are of degraded DNA samples. This can be corrected by the use of mini STRs as smaller sequences can be amplified much more efficiently than larger ones [13].

As a solution to the inhibition problem, the sample may be diluted in order to dilute the inhibitors (in turn minutely reamplifying the DNA), or excess of Taq polymerase may be added, so that it can bind to the inhibiting molecules and eat them out, while the excess molecules can aid in the amplification process as usual. Another solution consists of adding additives, like bovine serum albumin or sodium hydroxide that can neutralise inhibitors of the polymerase. Lastly, a separation step may be introduced before PCR to separate DNA from inhibitors with the use of agarose gel plugs [13].

6.14 Issues Related to Contamination

To *contaminate* is to accidentally transfer DNA. There are three possible ways in which contamination during PCR may occur; one is when the sample itself is contaminated with genomic DNA from the environment (which is entirely dependent on how the sample is collected, and how much care is exercised to prevent contamination), second is when samples get contaminated with each other in the preparation phases, and lastly when a sample is contaminated with amplicons of a previously performed polymerase chain reaction. The latter two can easily be avoided with proper laboratory procedures being adopted [13].

Laboratory-based contamination can be cross-checked by running a negative control/blank sample in parallel with the evidence, keeping all reagent parameters across both samples the same. Presence of PCR products in the blank sample is indicative of contamination, and the source must be found and omitted [13].

Contamination can adversely affect a case and its end results, though very rarely seen. In practice, most laboratories are very careful when it comes to processing evidence; so much so that all pre- and post-PCR formalities are performed as far away from each other as possible. It is usually during the collection process where carelessness is observed. Supposing a police officer has handled evidence without the use of gloves, his/her DNA gets mixed in the sample and may mask the DNA of the actual culprit, which may lead to a wrongful declaration of innocence. This adds to the pressure in laboratories as the sample is now categorised as “mixed” and will be analysed as such [13].

6.15 Mixed Samples and Their Analyses

A *mixture* occurs when there are more than two contributors of DNA in a single sample. These can be observed in cases of gang rapes, where the DNA of multiple assailants gets mixed with that of the victim, or when an injury causes a murder accused to bleed onto an already bleeding victim. As discussed above, a seemingly simple sample may become a mixture if not handled with care by police officials.

Mixtures—as the name suggests—are complicated to analyse and undo without experience and training. With progress in technology, PCR sensitivity has increased and along with the help of fluorescence detection can help spot minor components in the DNA profile. Statistical calculations to interpret mixtures have been thoroughly studied and are now in use in various laboratories across the USA [13].

In order to increase the chances of mixtures being detected, more loci and genetic markers with high count of heterozygotes are used. The degree of detection of varied DNA sources is directly related to the contributing percentage of DNA from each source, the specific combinations of genotypes, and the total amount of amplified DNA. Due to these parameters, all mixtures cannot be assessed using the same scale, as these vary from one to the other. By using highly polymorphic STR markers with more alleles, there is a greater resolution between two DNA sources. The more markers are examined in multiplex PCR, the higher is the chance of seeing multiple components in a mixture. The quantity, too, vastly affects detection. There is a higher chance of differentiating between two components that have a roughly equal ratio, as compared to if one is fairly larger than the other. Usually, components below 5% concentration go undetected. Peak heights from electropherograms or fluorescence scanners are studied to quantify the components of a mixed sample, as well as to determine the possible genotypes of the sources of the mixed sample [13].In order to determine whether a sample in question is a mixture or not, the answers to the following questions are sought after:

- Do any of the loci show two or more peaks in the allele size range?
- Is there a severe imbalance of peak heights between heterozygous alleles in a locus?
- Does the “stutter product” appear unusually high?

Should the answer to any of these be in the affirmative, it can be deduced that the DNA profile being studied has come from a mixed sample. Usually, a mixture is first identified by the presence of more than two peaks at one or more loci. At the same locus, sample with DNA from multiple sources can exhibit one to four peaks due to varied genotype combinations. When contributors share more than one allele, they become masked and the genotypes may not be distinguished easily. However, by examining the profile at loci where there are no shared alleles, it becomes easier to find a contrast between the contributors [13].

Once it has been determined that the sample being examined is a mixture, the next step is to designate the allele peaks, a process that becomes complicated with the presence of stutter products and other artefacts that emerge on an electropherogram.

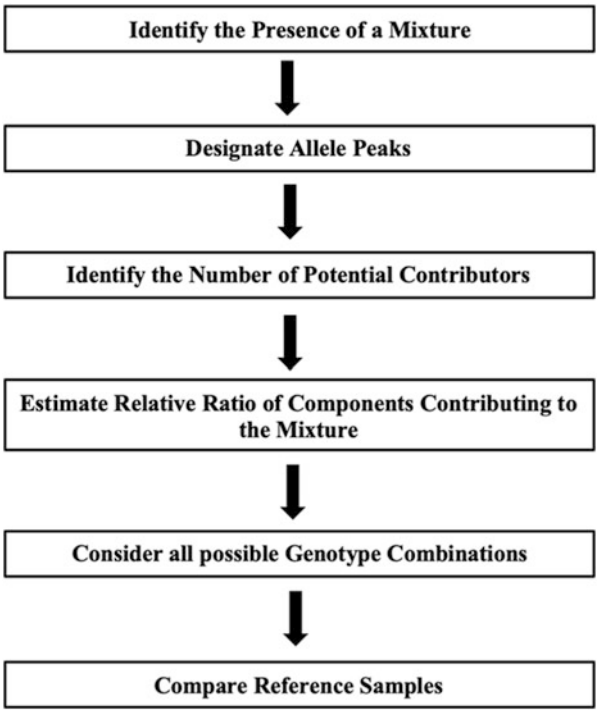

Fig. 6.7 Interpreting a mixture [13]

It is not always possible to exclude stutters since they are allelic products that differ by just one repeat unit. Generally, a stutter product is identified as being one repeat unit less and smaller than 15% of the area of the allelic peak. Still, confusion surrounding whether to consider a peak as a stutter product or an allelic peak gives rise to a bias within the examiner which may lead to an incorrect interpretation [13].

Next in line is to identify the number of contributors, as well as figuring out the ratio of contribution. The more the contributors in varying amounts, the more complicated the deciphering process. Studies have shown that the contributing ratios do not get affected by the PCR process *(Gill et al. 1998; Perlin and Szabady. 2001)*. In this way, peak areas and heights in an electropherogram can be related directly to the amount of DNA of each contributor in the mixed sample [13] (Fig. 6.7).

Once the ratio is estimated, genotype combinations have to be considered and drawn. Peaks that represent an allele are named alphabetically, starting from "A". Using the peak areas as reference, the genotype pairs are formed. Using the ratio estimate, along with the possible allelic pairing, the major and minor contributors can be estimated [13].

Finally, the genotype profiles are compared for possible contributors with the genotypes of reference samples provided. References can come from the victim or the accused. Once a match is found, the person cannot be excluded as a contributor to the mixed sample [13].

6.16 Conclusion

Biological evidence can be found in a plethora of crimes that are committed, and the use of forensic biology—study and testing—to analyse these evidence found and solve cases has proven to be a huge boon for the criminal justice system. However, the evidence found is only as reliable as the methods one would use to analyse it. If these procedures are not precise, or are done in a contaminated environment, it not only wastes a chance at analysing a minute quantity of sample but also wastes away kits and material that are quite expensive.

An improper collection, preservation, and handling of evidence has often caused a court to put forward a sentence that is contrary to facts put forward—innocent people have been wrongfully convicted, while culprits have gotten away scot free. The most famous of these cases, by far, is the OJ Simpson trial, where the defendant was given an acquittal solely on the fact that the police messed up the protocol that had to be followed while collecting a sample of his blood. The defence counsel argued that an entire vial of blood was collected, which could have been used to plant numerous evidence found at the scene of crime.

The onset of DNA analysis has helped many wrongfully incarcerated persons to be exonerated for the crimes they were held accountable for in an era where DNA testing did not exist. As many as 143 people (as of May 2004), most of whom were on death row, were proven to be free of any wrongdoing by help of advanced technology in DNA analysis. Cold cases, too, have been solved. Such was the case of Melanie Road, a 17-year-old who was raped and murdered in 1984. Her killer, Christopher Hampton, was caught and tried only in 2015—30 years after the crime—when his daughter got involved in a minor assault and her DNA profile made it to the CODIS (Combined DNA Index System), from where it partially matched with the DNA taken from Melanie's clothing, thereby proving that no crime can be perfect.

Despite these rays of hope in a pitch-black sky, there are many cases and evidence that are yet to see the light of a DNA analysis laboratory. Many sexual assault kits that are collected from a victim of rape have not been tested for DNA evidence, and are just tossed in a corner where the samples continue to degrade. There is an increased problem of funding as well, as most of the funds are put on high profile cases, leaving no equipment or funding to analyse the rape kits that keep coming in to police precincts. The same is also true for other cases which have run cold, have no record on the CODIS, nor have enough evidentiary samples, as those collected in the wake of the crime have severely degraded.

However, the biggest issue forensic science faces as a whole is what is known as the *CSI effect* that has stemmed from crime procedural shows like CSI: Miami, Criminal Minds, and Castle. The over-exaggeration of scenes portraying testing of evidence, such that a "100% match" is found by the databases, has made the courts—judges and jury—to incorrectly believe that anything less than a cent percent is not a match. This has caused jurors to ask for more evidence or rather higher matching evidence, thereby raising the standards to an extent that is just impossible to achieve. The credibility of circumstantial evidence too has lowered

due to the CSI Effect. In practice, it is common knowledge that there is always a high chance of a match with reference material in a database, and there is always room and scope for error. The portrayal of perfect matches that are obtained by typing a few keys on a computer are just facades of a good dramatic element in television.

As of today, *forensic genetic genealogy* has gained a lot of traction. The study stems from multiple companies running genealogical tests that can help people find their ancestry and pinpoint the start of their family trees. Most of the information stored in the databases of such companies have no scientific backing, except for the colour of hair, skin, and eyes. Though only recently emerging to be forensically relevant, a DNA profile uploaded on personal genomics website GEDMatch help nab Joseph DeAngelo, the Golden State Killer in 2018. He is known to have committed 13 murders, 50 rapes, and 120 burglaries between 1973 and 1986. Access to these databases is limited, given that these are run by private firms, and not by law enforcement.

As crime continues to increase and criminals become more aware of not leaving evidence behind, forensic science will evolve in order to continue putting justice at the heart of every scientific method followed. In a sense, forensic biology is a type of passport for biological evidence. The destination, however—either a courtroom as evidence or in a dull and dusty room while losing potency—depends on law enforcement officials at the crime scene and scientists at the crime laboratory.

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Sexual Offences: Recent Methods of Investigation

7

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Abstract

A sexual offense is a crime of violence against a person's body that causes physical trauma, emotional distress, and suffering of victims. The investigation of sexual crimes necessitates the involvement of a multidisciplinary team of forensic experts who are dedicated to the discovery, recovery, packing, and analysis of evidence. During the crime scene investigation, identification and recovery of biological evidence are of prime importance, since it is often the only way to confirm sexual contact and the offender's identity. The investigator must be able to detect proof that would otherwise go overlooked using characteristic technology and techniques. Traditionally, forensic science laboratories use established procedures to classify biological evidence and molecular methods to produce DNA profiles grounded on amplification and DNA sequencing that are laborious and expensive and not provide significant information in DNA mixtures samples. However, now there is a series of a modern method of investigation which overcomes the disadvantages of traditional methods and is very useful to identify the sexual offender in forensic investigation. This chapter explained about the sexual offenses, traditional crime scene investigation, and the recent investigative techniques like DEPArray for sexual offense evidence processing, bite mark utility, spermatozoa detection using ultrasound technique, and the forensic 3D/CAD supported photogrammetry approach which allows for a prevailing and reliable method of analyzing sexual offense data, allowing for unparalleled sensitivity and specificity while also removing the need for conventional human sperm confirmatory tests and qPCR-based DNA quantification.

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7.1 Introduction

7.1.1 Sexual Offences

When another person intentionally improperly interferes with your physical body, this is known as a sexual offense. In a sexual situation, an individual touches your private parts in any way, and/or by sexual activity, much of this is performed without consent or knowledge. Sexual abuse could also happen when an adult contacts a child under the age of 16 by phone calls, notes, or email, to communicate with the child and engage in sexual activity. Sexual grooming is another term for this [1].

Sexual assault (SA) is a crime of violence against a person's body resulting in physical trauma, mental anguish, and suffering for victims generating expenses for government-intended criminal investigation, medical care, and psychological attention. During the crime scene investigation, the identification and recovery of biological evidence (BE) are of utmost importance since sometimes these are the only way to prove sexual contact and the perpetrator's identity. With the help of specific technologies and techniques, scientific examiners must be able to find evidence that otherwise could go unnoticed [2].

Sex-related crimes are the most heinous and degrading of all the crimes. Children and women persist the most vulnerable group to this crime. The unprecedented increase in sexual harassment rates around the world is a major public health concern [3]. As per the National Crime Reports Bureau, a women or a girl is raped by a man in every 15 min [4]. However, the majority of rape reports go unreported. The perpetrators were known to the victims in nearly 94% of the cases and mainly belong to family, acquaintances, live-in spouses, employers, or others, according to the data. In 2018, 33,356 rape incidents were registered, affecting 33,977 victims, for an average of 89 rape incidents per day, and in 2019, 88,477 crimes were registered under assault on women with the intent to outrage her modesty. Since the abuse or sexual crime was perpetrated by a victim's acquaintance or relative against women were labelled under acquaintance or relative, the incidents were not registered in order to protect the family's credibility. Most of the crimes committed against women go unrecorded because families are pressured not to report the incident, resulting in an increase in the silent crime rate of rape [5].

Sexual offences are classified into different categories such as natural offenses, unnatural offenses, sexual paraphilias, and sex-linked offenses. These categories are further classified as

1. Natural offenses—rape, incest, adultery
2. Sexual paraphilias—sadism, masochism, necrophilia, exhibitionism, undinism, fetichism, frotteurism, transvestism, masturbation, etc.

3. Unnatural offenses—bestiality, sodomy, tribadism
4. Sex-linked offenses—trafficking, stalking, sexual harassment, voyeurism, indecent assault [6]

Sexual crimes are a rising problem for police, the general public, and the community, and they impact the citizens at all levels. The investigation process entails a collaborative effort between police officers, the investigating team, the victim advocate, and the medical examiner. There are a series of phases, from interviewing the victim to gathering data, analyzing it, and writing a final report.

The crime rate is growing every day, and the number of sexual assaults is increasing as well. Following the commission of a crime, the investigative process is followed; however, the traditional investigation procedure needs to be updated with advanced techniques for a better investigation and outcome. Recent progress in this area, like other fields, is desperately required. Several new investigation techniques have been established such as the DEPArray™ system for sexual offense evidence processing, bite mark documentation, and analysis: the forensic 3D/CAD supported photogrammetry approach, spermatozoa detection using ultrasound technique, etc.

7.1.2 Natural Offences

7.1.2.1 Rape

Rape is classified as consensual sex or touch if it's without the suspected victim's permission, where the act includes violence, force, coercion, or fear of injury. This description now encompasses dissemination against both men and women and even incorporates other males and females. Penetration forms are oral, interactive, and anal. The National Crime Victimization Survey describes rape as the illegal penetration or attempt of the act by an individual against the victim's will, with the use or attempted use of force [7].

Rape requires physical aggression and psychological manipulation, and involuntary sexual intercourse means the offender's vaginal, anal, or oral penetration. Incidents involving penetration of a foreign substance like a bottle, victimization of male and female victims, and homosexual and heterosexual offense are often included in rape [8].

States vary in their legal definitions of rape; however, most states share commonalities about what distinguishes this conduct. Second, rape occurs when non-consensual contact or penetration occurs between the penis and the mouth, anus, or vulva. This may also mean that a finger, hand, or penetration of an unusual object may have non-consensual contact or penetration. Rape can occur in several ways, including attempted rape, sexual harassment, statutory rape, and date rape.

Attempted rape requires the physical attempt to commit a sexual encounter as well as verbal threats of rape [9].

Statutory rape refers to sexual intercourse or improper sexual intercourse. For an individual under the legal age of consent, usually between 14 and 17 years of age,

irrespective of whether or not a minor person has engaged in sexual intercourse with an adult knowingly, voluntarily, and with mutual agreement, and failed to cooperate with the state, charges against the adult can still be filed. When a public official, such as a highly regarded educator, priest, or concerned citizen, engages in sexual activity with any child under the age of 18, more laws and penalties are enforced. Date rape is another type of rape, which usually occurs among acquaintances. Date rape is considered a sexual attack by a person with whom the victim has a relationship, and as the relationship, the assault takes place [10].

As per section 375 of the Indian Penal Code, rape is defined as a man who, except in the case hereinafter exempted, has sexual intercourse with a woman under any of the following six descriptions:

1. Against her volition
2. Without her approval
3. With her consent, whether her consent was gained by placing her or another person in fear of death or hurt that she is interested in
4. When the man knows with her consent that he is not her husband and that her consent is given because she acknowledges that he is another man to whom she is legally married or believes herself to be
5. With her consent, when, at the time of giving such consent, she is unable to understand the essence and effects of that to which she gives consent because of unsoundness of mind or intoxication or administration by him directly or by another of any stupefying or unsafe substance
6. With or without her permission, when she is under the age of sixteen

Explanation: Penetration is necessary for the offense of rape to constitute the sexual intercourse required. Exception: a man's sexual intercourse with his own woman, a wife not under the age of fifteen, is not rape [11].

7.1.2.2 Incest

Incest is a specific form of child sexual exploitation that is often serious implications for the infant, the abuser, and the family. The Webster Dictionary describes incest as "sexual intercourse between persons too closely related to marry legally." In the area of child sexuality, abuse refers more frequently to any sexual contact between a child and a close relative [12].

The most common type of child sexual exploitation is incest. Incest is described as any sexual contact between a child or a teen and a person who has intimately involved particularly stepparents and live-in spouses of parents, related or considered to be related. Many perpetrators are female; most perpetrators are male.

In most cases, it is the dad or the stepfather who misuses the kid. Incest likewise incorporates sexual action by kin, cousins, moms, uncles, aunties, or grandparents. Incest includes sexual activity that happens just a single time, just as a movement that happens throughout an all-inclusive timeframe, frequently quite a long while. Fondling, oral sex, anal sex, and intercourse are among the forms of sexual activity. The disintegration of loyalty within the family increases the mental harm associated

with sexual abuse. About 50% of women don't remember their adulterous experiences until something stimuli adult memory. Many women who still do recall may be saddened to learn that the crucial steps of understanding and exposing incest to others do not offer immediate relief from the burden of pain and issues left behind by the incest. It does not make the timidity go away to understand why one has always felt shy undressing in front of others. Similarly, resolving other concerns, such as rage and sorrow, while important to the work of incest, does not inherently contribute to the resolution of sexual issues [13].

7.1.2.3 Adultery

Adultery is an infringement upon the spouse's right over his better half. It is an offense against the sacredness of the marital home and an act performed by the male. It is an opponent of social and illegal agitation. The extent of the offense under the segment is restricted to infidelity submitted with a wedded lady, and the male guilty party alone has been made responsible to be rebuffed with detainment, which may broaden up to five years, or fine or with both. A woman's consent or willingness is no justification for the crime of adultery. Adultery, therefore, is a crime performed by a male against a husband concerning his wife. It is not committed by a person who has consensual sex with a single woman or a prostitute, or a widow, or even a married woman whose husband or wife consents to it [14].

Adultery is limited in scope as mentioned under Section 497 of the IPC, opposed to the violation of adultery as known in divorce proceedings. The crime was committed only by a male who had sexual intercourse with the wife of another man and without the latter's permission or connivance. Evidence of sexual activity, moreover, is an integral element of the crime, but clear evidence is rarely accessible and must be derived from the entirety of circumstances in most instances. A married woman whose husband consents or connives to the sexual relationship must be the perpetrator. This will not be a crime of adultery and, thus, will not be subjected to Section 497 IPC. In case of adultery, the offended person is the husband whose wife has consented to have sexual relations with an individual other than her spouse. Section 198(1) of the Code of Criminal Procedure, 1908, states that no person other than the wife's husband shall be found to have committed an offense of adultery under Section 497 or Section 498 of IPC, if, in the absence of the spouse, any person who, on behalf of the aggrieved husband, took care of the woman, with permission of the court, complains against the accused. However, the consenting woman (the aggrieved husband's wife) cannot be convicted in the event. Section 497 specifically states that a woman who is a party to the crime of adultery will not be charged as an abettor or co-accused because she is considered a victim by the statute and not a perpetrator of the offense. Although the Indian Penal Code (Amendment) Act 1972 was passed, it was suggested that this right imposed on women about an adultery offense should be scrapped given the transformation of Indian society and change in sociolegal as well as ethical standards with the advancement of the period and democratic response, but the proposal did not collect the requisite support and must be released [11].

7.1.3 Sexual Paraphilias

Sexual paraphilias is an emotional dysfunction in which a person is having repeated extreme sexual desire and fantasies and may also involve non-human objects.

Characteristic features of sexual parphilias are as follows:

1. Periodic extreme sexual imagination, sexual desire
2. Behaviours involve non-human objects, oneself sufferings, and humiliations
3. Suffering and humiliations of oneself and one's partner
4. Recurrent desire for children and other nonconsenting individually

This behaviour and sexual desire are responsible for significant sufferings and deterioration in social, occupational, and other important settings. As per DSM-IV, there are eight different types of sexual paraphilic disorder like sadism, masochism, necrophilia, fetishism, exhibitionism, masturbation, frotteurism, and undinism [15].

7.1.3.1 Sadism

Clinically sadism is characterized to experience pleasure from others people psychological and physical suffering. Sexual sadism paraphilic is mainly focused on getting sexual gratification by violently dominating and inflicting pain or by the act of cruelty on the opposite partner suffering including humiliations of the victims.

A few people with this paraphilia are annoyed by their perverted dreams, which might be summoned during sexual action yet not in any case followed up on; in such cases, the vicious dreams ordinarily include having unlimited oversight over the person in question, who is unnerved by expectation of the looming savage demonstration. Others follow up on the perverted sexual desires with a consenting accomplice who readily endures torment or embarrassment [16].

Some scholars propose that necrophilia and other posthumous practices are twisted in nature. The suggested standard, in accordance with Dietz et al., reconfirms that no conduct occurring after a casualty is oblivious or perished and meets the weight needed for the utilization of the descriptor perverted. If the victim suffered, whether the suffering was purposely induced, and if the suffering sexually attracted the offender are the key issues, and this is the reason neither sexual nor cruel acts performed on an unconscious or deceased casualty are essentially proof of sadism [17].

Typically, sadist persons are the men who are excited as much by the power and force as by the sexual demonstrations. They may likewise participate in unusual formal conduct and related to the sexual ensnarement of victim and might be sexually excited by the oblivious or dead body [18].

7.1.3.2 Masochism

Masochism is a psychosexual condition in which the suffering inflicted on oneself achieves sexual relief. Masochism term gets from the name of Chevalier Leopold von Sacher-Masoch, an Austrian author who expounded broadly on the fulfilment he acquired by being beaten and oppressed [19]. It is purposeful interest in an action

that includes being embarrassed, beaten, bound, or in any case manhandled to encounter sexual excitement. Sexual masochism issue is sexual masochism that causes huge trouble or essentially weakens working. The disease of sexual masochism is sexual masochism that causes severe discomfort or seriously impairs functioning. The amount of pain involved will range from ceremonial humiliation with little abuse to extreme whipping or beating; the masochist normally maintains some control over the situation and, before being seriously hurt, can end the violent conduct. Although pain in certain individuals can induce a certain amount of sexual excitement, it becomes the primary end of sexual activity for the masochist [20].

Masochistic operations might be the liked or restrictive method of creating sexual excitement. Individuals may showcase their masochistic fantasies on themselves—for instance, by restricting themselves, penetrating their skin, applying electrical stuns, and burning oneself. They may also search out an accomplice who might be a sexual sadist and include the activities like leaping, blindfolded, hit, flogged, mortified by being peed or crapped on, compelled to dress in drag and part of a re-enacted DSM- 4 as a paraphilia; this scheme is not otherwise defined. It is a one form of sexual masochism disorder. Most persons who indulge in autoerotic asphyxiation are males, although this activity has also been recorded in teenagers, older adults, and women. The person engaged in autoerotic asphyxiation limits their breathing to improve their sexual experience at or near time of orgasm usually, or herself, by strangulation or suffocation, neck ligatures like scarves, underwear to choke themselves, plastic bags put over the head, suffocating masks, chest compression, and simultaneous masturbation. It is believed that the establishment of a hypoxic state increases orgasm and sexual gratification. Tragically, there is a slim line isolating hypoxic rapture from unconsciousness and death. Loss of consciousness can occur rapidly because, even before hypoxia and hypercarbia become important, obstruction of venous return from the brain impairs cerebral perfusion [21].

Individuals who asphyxiate themselves in such a way that if they lose consciousness, the ligature does not release may accidentally have permanent brain damage or death. Not surprisingly, that is, the most dangerous of autoerotic asphyxiation is considered practitioner's paraphilia [22].

7.1.3.3 Necrophilia

Necrophilia is a term derived from the Greek words philios which means appreciation for/affection and nekros meaning dead body. Necrophilia is also called as necrolagnia, necrocoitus, necrochlesis, and thanatophilia. It includes the sexual fascination in a dead body. Necrophilia refers to a recurrent sexual arousal or sexual contact with the deceased, associated with the dead. It could be seen alone or in conjunction with a variety of different paraphilias, including sadism, cannibalism, vampirism (the drinking of blood from an individual or creature), necrophagia (eating the substance of the dead), and necropedophilia (the sexual attraction to children's corpses) [23]. It has even been seen in wildlife creatures known as necrozoophilia. Very often, grave diggers and mortuary attendants were found rehearsing necrophilia. They participate in this action maybe in light of the fact

that of their depression, combined with simple admittance to corpses. It is likewise conceivable that they picked this career, since that they were necrophiles, which also permits them free and unhindered access to dead bodies. DSM-IV-TR does not assign necrophilia to any particular or special code. Instead, along with many other rare paraphilias, it is grouped under Code 302.9 [23, 24].

7.1.3.4 Fetishism

Fetishism is recurring, intense, explicitly sexual fantasies, sexual desires, or gratification including the utilization of fetish, for example, leather and rubber garments, women panties, stockings, bras, Boots, and shoes. This is called 'transvestic fetishism' when the fetishes are restricted to female clothing that the person uses to cross-dress The sexual attraction to uniforms is a special kind of fetishism, even though it is hardly ever a clinical issue [25].

7.1.3.5 Exhibitionism

Exhibitionism is characterized by the individual having repetitive, exceptional, explicitly stimulating fantasies, or practices, including exposing one's private organs to unwilling audience. Exposing clearly a very persistent and incessant behaviour among these sexual offenders [26].

7.1.3.6 Masturbation

Masturbation is an effort to accomplish self-pleasuring by contacting, scouring your own body parts which lead to the erection and sexual arousal through genital organs stimulant physically by hand or mechanical incitement. It might include two people, which is known as mutual masturbation. It is common in both men and women. Male largely involve rubbing of penis against a bed or other objects and may use hollow articles like test tubes, bottles, and rubber, whereas females may rub the genitalia against a pillow or bed. They can also move fingers over clitoris or labia minora to stimulate sexual arousal. Sometimes, women can use wooden rods, glass tubes, bananas or insert fingers in to the vagina for experiencing sexual pleasure. Masturbation is not a sexual intercourse, but it is an offence when practiced openly.

7.1.3.7 Frotteurism

Frotteurism is derived from the French word "frotter," which means "friction." It is also known as "frottage." It is characterised as "a person who becomes sexually aroused by the act of rubbing up against a non-consenting person for sexual gratification". It is an atypical paraphilia and added in to DSM -III in 1980. It's a paraphilia that often coexists with other paraphilias including exhibitionism and voyeurism, all of which are courtship disorders. A frotteuristic act has legal consequences for the perpetrator, and victims report a variety of negative consequences as a result of victimization [27].

7.1.3.8 Undinism

Undinism is a paraphilia in which sexual excitement is associated with the sight or thought of urine or urination.

7.1.4 Unnatural Offenses

7.1.4.1 Sodomy

Noncoital carnal copulation is called sodomy. In history, literature, and law, the term is understood in many ways: (1) as indicating any homosexual activities among men, in contrast to the biblical tale of Sodom (2) as signifying anal penetration, (3) as associated with bestiality or zoophilia (i.e. sexual relations among animals and humans), and (4) covers a variety of other sexual behaviours, ranging from sexual intercourse with juveniles to oral-genital intercourse and adult-to-adult intercourse [28].

In some countries, sodomy is a felony and is rejected in many others as a sign of the disorder. Some criminal systems, even though the relationships are mutual as well as between lawfully consensual adults, include punishments as serious as life imprisonment for homosexual intercourse. As such, sodomy laws tend to extend even to married couples, simply outlawing several sexual contacts.

No such legislation is identified in the laws of Sweden, Switzerland, Denmark, Italy, and France. The Wolfenden Committee in England and the American Law Institute in the United States have suggested that criminal laws in this area be eliminated, except cases involving crime, child abuse, or public appeal to commercial defectors [29].

7.1.4.2 Tribadism

Tribadism is also known as lesbianism or sapphism. Female homosexuality in which females receive erotic desires from another woman by embracing, body touches or frictions of genital organs, etc. This form of action is part of India's LGBT rights, which were legalized by India's supreme court, claiming that homosexuality in ancient Indian and traditional codes was never unlawful or a federal crime but was criminalized by the British throughout colonial rule in India [29].

Tribadism is a type of non-penetrative sex in which a female rubs her vulva against the body of her partner for sexual arousal, particularly for sufficient clitoral stimulation. Tribbing, widely known for its scissoring role, is a form of non-penetrative sex.

That could include woman-to-woman genital contact or a woman rubbing her vulva against the thigh, chest, buttocks, arm, or other parts of her partner's body except for the mouth [30] [31]. Several sex positions, including the missionary position, are recorded [32].

Lesbian sex generally included social assumptions regarding the capacity of women to be penetrative sexual partners [30, 33]. Women suspected of having been penetrative during sexual activity were subject to mockery or punishment [30, 34]. The term applies to different types of exhibitionism without any derogatory connotations amongst women in modern times. Via the use of fingers, a dildo, or a double-penetration dildo, it can also include vaginal dissemination or apply to a masturbation method in which a female squeezes her vulva against an inanimate object such as a bolster to feel pleasure [35].

Section 377 IPC stated that whoever knowingly has lustful intercourse with any guy, female, or animal against the order of nature shall be punished with life imprisonment or imprisonment from either form for a period that may expand to 10 years and sentenced to fine. Penetration is appropriate to constitute the carnal intercourse required for the offense mentioned in this section [11].

7.1.5 Sex-Linked Offences

7.1.5.1 Stalking

Stalking is much more prevalent in today's scenario than any other form of sexual violence, and most of the time, it's gender-specific. It is a course of conduct that includes infliction of one individual by another individual through continual unsolicited intrusions/interventions and communications to a level that the individual fears for his/her protection [36, 37]. It includes actions such as following a person and visiting at home/office or business place. Frequent distressing phone calls or messages, sending written messages or objects, or destroying property of that person. In some instances, these courses of action may be accompanied by serious harmful threats and may or may not be the indication of any form of assault or homicide [38]. Stalking has arisen as a form of human behaviour which takes attention from the public, as well as attracting the interest of researchers among various mental health professional. The development of stalking as a major social issue has to lead to in its categorization, in much western law-making legislations, as a particular form of a criminal offense [39].

7.1.5.2 Voyeurism

The definition of voyeurism has been changed many times; in many places, it has been described as a pathological indulgence in looking at some form of nudity as a form of sexual gratification despite normal sexual acts [40, 41]. In voyeurism, the gratification is observed without coming in sexual intimation with any individual who is being noted. It is a common form of paraphilia and is mostly seen among men. Voyeurism generally starts at the age of puberty and phase of adolescence and maybe initial stages of adulthood. Several degree of voyeurism is seen more among teenage boys and men but gradually increasingly between women. It is taken as normal behaviour in society if the mild form of voyeurism is seen when consenting adults are involved. Sexual content available on Internet freely is not considered a form of voyeurism because the element of voyeurism involves secret observation.

7.1.5.3 Sexual Harassment

There was a time when the study of sexual assaults/harassment majorly focused on identifying its importance in being a social problem or not and sufficient of research for its prevalence; later on, due to increase in reported cases of sexual harassments, the study is shifted towards finding the causes and effects of it on individual and society.

In a legal point of view, sexual harassment falls under a type of sex discrimination that have two major forms of behaviour; first is quid pro quo, and the second one is hostile environment harassment. The former form of harassment includes sexual intimidations or bribing someone that is made a condition of employment or used for the basis of employment decisions. The hostile environment includes those behaviours like having comments, sexual jokes, and inappropriately touching that interfere with a person's ability in doing the job or that lead to a "stressful/unfavourable hostile or offensive working condition and intimidating environment".

There is significant variation in the approximate proportions of women reporting incidents of sexual assault. It has been observed that around 90% of women experience some form of sexual harassment at the workplace [42].

Sexual harassment severely affects the mental health of women and is a matter of concern because taking a woman as the object of sexual attention severely undermines her self-confidence and self- esteem as a potential worker. A profound aspect of this sexual harassment includes realizing women that they are not welcomed at the workplace and a not respectable member of workgroups, which hampers many career opportunities for them.

7.1.5.4 Sex Trafficking

Sex trafficking is a form of human trafficking, including sexual slavery, and is taken as a form of modern slavery for sexual exploitation. According to an International Labour Organization report of 2012, 20.9 million people were subjected to forced labour, with 22% (4.5 million) being victims of forced sexual exploitation. However, it is difficult for researchers to obtain precise, credible figures due to the hidden nature of sex trafficking. According to the ILO, the global economic profits share of sexual exploitation are projected to be $99 billion [43–45].

A victim is coerced into a condition of reliance on their trafficker(s) in one of several ways and then exploited by the trafficker(s) to provide customers with sexual services [46]. Sex trafficking crimes may include acquisition, transport, and abuse [47]. This also comprises domestic minor sex trafficking (DMST), child sex tourism (CST), or any other forms of commercial sexual exploitation of children [46].

7.1.5.5 Indecent Assault

Indecent assault in many jurisdictions is an act of aggravated assault. It is defined as a crime of sex. Under Sections 14 and 15 of the Sexual Offences Act 1956, indecent assault was an offense in England and Wales. Under Section 3 of the Sexual Offenses Act 2003, it was repealed and replaced by a related crime of sexual harassment. Any unwanted sexual activity or touching was identified as that which is against their will imposed upon individuals. The definition of indecency relies on dominant perceptions on what is improper conduct. This may involve actions such as pressuring others to watch masturbation or pornography. This is often linked to cases of sexual abuse, including, but not necessarily, forced oral sex.

Some of the forms of indecent assault widely used include—groping someone in an uncomfortable way to arouse some sexual desire, touching people or

inappropriately grinding them against their bodies, teasing others poorly, and shaming the other person's body

7.2 Investigation in Sexual Offenses

Sexual offenses are a major growing concern for police, the public, and society, and at all levels, it affects the people. The investigation in case of sexual crimes is of at most importance. The primary sources of evidence and information are mainly gathered from the victim's body, the suspect and the scene of the crime, and also the witnesses. The investigating officer should carefully investigate the sources and also any other available source of information and evidence.

Before beginning to examine the crime scene, police should collect as much information about the type of crime scene: its surrounding, environment in order to avoid the degradation or deterioration of important and/or vulnerable evidence such as shoeprints, trace evidence, and so on. The main areas of inspection are the rugs, floor, bedding, toilet, and trash or dustbin where things such as condoms may be discarded by accused while the inspection could be also extended to the neighbourhood. For searching the evidences that indicate that sexual contact taken place, naked eyed observation is important, but using light sources for identification of biological evidences is important . Because of their properties, such as light absorption (blood) or fluorescence radiation, these components of biological evidence necessitate the use of forensic light sources for identification (semen, saliva, and urine). This is a simple, presumptive, and non-destructive examination.

These cases are more complex because of higher chances of contamination or degradation of evidence (like DNA, blood, hair, fibres). It depends on the case and type of sexual offense, and accordingly, evidence needs to be collected, in alcohol and drug-facilitated sexual assaults, rape cases, and unnatural sexual offenses like sodomy.

For proper investigation of sexual offenses, appropriate training, preparation, and establishment of jurisdictional policies. The examiners and other related healthcare professionals, 911 dispatchers, law enforcement officials, prosecutors, emergency medical technicians, advocates, and judges are given education and training on alcohol and drug-facilitated sexual assault, also on the management of these type of cases where sexual assault is suspected [48].

7.3 Investigative Procedures

Officers and paramedics respond to sexual harassment calls in a variety of ways. Personnel in charge of communications must make every effort to keep the lines of communication accessible. Keep an open mind and don't pass judgment on the details obtained from the perpetrator or the party that reported the crime. Each victim is unique and, depending on his or her circumstances, will react differently.

Officers or communications staff responding to sexual harassment calls must make an attempt to have an open mind and refrain from interpreting the facts received from the witness or party reporting the crime. Based on his or her history, attitude, and the circumstances of the attack, each survivor will react differently. For example, the majority of sexual harassment victims are female, but if the caller is male, stereotypes should be avoided. Victims of drug-assisted sexual abuse, in particular, can experience blackouts, gaps in recollection, and overall confusion about occurring of assault.

7.4 Response by Communications Personnel

Communications staff should meet standard emergency response protocols when a caller reports a sexual assault. This involves gathering the basic details of the situation, looking for if the victim or any other person is in imminent danger situation and in need of immediate medical help, and also keep dispatching emergency medical assistance as required. If the call is about a current or recent crime, communications staff should collect information from the caller to help in locating and prosecuting the crime and apprehend the suspect.

When a sexual assault is reported by a caller, emergency response procedure should be followed. This comprises of explaining about the initial situation, understanding the seriousness of the situation, also looking for any danger or life-threatening condition victim may be in, or any requirement or emergency medical help needed. If call related to an ongoing or recent incident, communications officials should collect details from the complainant to aid in locating and detaining the suspect. Often, you should be able to determine if or not there is a connection between the victim and the perpetrator, whether there has been some previous history of abuse, and whether a weapon is involved. To minimize victim frustration, the personnel of communication response have to explain that the questions being asked will not hinder the arrival of an officer to the location of caller. The communication personnel have a duty to be on the call till the officer reaches to location. It becomes almost important in sexual assault cases to determine whether a victim has urinated, bathed, douched, or any such form of activity that potentially may damage probative evidence like hair, semen, and saliva. Precautions should be taken while collecting this information that it may not cause any emotional trauma and keeping in mind that the victim may feel guilty or ashamed. Victims may avoid such types of questions or give a negative answer because of internal fear that they did something wrong. In a situation where the victim has taken such actions, he/she should be informed that more evidence still might be recovered or identified.

If the victim has not done any of these things, communications staff must request the victim not to do so that he or she withdraw and explicitly clarify why—the reasons for the request if the suspect, for example, has to urinate and can't wait for the cops to arrive. He or she should be told where to put the urine collection tube to pee in a clean jar or container.

However, if the victim has not done any of these activities, it is necessary for personnel to explain that he or she not do so and to elaborate the reason for doing so. In situation where officer is not able to respond with a urine collection container and victim has need to urinate, in such situation, a clean jar or container having a lid should be used till officer responds.. Additionally, the victim should be asked not to wash cloth and to collect the clothing that was worn at the time of assault; if possible, keep it inside a clean paper bag.

7.5 Investigating Officer Response

Officers should follow standard emergency management protocols when responding to a sexual harassment scene, including ensuring the victim's safety and, where necessary, also beginning of search of the perpetrator. After ensuring the victim's safety, officers can make appropriate attempts to enable the survivor to ascertain the scene, location, date, and time of the incident, where the preliminary report will be made. The officer should specifically clarify the report process, including the functions of the first responder, investigating team, and everyone else the victim will interact with.

The officer is frequently in charge of initiating the investigation. After safeguarding that the survivor requires no urgent transportation to a health centre, officer can proceed with the initial interview. The interview can specifically have questions which would determine the necessary details of the assault and comprise the evidence required for the investigation, like perpetrator identity, name, and characteristics of crime; the mental and physical state of the victim should be taken into account while asking questions. The victim may be told that a second interview with a professional investigator will take place at a later stage. During the first questioning, the victim may be asked about anyone he or she reported about the sexual attack. The first individual the victim talked about the assault should be identified and examined in particular because this "outcry witness" will be helpful to validate the legitimacy of the survivor. This is especially relevant if the suspect raises a consent defence or in delayed reports. Officers should record any type of physical injury and inquire about any indication or signs and symptoms of injury around neck area or strangulation. The signs may be like petechiae, any defence marks like fingernail mark on neck and face, abrasion, bruises, any sort of discoloration on the neck area, swelling of the tongue, difficulty in eating, and breathing; all these things should be taken into account [49]. On the requirement of forensic medical examination and the victim approves for it, the officer needs to make arrangements for travelling to the specified facility. Proper chain of custody should be maintained. During transportation of victim, the victim's body should be considered like a crime scene whose protection is a priority.

7.6 Drug Facilitated Sexual Assault

This type of sexual assault is much more complicated due to the use of alcohol and date rape drugs. Excess alcohol consumption by the victim leads to a state of unconsciousness, and thereby, offender sexually assaults the victim. In cases of date rape drugs, the victim becomes unconscious and in a state of deep sleep unaware of the condition and situation. The investigation is much more complicated in drug-facilitated sexual assaults reason being drugs like Flunitrazepam, Rohypnol, GHB, which affect the nervous system and lead to memory loss; the victim is not able to recall the event that occurred in the last few hours. The victim may or may not be able to recall that something has happened with her; if she suspects something, maybe after few days, it becomes too late because of the high chances of loss of evidence during that time [50]. First responders must understand that while Rohypnol and gamma-hydroxybutyrate (GHB) are commonly publicized in drug-facilitated sexual assault as the "drugs of choice", assailants can use various other substances to facilitate sexual assault (including alcohol). They need to know the significance of collecting samples of toxicology as medically required or suspected of alcohol or drug-facilitated sexual abuse, as well as the value of obtaining patients' informed consent before collection of the sample. They should also be aware that toxicology sample processing is usually separate from the forensic evidence collection kit for sexual abuse, and toxicology analysis procedures can be different from other evidence analysis procedures [51].

7.7 Delayed Report

Some victim of sexual assault may not report the incidence; the causes are special to each person and can comprise the victim's state of mind or feeling of guilt, humiliation, denial, shock, self- blame, and ambiguity about the incident—if it is a sexual assault or not, fear of nobody will believe, and anxiety about family and friends finding out about the incident, and mistrust of the judicial system both apprehension over the effects and in what way they would affect the life. Officers must be cooperative with all the parts of the victim where she fells hesitancy. Officers must be mindful that concerns related with delayed report should cause victims to think the officer does not believe her version of events or may blame her for the incident. Although the details for a delayed filing must be reported, a delay in reporting the incident should be regarded as genuine and should not be seen as proof that the victim is falsifying about the incident [52].

7.8 Role of Supervisor

When a sexual assault is reported, the standard incident response protocol is followed; a supervisor is needed to confirm about the investigation that it is done appropriately with a victim-centric/oriented approach and to respond to support

officers conducting sexual harassment cases where possible or when ordered by an officer.

To make sure the polygraph tests are never conducted on victims of sexual harassment, agencies may be penalized under the Violence Against Women Act. Conduct after-action investigations and sexual harassment investigation audit on all sexual assault cases to ensure their credibility and continuity. Encourage police to look for simultaneously occurring and interconnected crimes/incidents. Ensure that the investigating officer and other investigators in case understand case coding and that sexual harassment reports are properly coded.

7.9 Victims Advocate

Victim counsellors will be particularly supportive in sexual harassment cases and can be contacted as quickly as possible for encouragement or moral support also to help the victim in taking rational decisions during the investigation procedure. In seeking an advocate, the cop should relay information about the victim's medical needs, such as linguistic problems or mental health concerns, or physical disability, as well as the requirement of an advocate to the victim to be of same gender. Victim advocates operate within and outside of the criminal justice system. Because of their links to the administration, most advocates in the criminal justice system are not given counsellor privilege. Community-based advocates, on the other hand, generally do have counsellor communication authority. Specifically, privilege laws may halt counsellors from appearing or being necessary to testify in court and several even ranges that ban/restriction to written records. The victim has the opportunity to refuse the advocate's services, and this decision must be accepted by members of the responsible party, including the officer in charge of the investigation. If the survivor refuses help, written referrals for alternative advocates should be provided for assistance. Victim counsellors should not be used as interpreters when doing so puts them in the role of helping with the law enforcement case rather than working on their victim supporting and assisting role. Law enforcement authorities must collaborate with victim advocate groups and others to find new approaches to deliver robust treatment for unique groups such as impaired hearing and speaking and who do not have English as their first language

7.10 Collection of Evidence

When referring to a complaint of a sexual assault, police must adopt their agency's evidence gathering protocol in the investigation. This includes adhering to the established procedures for collecting DNA evidence and maintaining the chain of custody, ensuring proper collection, labelling packaging, and forwarding of evidence. Clothing worn during the assault should be firstly collected by the officer and immediately after that, particularly clothing. Closest to the vaginal region, comprising undergarments, shorts, skirts, or trousers—every piece of garments should be

packed individually in a paper bag. Also records of photographs and videos of the victim's injury can be made. Photographs can be taken as part of the forensic investigation of most cases by a licensed medical practitioner. However, if the victim refuses the medical forensic test, photos and videos of injuries can be collected and viewed in the context of the investigation by law enforcement. When taking the photograph of victim in these cases, firstly, the officer should obtain permission to photograph any apparent bruises or injury and should prefer drapes or other methods appropriate in protecting the victim's privacy. The person of same sex as that of the victim should be considered wherever possible for photographing. The photography of private parts like genitalia should be taken specifically to be a part of the forensic or medical examination and should never be taken by officers of law enforcement. The confidentiality and integrity of these photographs should be controlled and maintained properly.

Furthermore, officers should concentrate on gathering information or evidence to track the suspect's use of alcohol or drugs to establish vulnerabilities to victim and threats posed by the accused/suspect, as well as the setting in which the attack happened, including signs of alienation and soundproofing. Investigators also consider or take into account other evidence probably encountered in drug-facilitated sexual assault cases [53]. Such type of evidence can potentially be located at the suspect's residence, at the home, locker, at employment place, or in a vehicle. This evidence can be in the form of drugs (tablets, capsules, powder), raw materials for making drugs, recipes, and literature related to the use of date rape drugs. From electronic gadgets of suspect, the victim's related information like photographs and videos can be obtained. Additional to that, the officer must also search for the person who may have seen the suspect and victim together or using drugs or alcohol.

In cases where suspects accept that sexual contact existed but simply argue that it was done with the consent of the victim, officers should concentrate on gathering evidence of prior attacks or violence, intimidation, threats, or stalking. Other evidence need to be collected to record the perpetrator pattern of action, as well as selection and grooming procedures, false conditions, behaviour before and after the assault like any form of communication, or any suspicious manipulative behaviour by the suspect. Officers handling cases in which the sexual assault is done by a strangers, on the other hand, should base their forensic activities on the identification of trace evidences and majorly to get DNA profilling done that can be used to track down the suspect. Events that occurred before and after the attack should be documented to identify any additional witnesses and forensic evidence, locations that may help in getting more evidences.

7.11 Victims Interview

In cases where the accused is familiar to the victim, the officer should ask as to how long the survivor has known the accused, the situations of their encounter, and the severity of their prior partnership, and what, if any, behavioural problems they had

changed caused the position to shift from one based on consent or permission to one based on need/force or intimidation.

Following questions should be asked by the officer:

1. The site of the attack, as well as any isolation techniques used by the suspect like isolating the victim from relatives, taking the victim to a distant location, or requesting that the survivor travel somewhere private
2. The suspect's actions, threats (real, assumed, or indicated), movements, intimidation, and other activities.
3. Methods with which the victim refused or implied non-consent, whether verbally and nonverbally. Nonverbal cues may include standing still, resisting contact, and being silent.
4. The victim's behaviour before and after the sexual attack, as well as his or her emotional health during the assault.
5. The feelings and thoughts of the victim during the assault.
6. Peripheral details and sensory evidence which the victim has seen.
7. The victim's post-assault behaviour and emotions, including shifts in routine, exhaustion, mood fluctuations, sleep and food disruptions, flashbacks, nightmares, and tremors.
8. Any form of pre- or post-assault communication, tracking, harassment, or other suspicious activity.

Investigators should often question victims of sexual harassment about any conditions which indicates the involvement of a substance for doing the sexual assault (e.g., at the time of the abuse, the victim was under the effect of drugs, any memory loss, disorientation, or confusion encountered by the survivor, serious illness, or hallucinations). Usage of any drug prescribed should also be taken into notice. However, investigators must make it familiar with the survivor that drug misuse do not confirm the allegations of sexual assault have occurred. When drugs or alcohol are used, the victim memory about the sexual assault is very little. The victim's statement about the event might have significant gaps, making it very difficult in explaining what has happened with her, to the officer in charge of the inquiry. Because of this, the victim might be enormously anxious or nervous during the Interview. Investigating officer must be cooperative and be patient and polite and with an open mind listen to the victim as she recalls the scene. Also, investigators must keep in mind that a victim whose memory is compromised as a result of a drug's pharmacological impact can be fill up the blanks or gaps in recall in the event unintentionally in one's mind. As a consequence, investigators or examiners must stop asking any suggestive or leading questions during the interview.

Victims must express how they feel or what they were doing before becoming unconscious. It is also important to interview any person who may have heard or spoken or seen before or after the attack on the victim. Frequently, it is that person who could act as a witness and who determine time period, confirm suspicious or abnormal conduct or behaviour, include vital information, and identify the important piece of information. This thing should be taken into consideration that that in few

cases of sexual harassment, victims may withhold information from their original account of the crime that they consider humiliating or inappropriate or intimate. As a result, investigators should anticipate any hesitation on the part of the survivor to explain uncomfortable evidence. Additionally, the investigator specifies the relevant details that need to be discussed to determine the legal aspects of the crime and proceed the investigation and start the trial.

7.12 Approaching Suspect and Interviewing

Officers can perform a background search before contacting the suspect, primarily checking for allegations, criminal records/charges, and punishment given for similar type of crimes, especially violent crimes and breaches of security orders. The investigator may also try to connect with staff from other departments within the department, as well as those from other organizations, to establish correlations or evidence or patterns that are similar to other types of violence, such as indecent exposure or voyeurism.

Depending on the jurisdictional laws, officers may also consider of making a pretextual or confrontational call or texting. There are registered phone calls. Typically, the victim initiates the process while being supervised by a group of law enforcement officers with the mission of obtaining the suspect's incriminating comments; all these actions depend on the mental and physical state of the victim and preferred to be done in the presence of an advocate of the victim for support.

Officers must follow their agency's protocol when questioning offenders, which should include instructions when performing custodial and noncustodial interviews as well as interview recording. Officers should question the suspect how a person should indicate or express non-consent for intercourse. The explanation by the suspect may be helpful in further proceedings mainly if they try to the consent defence.

7.13 Protection of Victim

To the extent possible by law and policy, law enforcement officers must assure to preserve the privacy or confidentiality of the victim's records. The duty of investigator also includes that they must ensure that victims are aware of their rights as a crime victims as per state law—that they have the right to remove their name from public record and to be available and give a statement at the proceedings. The survivor, witnesses, and the third parties have the right not to get harassed or intimidated by the perpetrator/suspect. If such rules are broken, the investigator may clarify how to call law enforcement, including telling the client to dial 911 in emergency. The victim should be given the report number, including contact details of the officer in charge of monitoring and chief investigator the subsequent person handling the case. Finally, victims should be informed about decisions on arrest, victim benefits, special restraining court dates, orders, and releasing date parole. Additionally

officers may also notify the survivor of the likelihood of public attention, including the information provided to the media houses about sexual harassment victims and media attention. By developing multi-agency holistic guidelines for responding to sexual harassment, organizations should make sure that the victims are safe and get the best care and support.

7.14 Medical and Forensic Examinations in Sexual Assault

In sexual assault, forensic investigation and medical examination have the potential to address number of the questions of sexually assaulted victims and will raise the probability of help from collecting evidence and aid in the investigation and conviction of the perpetrator.

7.14.1 Time Guidelines

While many jurisdictions historically take a time span of 72–96 h for gathering evidence after a sexual assault, numerous have stepped away from this procedure, toward a longer time span of 120–168 h. Evidence collection, medical treatment for STIs, pregnancy, and the recording of injury are frequently likely even after that time span (up to 10 days after the sexual assault) and should be done to document injuries. Toxicological evidence in sexual assault cases should be collected up to 5 days after the assault [54].If the sexual assault is done by a stranger in such case, DNA and biological evidence may have a huge influence on the likelihood of identifying the assailant and make him responsible for the assault. In such cases, an evidence collection by forensic examiners may be done up to three weeks after a vaginal sexual assault; subsequently, potential evidence can still be recoverable.

If there is a suspicion that the attack was committed in such situation where victim was unable to give consent, or unable to recall, unable to explain lapses, or unaware of a complete description of the attack, complete toxicology tests must be carried out. The victim should be told of the importance of getting a toxicology test to determine the effects of alcohol and medications at the time of the attack. Such kind of medications along with alcohol may have harmful effects on the victim's body.

Where a drug-assisted sexual attack is alleged, a urine sample must be taken as early as possible, although this can be obtained later up to 120 h after the incident. A blood sample can be taken if it has been less than 24 h since the assault. Many sexual assaults go unreported/delayed in reporting the department should collaborate with laboratories that are accomplished in detecting very low amounts of medications/drugs widely used in sexual abuse in collected urine and blood. Samples, victims' illicit drug abuse, like underage alcohol, should not be used to dishonour or deter the victim from reporting the incident. For the officials of law enforcements, the priority should be to thoroughly investigate the victim, not prosecute the victim for violating rules.

7.14.2 Collection of Samples

Urine has a wider detection range for medications widely used in these situations than semen. The earlier a urine test is collected after an attack, the better the odds of finding contaminants that are rapidly degraded—removed from the body.

(a) **Urine samples from victim**—If patients might have taken a substance used to facilitate sexual abuse during the previous 96 h, a urine sample of at minimum 30 mL, ideally 100 mL (around 3 ounces), should be collected in a glass jar or sterile plastic (as per protocol)—not sterile the urine sample (e.g. bacteria present in the urine would not interfere with analysis or results). In situations when patients are unable to wait until they arrive at the exam centre to urinate, first responders can ask them to supply a sample to carry it to the facility, thus recording the chain of custody [48].
(b) **Blood samples from the victim**—When necessary, take a blood sample. If medications used to promote sexual abuse were consumed during the previous 24 h, a blood sample minimum of 20 mL should be obtained in a gray-top tube (having preservatives potassium oxalate and sodium fluoride), as per jurisdictional guidelines [48].
(c) **Sample collection from the suspect**—The medical examiner should collect the samples specified in the warrant during the examination. This may include penile swabs, combing of pubic hairs, and other sources of DNA evidence. Additional trace and biological evidence should be gathered in compliance with the agency's evidence collection policies. In comparison to those biological specimens, as well as trace proof, the forensic investigator can keep track of information of the suspect's clothing, tattoos, scars, piercings, and any other distinguishing/unique features.
(d) **Preservation of evidence and chain of custody—**The toxicology lab's specifications for specimen processing, packing, marking, preparation, handling, and distribution should be known by all concerned healthcare staff. Policies for storing these samples while patients are not present should be in effect. The chain of custody, as with all forensic evidence, must be maintained.

7.14.3 Recent Investigation Techniques in Sexual Offences DEPArray™ System for Sexual Offense Evidence Processing

The understanding of DNA mixtures from heterogeneous samples remains a key challenge in forensic community. Identification, collection, and analysis of a single cell or cluster of cells possess a widespread inference in the examination of forensic evidences. So, while processing and interpretating heterogeneous DNA mixtures obtained in sexual assault cases, deconvolution is necessary. Within the forensic science community, there is a great need to find creative, stable, and reliable methods for physically separating these heterogeneous sources in order to minimize an already backlogged system.

While DNA analysis, processing of DNA evidence and serological analysis are much focused. However, to increase the efficacy and augmentation of downstream DNA, analysis development of improved serological techniques shows a positive result. Many techniques like light microscopy, differential extraction method, fluorescent-activated cell sorting (FACS), or laser capture microdissection (LCM) are being investigated for the separation of distinct cells from the mixtures of DNA, but none of these shows a promising result in the separation of different types of cells. These techniques sometimes fail to separate adequate human sperm cells from mixtures of epithelial cells and other cells.

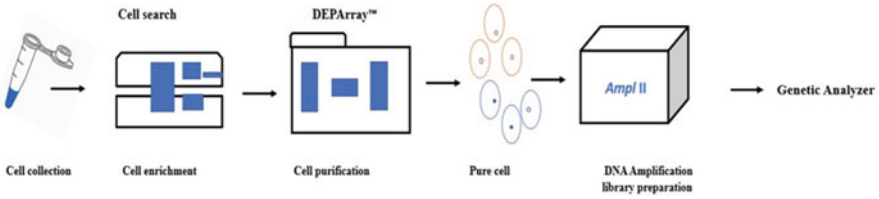
A semen is the most frequently found evidence of a sexual offense. Its detection is necessary in investigating the sexual crime; affirming its essence on samples obtained from the body cavities gives almost verifiable proof of direct sexual contact. Nonetheless, the utmost important objective of forensic examination is to determine a genetic profile of a sample to identify the culprit. The standard protocol of genetic profiling in forensics includes isolation, quantification, STR amplification, and analysis of DNA sequence by means of various commercially available kits which are extremely efficient in generating genetic profile from trace amount of DNA sample. In spite of high efficacy, these technologies cannot produce promising results in case of DNA mixtures, having biological fluids from two or more individuals and lead to the production of mixed genetic profile. One of the major glitches of forensic genetics is biological mixtures.

Nowadays, to overcome the limitations of the above-mentioned techniques, DEPArray™ image-based and highly automated technology successfully allows the isolation of pure cells with single cell resolution from 100% homogeneous samples. It is highly robust and sensitive technique. DEPArray™ technology ensures genomic and expression analysis at the single cell level and enables the forensic scientist to successfully analyses mixed biological evidences [55].

DEPArray™ technology includes a benchtop instrument, DEPArray™ cartridge, and DEPArray™ analysis platform. DEPArray™ cartridge is a microfluidic cartridge that consists of array of individual electrodes with embedded sensors that allow dielectrophoretic (DEP) cages to be built around the cells. It is based on the electrokinetic theory that produces an electric field force on neutral and polarized particles such as cells suspended in the liquid which in turn control manipulation and collections of individual cells of interest ranging from small sperm cells to large epithelial cells. Isolated cells are then transferred to a field cage with stable levitation due to the change in electric field pattern. The fluorescent microscope is attached to the DEPArray which acquires high-resolution cells images and measures cells intensity and morphological parameters [56].

Collected data are displayed in the CellBrowser™ software, which provides multiple analysis tools for cell identification and selection: the high level of technology and automation minimizes the operator interaction with the DEPArrayTM system to the selection of the desired cells. Selected cells are collected in homogeneous groups or as single cells, with clean drops of buffer into a 0.2 mL tube, and are then ready for further genetic analysis. The major advantage of DEPArray includes the (a) identification and isolation of specific sperm cells before the DNA extraction,

(b) being highly sensitive in the detection of sperm cells from the biological mixtures, (c) DNA quantification takes place directly by cell counting which further exclude the requirement of quantitative PCR, (d) potential inhibitors being shut off which leads the way to enhanced amplification, (e) low template DNA samples showed improved resolution and interpretability [57].



7.15 Procedure

7.15.1 DEPArray™ Sample Processing

DEPArray™ forensic sample preparation kits are available for the sample preparation which permits the recognition, identification, and extraction of different cells such as human sperm, epithelial cells, and white blood cells from the collected biological mixtures swabs. Cells are concentrated by centrifugation and stained using specific antibody conjugates like fluorescein, allophycocyanin and phycoerythrin, and 4,6-diamidino-2-phenylindole [56].

7.15.2 Differential Extractions

All the samples are suspended in digest buffer, proteinase K, and incubated for 1 h at 560 °C. After that, swabs of samples are spun where the epithelial fractions of cells are removed and remaining cell pellets will be resuspended and washed in digest buffer. Epithelial and sperm cell-obtained fractions are then processed using DNA IQ™ Casework Pro-Kit and Maxwell® 16 (Promega) which contain 1-thioglycerol to extract sperm cells. Aging extracted sperm cells are resuspended in proteinase K, 1-thiogylcerol, and casework extraction buffer, further incubated for 30 min at 56 °C. Subsequently, lysis buffer is added in incubated each fraction and analyses under Maxwell 16 which is preprogrammed with DNA purification procedure. Extracted samples are concentrated using millipore filters and quantified using qPCR assay to determine male and total human DNA concentrations and also at the same time quantify autosomal and Y-chromosome DNA [55, 58].

7.15.3 Amplification and Analyses

All the samples are amplified using an amplification kit which allows co-amplification and fluorescent detection of the 18 autosomal loci in the expanded CODIS core loci and further analyzed using genetic analyser [58].

7.15.4 Bite Mark Utility in Sexual Offences

Bite marks can be found in sexual assault cases while not very prevalent in other attacks. The adequate information of the teeth is transferred to the bite marks; it can be useful in identifying the human [59]. One of the good techniques for obtaining the evidence of bite marks is photography. The role of ABFO scale II in photography has been well known for the compilation of evidence of bite marks [60].

During photography, if ABFO scale II is used, photography can effectively be used during the Gorea and Jasuja process to translate to life size [61]. There have been various approaches to equate the teeth with the bite marks, but most of the bite marks are compared using simple methods such as acetate translucent overlays [62].

For the measurement of bite marks as a 3-D structure, few new methods have been designed using electron microscopy, stereomicroscope, and optical overlays of bite marks for the DNA fingerprinting using saliva found on the bite marks [63]. Bacteria found on the bite mark can help in identification by its genomic profiling [64]. Depending upon the case, a particular method can be used for bite mark analysis as acetate sheet can be used for the overlay of bite marks as it is a easy, helpful, and cheap method for the identification [65].

7.15.5 Bite Mark Documentation and Analysis: The Forensic 3D/CAD-Supported Photogrammetry Approach

The forensic 3D/CAD supported photogrammetry is a technique used to document and record the small objects from the surface. The technique makes it possible to analyse patterned skin, soft tissue or bone injuries to match potentially incriminating instruments in form, size, and angle [66, 67].

Taking a series of images or scanning involves three-dimensional recording of items to be studied. The examination of 3-D recorded objects needed a series of photographs. A computer system then determines the location of key locations on the items' surface in space and then generates three-dimensional representations of the items. These data models are used to create graphical true-to- object volume models using a 3D/CAD program. The objects in question, such as the bite mark and the teeth of the dental cast of a perpetrator, may then be randomly pushed against each other on the 3D screen for contrast and measurement and likely to assess their coherence [67]. The visual properties of the representation may further enhance the additional outline of the photogrammetric 3D model of the artefact with a two-dimensional image of the bite mark.

In this method, the implicated cast does not obscure the patterned structures of the injury site, as is common when the teeth or the cast is in the focus and the bite mark is in the periphery. Curved illustration, or a reversed reflection of the bite mark, shows accurate visualisation of the bite mark's progression. The cast is depicted here as penetrating the bite mark from behind, which showed the virtual viewpoint from beneath the skin [68].

7.16 Spermatozoa Detection Using Ultrasound Technique

The variety of discipline are involved in the study of the sexual offence in criminal context which helps in reconstructing the criminal act by examining different forensic evidences found at the crime scene and victim. The biological evidence like semen, blood, and vaginal secretions found at the scene of crime are analyzed by forensic biologist using various biological methods and procedures applied to a criminal context to determine if a sexual crime has been committed which aid in the confirmation or refutation of potential aggressors. Among all the biological evidence while investigating the alleged victim of sexual offence, semen is the most vital evidence. The spermatozoa present in the semen can be detected using various staining techniques such as Christmas tree test. This includes the extraction and dilution of sample in distilled water and buffer so that the spermatozoa cells get freed in to the medium. It is a conventical method of sperm cell detection but requires to ensure that sperm cells must be freed in to the extraction buffer without any degradation. To increase the efficiency of sperm cells recovery, a technique based on ultrasound waves has been revolutionized. This technique utilizes ultrasound waves to extract the sperm cells from the sample without affecting their morphology and quantity. The sample is added in a diluting medium and placed in an ultra sonicator. The sound waves in the sonicator travel through the aqueous medium and free all the sperm cells present in the swab or cotton. With the application of this technique, it is possible to recover higher number of sperm cells in negative samples like garments [69].

7.17 Conclusion

Sexual crimes are a major concern for enforcement, the general public, and community, and they impact citizens at all levels. Sexual offences are the most horrific and degrading. The sexual crimes considered as a crucial criminal act. The evidences from the crime scene must be collected in a proper way under the supervision of investigating officer. While interrogating the victim of sexual assault, the medical practitioner and investigating officer must take precautionary measures to not hurt the victim's sentiments and mental peace in any manner. The examination of victim should be done by a medical practitioner, and all the relevant evidences like clothes, vaginal swab, urine sample, or any other relevant sample must be collected for the analysis.

A wide range of sophisticated investigative and research techniques are now available. Among them, the DEPArray demonstrates the interpretation of DNA mixtures, which remains a major challenge in forensic evidence research. It allows forensic biology/DNA analytical laboratories to improve their normal workflow. DEPArray workflow results in fewer mixture samples, allows for the purification of sperm and epithelial cell fractions without the need for differential extraction, increases sample amplification success rates, and improves the analysis of low template DNA samples. It also removes the need for additional human sperm confirmation tests and allows for direct recognition of the type and number of cells being tested, removing the need for qPCR-based DNA quantification. The DEPArray has a clear role in the processing of sexual offence data, but it may also be used to process other forms of evidence such as trace or "touch" evidence. It also provides many advantages over other traditional methods of DNA extraction and possesses high specificity and sensitivity.

Extraction of spermatozoa from biological samples using ultrasound wave is another emerging technique. The application of ultrasound waves on biological samples aids in the liberation of cells in the diluting medium as these sound waves pass through the aqueous medium and free the sperm cells attached to the cotton swabs and recuperate a high percentage of sperm cells without being destroyed. So the use of ultra sound techniques increase the sperm cells recovery, without affecting their morphology and quantity.

Bite marks are the common evidence in sexual assault cases which might be helpful in the identification of an individual. The information related to the teeth needs to be recorded by a proper method. The forensic 3D/CAD supported photogrammetry approach helps in documenting and recording the small objects from the surface. This technique helps in analyzing the patterned skin, soft tissue, or bone injuries to match potentially incriminating instruments in form, size, and angle by taking 3D images. Bite marks are also recorded as the 3D structure that later can be compared with the dental cast. The 3D images give the curved illustration that helps in getting the progression of the bite marks. This method serves better-curved photographs for the comparison of bite marks.

Conflict of Interest There is no conflict of interest.

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Investigation of Disaster Victim Identification

8

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Abstract

In any mass disaster case, irrespective of type of disaster, victim identification is very substantial. This procedure is culmination of distinctive fields of forensic science including anthropology, odontology, radiology, and fingerprint and DNA analysis. All experts of these fields collaborate together and manifest their knowledge and expertise to identify the victims in a disaster. The review focuses on the discussion on identification of victims using different forensic tools and methodologies. The December 2004 tsunami disaster is used as an example to illustrate the working of forensic experts and methods and techniques used in identification of the victims. The newer methodologies and advancement in victim identification techniques are also discussed.

Keywords

Disaster victim identification · Forensic odontology · Forensic anthropology · DNA · Forensic radiology · PMCT

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8.1 Introduction

The fundamental part of forensic investigation is identification. This process completely relies on the information gathered from antemortem and postmortem records along with DNA, medical, or dental records [1]. The use of identification for legal requirement is practical to infer the fatality incident (event or disaster).

Human identification investigators have a humanitarian and legal responsibility to identify every individual where possible, so that they can be returned to their families [2]. These investigations are needful for socio- and medico-legal purposes and are performed in cases such as natural and man-made disasters or in case of highly decomposed or dismembered body to conceal the identity of the deceased. Disaster victim identification (DVI) is an acute and challenging task which involves specialists from various fields including pathologists, archeologists, anthropologists, odontologists, radiographers, biologists, etc. [3] DVI can also be described as course of action used to favorably identify deceased victims of mass fatality incidents (MFI) [4]. These incidents give rise to a large number of victims. On the basis of number of deceased, MFI can be subdivided into major, mass, or catastrophic [3].

The procedure of DVI consist of five phases: [4, 5]

1. Scene
2. Mortuary
3. Antemortem data
4. Reconciliation
5. Debrief

Mass fatality incidents (MFIs) take place in many forms and can be categorized into two categories, namely, natural and unnatural. Natural incidents include tsunamis, earthquakes, and hurricanes, while unnatural incidents are terrorist attacks and air crashes [4].

8.2 International Police Organization (Interpol)

Interpol is an international organization that addresses the issue of DVI. This organization has laid guidelines according to which the entire procedure of victim identification should be followed. The first DVI manual was published in 1984 [3]. The Interpol philosophy aims to stimulate DVI teams to make use of “best practice” to obtain maximum results in DVI operation [5].

This Interpol organization claims that there should be permanent disaster victim identification team. Interpol recommended that the 187 member countries should adopt common procedures and protocols for victim identification in any disaster irrespective of cause of disaster or number [3]. These recommendations are made to achieve and maintain improvement of standards [6]. The Interpol standing committee made three working groups: Forensic Pathology, Forensic Odontology and Police [7].

The Forensic Pathology working group is subdivided into:

- DNA anthropology
- Imaging
- CBRN-E

The Forensic Odontology includes:

- Dental forms
- Expert qualification
- CT imaging

Police work includes:

- Victim recovery
- IT/large data
- International DVI management exercise
- AM data collection

DVI guide aims to contribute to the efficiency and effectiveness of disaster handling [8]. The methods for identification process are divided into groups: primary and secondary identification methods [5].

The Disaster Victim Identification is part of the INTERPOL operations police support the Directorate. This DVI unit consists of a team of police and scientist and helps in preparing countries so that they can respond to catastrophic events involving mass fatalities [9]. The three major or primary identification include:

- Comparison of dental traits and characteristics
- Fingerprints
- DNA profile

Other traits including tattoos, body piercing, clothing, or jewelry can also be recorded and are useful for establishing identification. Interpol has contracted a Danish IT company for its data software which is used to compare data entered into it from both antemortem and postmortem records of missing people [3, 5].

A vast number of discipline can be utilized to reinforce the identification of victims. Techniques include visual identification of both the body and the belongings recovered from the body, fingerprinting, radiology, inspection of unique medical features, anthropology, and dental comparisons [4].

8.3 Working Conditions

A temporary morgue is usually constructed where the victims are taken for examination. Bodies are firstly cooled with dry ice, then packed in body bags, and tagged with labels or tracking number. Body bags are usually stored in containers having temperature below 0 °C. Cooling is done to prevent putrefaction of the body.

For identification of human remains, which are highly decomposed or partially skeletonized, certain methods are used. Forensic dactyloscopy, forensic odontology, forensic anthropology, and pathology are disciplines which assist in identification of the deceased. Fingerprints and palm prints along with the external body detail are recorded first. Person's belongings like clothes, jewelry, tattoos, scars, etc. are also recorded [10].

Autopsy is performed latter to external analysis to expose prior surgery. The odontologist checks for dental records and prepares the radiographs which assist in age and gender estimation of victims. Anthropological findings are also recorded side by side. Postmortem findings are mentioned on pink Interpol form, whereas antemortem findings are mentioned on yellow Interpol form [8, 11].

8.4 Key Tasks for Victim Identification [5]

8.4.1 Evidence Collection

The evidences are recovered from the disaster site. These evidences include fragmented or complete evidences like fragmented or complete jaws, teeth, or bones. The bodies recovered from the site are usually mummified or dismembered. There is rarely any case in which the entire body without any injuries is found. Documentation and photography of the evidences are a must while collecting the evidence.

8.4.2 Examination and Recording

The officials from different disciplines employed at the site are responsible for maintaining safety standards and occupational health during analysis and documentation. They analyze the samples and make proper record of the information gathered from the evidence.

8.4.3 Interpretation

Forensic officials search and compare antemortem and postmortem data and compare the records. Once the comparison is done, forensic experts discuss and re-examine the information to generate a final report.

8.4.4 Reporting

Reports are imperative and are used for making a final decision. Reporting starts right from the initial assessment when officials reach the crime scene. All the minute things are noted down including the tagged labels of the bodies recovered. Separate DVI forms are available for recording the information. For instance, yellow Interpol forms are used for recording antemortem data, while pink Interpol forms are used for recording postmortem data. The information provided by the family members are also recorded, as it is also very beneficial in the investigation. All the reports are compared and studied, and a final decision is made by the forensic experts and lead investigator official.

8.4.5 Presentation

Officials are recommended to use a standardized format for data presentation. Standardized data ensures accuracy, clarity, and factual summary of collected and interpreted evidences. Proper guidelines are provided to officials for presenting the information they collected.

8.4.6 Protocols

Use of protocols is mandatory while dealing with any disaster case. The changes are made in the protocols accordingly; it depends upon the type of disaster. The officials along with the lead officers discuss the conditions and make changes in the protocols. The employed staff has to follow the guidelines and work according to them. For example, Interpol has given guidelines for working in different disaster cases. The guidelines provide information regarding sample collection, handling, analysis, and storage of information. Moreover, the guidelines are updated according to the recent developments. [22]

8.5 Forensic Odontology

Teeth being insusceptible to the unpropitious conditions of the disasters can be used as a great investigative tool; hence, forensic odontology has played an imperative role in identifying large number of victims [12]. Forensic odontology is the study of teeth for legal issue and is considered as one of the primary identification method in identification of victims in mass fatality incident [8]. Dental teams are implored in collecting and recording both antemortem and postmortem data which are further compared and noted [5].

Forensic odontology comes into play in all of the five phases: [12]

- Scene
- Postmortem
- Antemortem
- Reconciliation
- Debrief

8.5.1 Scene

At the crime scene or disaster scene, the odontologists are guided to collect and locate as well as document the image before retrieval. Protection of evidences is an imperative point to keep in mind during the collection procedure [12]. Proper equipment including overall suits, boots, rubber gloves, and helmets should be used, and guidelines should be followed. Dental remains which can easily get carbonized are reduced to fragile components [5]. Protecting these fragile evidences at the scene and during transportation to the mortuary is censorious and leads to difficulty in obtaining PM evidences and further identification procedure. The recovery method or procedure require accounts mapping including GPS and aerial photography of the disaster area [12].

8.5.2 Postmortem/Mortuary Team

The mortuary team collects the information which is required for investigation. A proper area is constructed with all facilities for body examination. Different specialists including fingerprint expert, police, pathologist, DNA expert, and odontologist examine the body and register their findings on the pink Interpol form. Usually, forensic odontologists are employed to examine the body for confirmation of their findings [8].

Photography and documentation are done side by side. Personal belongings, clothing, and external body of the deceased are photographed. Other items such as watches and pocket contents are not used as direct proof, but these things act as a corroborative evidence [12]. The postmortem lead officer or in-charge should record all the findings in DVI form for each body. During dental examination, all the details are registered on the postmortem F1/F2 Interpol form [13]. Certain parameters followed by experts include the following: Jaws should not be removed by the odontologist until and unless more specific examination procedure is required. Use of computer technology and database software saves time and labor and reduces the risk of transposition error. Equipment used for examination includes dental mirrors, cheek retractors, probes, alcohol, or other chemical solvents for washing away the debris, tweezers, and toothbrushes. All these equipment should be properly sterilized because this might become a barrier in DNA cross-examination [12]. Battery-powered X-ray machines are usually used for dental radiographs. The use of nondestructive techniques should be done to get more access to dentition. Mandibular dissecting technique is recommended because it is nondestructive and it allows

easy access to both the maxilla and mandible by helping in repositioning facial tissue after autopsy [5, 8, 12].

Dental age estimation is an imperative parameter for identification [13]. Postmortem dental age estimation allows forensic experts to match the questioned dental evidences to that of suspected ones from the missing persons list. Morphological and radiological age-related parameters are used for age determination from teeth. Secondary dentine formation, enamel wear, cementum incremental lines, etc. are some of the points which provide aid in dental age investigation.

If the teeth sample is recovered as such, without any damage, then 2-D radiographs are used to apply Kvaal technique. After the development process, extraction methods are performed for examination. Genetic identification proves to be a commendable tool in identification of disaster victims [8]. DNA extraction from teeth can also act as complement method. Dental pulp material is a good source of DNA and is used for analysis purposes [5]. Canines and premolars are two vital teeth which can be extracted and sent to forensic DNA laboratory [8].

8.5.3 Antemortem Team

Antemortem information is obtained from family members of missing people. Family members can tell the information regarding healthcare centers from where the deceased went for treatment and other medical or dental information. All the dental records, CT scans, x-rays, dental models, mouth guards, and dental photographs should be collected. The content and source of original dental record can be analyzed and are noted on Interpol forms [8]. Some dental forms might be of poor quality because of abbreviation, nomenclature, discrepancies, or poor handwriting. In this case, forensic odontologists can also visit the treating dentist to gather information and clarify the problems of deceased searching, collecting, receiving, quality assurance transcribing, and analyzing which are crucial steps in quality system of DVI process [5, 12].

The dental teams elucidate and decipher the records onto yellow Interpol form, using FDI notation. Accuracy is paramount; hence, disparity should be classified, and further doubt should be referred to an antemortem team leader for arbitration. Care should be taken during elucidation of radiographs. After transcription, one must check the quality which is part of the protocol to be followed to avoid jeopardizing the quality of data gathered [5, 8, 13].

8.5.4 Reconciliation

In the reconciliation phase, odontologists compare the antemortem dental record with the postmortem record and make a record of findings.

Software likes DVI system international is a useful application for comparing large number of records. The end decision is taken by odontologists, who manually check and discuss the findings. Certain discrepancies are usually found in the

comparable data. Radiographs and anatomical data are usually checked, and individualistic features are noted. Superimposition technique is also performed on dental structures. Other information like dental implants and radiographs of long shapes aid in comparison procedure.

For quality assurance, the report made by the officials should be standardized, unbiased, clear, and impartial; all the conclusions should be made in layman language without using technical terms [12].

8.5.5 Debrief

In debrief stage, the major focus is to review all the aspects of identification of disaster victims. This is done to check whether the earlier procedures performed are accurate or the protocols need to be improved for further use. All the details gathered in the above stages are checked and presented to the odontology coordinator; all the information including staff rosters, occupational health safety, management, security, reports, quality assurance, methodology, and future needs are also recorded and presented [13]. Every stage right from the commencement of disaster to debrief is checked and analyzed. Proper checklist is suggested to be made. DVI record register consists of all the aspects of quality management in DVI cases and is available without any cost if needful [8].

If the disaster occurs at a large scale causing immense destruction to the population, then it is very difficult for forensic odontologists to deal with the situation. This may cause error because of time and cost for examination, analysis, and sampling of deceased bodies. Byard and Winskog suggested that one must focus on the number of cases that need to be re-examined [5].

Proper SOP (standardization operating procedures), forward planning, standardization, and international cooperation are important to provide an effective response to any DVI incident [5].

Forensic odontology is an imperative method which has been developed enormously since 2004 after recent developments in odontology in the tsunami case which include computer-aided 3-D imaging, forensic radiology, forensic craniofacial reconstruction, and virtual autopsy. Newer diagnostic tools in forensic studies and analysis are developing, and the protocols for DVI operation are also optimized timely [5, 12].

8.6 New Methodologies in Forensic Odontology

There is much more specificity, sensitivity, and speed in odontology field because of newly introduced advancements and methodologies. New methods are used for analysis and interpretation of dental evidences and had made the investigation much easier and quicker than before [14].

8.6.1 Photography

New cameras and photography tools are invented which assist in analysis. With the addition of measurement feature, there is no need to manually calculate the dimensions of the teeth or any dental sample. The software itself provides the entire details of the evidence making the analysis frequent and specific.

8.6.2 CAT Scanning

Teeth and jaws can be scanned by unique features. The data is stored and can easily compared with the antemortem records.

8.6.3 Barcoding

This technique has been used in the past, but newer modifications has increased its specificity. It can store more information regarding the person as compared to prior ones.

8.6.4 RFID Tags

RFID stands for radio-frequency identification. This is used for locating and identifying tagged living or non-living things. A microchip is used which stores information and tag number. The digital information is converted to interpretable information by the use of antenna.

8.6.5 3-D Scanning

Documentation of evidences can be done by 3-D laser scanner. The scanner is a very effective tool as it can measure the level of bite marks. Moreover, one can obtain tool impressions by using this 3-D scanner. Intraoral 3-D maps are also useful as they have high resolution and, hence, assist in investigation.

8.6.6 3-D Printing

Re-creation of dental images in an accurate manner is very helpful in testing the missing fragments of the jaw found at the disaster site. The jaws are printed via 3-D printers, and hence, the missing fragments are compared.

These evolving technologies will provide great aid in investigation of disaster victims. These methodologies help in collection, interpretation, and storage of dental data. Results are usually specific, quicker, and accurate.

8.7 Case Studies

- The first dental identification was done in the year 1849 in the United States. Dr. Oscar Amoedo was the first person who used dental evidence for identification in mass disaster. He analyzed the samples in the fire disaster that was held in a charity bazar in Paris in 1897; hence, he is considered as the father of Forensic Odontology.
- In 9/11, thousands of people lost their life in the attacks on the World Trade Center in the United States. Victims were identified using the DNA extract found in their toothbrushes.
- In India, the first case of dental identification was done in 1191. The first human identified using dental evidence was M. Raja Jayachandra Rathore, a native of Canouj. He passed away in battle and was identified on the basis of his false anterior teeth [14].

8.8 Forensic Anthropology

Forensic anthropology is the study of the evidences or material believed to be of human origin to answer the questions related to medico-legal investigation or identification [15]. Anthropologists have been part of disaster victim identification for a century. Earlier anthropologists were employed to generate biological profile from dead remains [3]. Nowadays, forensic anthropologists can develop and implement quality assurance and quality control. The bodies recovered from the disasters are usually decomposed, cremated, distorted, partially burnt, or fragmented on combination of such conditions. The officials employed for locating, recovery, and analysis must have skills and expertise to deal with such cases. They must know how to distinguish the bodies on the basis of their remains, so anthropologists deal with several such cases and play a vital role in disaster victim identification process. Forensic anthropologists work in some phases of the DVI process [5, 14, 16, 17].

8.8.1 Scene

Generally, forensic anthropologists and pathologists are not part of the crime scene investigation, but in mass fatality incident, they might need to visit the crime scene to identify the remains. They overlook the preservation procedure at the site; in addition to that, anthropologists can identify, collect, and recognize the evidences which they can analyze further. Poor management or preservation can affect the integrity of the evidence and might lead to barrier in reconstruction of events. Anthropologists can also recognize and identify spatial temporal relationship b/w bodies and associated evidences. This also helps in identifying the number of individuals. Mapping of the scene is an imperative step during the investigation. The scene should be photographed and documented properly [16].

8.8.2 Mortuary

The DVI mortuary involves various specialties including radiology, fingerprint, pathology, photography, and DNA collection. After the collection of the evidences, they are sent to the mortuary for analysis. Separation of anthropologist samples takes place in this case. The bone samples are distinguished, human or non-human, recognizable or non-recognizable, and amalgamated remains separated. After separation, the skeletal anthropologists analyze to infer the location of the bone inside the body and position (left/right) of the bone in skeleton and soft tissues [14].

All these evaluations can aid in determining the number of deceased and generating the biological profile including age, gender, and stature of the individual. Proper reports are made by anthropology experts regarding ante-, peri- and postmortem findings [13, 15, 16].

The bones recovered from the affected area initially appear to belong to a single individual but after closer examination can reveal that minute fragments of other individuals also commingled with them. The muscles and bones of different individuals can be collected or analyzed as a whole and can be predicted to be of different individual after analysis. Before analysis, it is imperative to sort or separate the commingled bones before X-ray, photography, and DNA analysis.

These coalesced evidences are responsible for inappropriate assessment, and hence they need to be separated after discovering such samples; it is important to re-examine the samples and make reports accordingly and split into multiple new cases. For instance, if the X-ray is taken before the split, it will contain all the merged remains, and the results will not be accurate.

Other information that forensic anthropologists can provide are details regarding skeletal pathologies, skeletal anomalies, and variation. This information is potentially useful and aids in identification procedure.

Forensic anthologists have knowledge regarding bones, bone remodeling, and taphonomy. Forensic archeologists and anthropologists cooperate with each other and show their expertise. Forensic archaeology has developed advancement techniques in mapping, searching, and processing a crime scene. Interpol DVI guide helps in the mapping procedure and provides labels for all the recovered evidences or bodies found at the crime scene. The recent trends include use of electronics mapping equipment such as drones and GPS. Such devices help DVI team map a disaster site. Geographical information system provides useful information and helps in recording the location of human remains. The location and evidences can be electrically recorded in mobile phones or other GIS-linked devices [17].

The identification of deceased migrants is not possible by using techniques such as DNA, odontology, and fingerprints. There is a need for development of alternative methods for identification such as forensic anthropological biological profiling. Having knowledge regarding human anatomy and variability, anthropologists are considering new advancement methods to analyze decomposed and skeletonized samples. Anthropologists can help in identification as the ideas and newer methodologies are evolving.

The skills and expertise of forensic anthropologists are usually underutilized. These scientists can play an important role at disaster site during initial site assessments. They can generate bacterial profiles during postmortem from skeletal remains. During reconciliation, anthropologists check quality assurance and quality control protocols. Proper reports and pink integral DVI forms are [checked] made, and errors are checked. This field is essential for identification of maximum number of deceased in disasters [3, 13, 15].

8.8.3 Case Study

In February 2009, Victoria situated in southeast Austria experienced the most destructive bushfire. This bushfire caused loss of several lives and properties. The forensic team including anthropologists, odontologists, and pathologists reached the site within 48 h of the disaster. The analysis and identification of deceased was carried out in the Victorian Institute of Forensic Medicine in Australia. Anthologists played an essential role in initial site assessment and contributed a lot in identifying and recovering the skeletonized remains from the disaster site. Forensic anthropologists differentiated humans and non-human skeletal remains and generated biological profiles including age, gender, and stature. Moreover, they assisted in inferring the number of individuals at the crime scene [15].

8.9 DNA DVI

Deoxyribonucleic acid or DNA is an essential tool for identification in case of missing person, paternity disputes, and other criminal cases. Interpol has recommended DNA profiling as an imperative technique along with dactyloscopy, odontology, and pathology [4, 6, 10, 18].

8.9.1 Antemortem Samples

Antemortem data can be collected from three sources as stated by Interpol guidelines:

1. First-degree relatives
2. Body fluid or autopsy sample of the victim
3. Personal belongings used by the deceased/victim

Buccal swabs, blood samples, or FTA cards can be used for collecting samples from the blood relations or families of deceased. Guthrie cards are also considered as antemortem samples.

First-degree relatives are usually considered because they provide good profile for identification person. A person with a forensic genetics background is also

required at the crime site. He would assist in collecting antemortem samples. Proper documentation of relationships and samples collected along with chain of custody is required. Interpol DVI forms should be filled out side by side as they ensure that all protocols are followed. Forms are considered as an essential thing for identification procedure. They are considered as an efficient way of collecting important and accurate data.

Family details provided by the witnesses and analyzed by the geneticists are documented by the sample collector. This information is required for pedigree.

A proper record of genetic and contact information should be maintained including sample type name, date of birth, gender of missing person, first name of person providing sample, relationship to missing person, etc. Buccal swab and other specimens collected are received and sent to the laboratory where they are stored at 4 °C. Interpol regards DNA sample as bio-bank samples. Bio-bank samples are either medical specimens or blood stain specimens. These specimen are used for screening purposes. These specimen are used for storing samples and preventing them from contamination. They are labelled with identify of donor. These stored samples are directly analyzed through DNA technique.

There has been an evolution in the use of cards for collecting samples for analysis. Some countries use Guthrie cards for identification. PKU cards were also used in Southeast Asia when identification of tsunami victim was performed in 2006.

Some DNA laboratories make an Excel database including all the information of the deceased, missing person.

AM data includes the personal belonging of the victims. The profile generated from personal objects can be easily compared with DNA profiles. Certain limitations might occur regarding the owner of that object or who has handled that thing. These parameters may generated mixed DNA profiles [6, 18].

8.9.2 Postmortem Samples

The collection, method of handling, and maintenance of specimens is dependent upon the type of disaster and the circumstances at the site of disaster. If proper record is not maintained, then it creates a barrier in identification. So, the official should make sure that they should follow Interpol protocols right from the initial assessment and cover all the procedures they follow and note down the findings.

Proper labeling, photography, written notes, and chain of custody should be maintained; kits for assessment including tubes, scalpels, and labels are used. Specific 1 M numbers should be provided or labelled to everybody. Forensic pathologists sit together and decide at an early stage which part of the body needs to be analyzed and needs to be deported.

The DNA gets easily degraded by temperature, humidity, chemicals, and UV radiation. As stated earlier, samples should be kept at 4 °C. Preservatives like Genofix, common salt, and alcohol can be used; white rum can be used as a substitute if absolute alcohol is not available.

The samples are collected according to the condition of the body and the circumstances. In general, blood or soft tissues are considered for DNA analysis. In putrefied bodies, bone or tooth samples are considered for genotyping process. Putrefied bodies do not contain soft tissues because of degradation. Also, the quality of DNA decreases with time, and soft tissues are generally collected with swabs. Analyses done with soft tissue/fresh material are less time-consuming and less laborious. If the integrity of the sample is maintained, only then the DNA genotyping is easy. In mass disaster cases, generally, in postmortem process, the samples like blood or other body fluid or tissue samples are limited and have less integrity. Degraded DNA inhabits the extraction of large DNA fragments thus reducing the chance of victim identification.

The main study thing which affects sample quality is contamination; proper guidelines are needed to follow during sample collection from bones and teeth:

1. Protective clothing and clean sampling areas
2. Sterilized instruments
3. Storage in appropriate containers and in adequate temperature conditions

All the samples should be packed in sealed containers, and they should not leak. Every sample should be labelled properly, and proper chain of custody should be maintained [6, 18].

8.10 DNA Analysis

DNA analysis is done by officials.

- Sample processing
- Isolation of DNA
- DNA profiling

8.10.1 Sample Processing

Processing of the sample collected from the disaster site is mandatory. All the tissues, blood samples, and mouth swab sampling cards need to be processed prior to extraction.

In case of disasters, there is a high rate of contamination. Hence, it is required that the officials should clean the samples and remove all the debris and other contaminants from the samples. For an instance, if a bone is recovered from the disaster site, it should be washed with tap water and then rinsed with ethanol. Tissue samples collected from the crime scene, either normal or burnt, are cut into slices. Blood samples are generally spotted on a sterile cloth and dried completely. All samples are treated separately to avoid contamination. The area where all these samples are processed is cleaned with sodium hypochlorite solution before and after

treatment of every sample. To deactivate nucleases and to destruct extraneous DNA, UV irradiation is used. [18, 23]

DVI manual provides people guidelines regarding the same collection; officials follow that instructions and work accordingly.

DNA qualification is performed by kits like Quantifiler™ [18].

8.10.2 Isolation of DNA

DNA extraction is performed on the basis of type of sample and the guidelines followed. Certain variations in the protocols are made at the disaster site according to the situation and type of case. PM samples recovered from the site include bones, tissue, blood, or other body fluids. DNA extracted from the samples are then quantified, and degradation is checked. Certain preservatives like from alive paraffin are used to store bio-bank samples.

During extraction procedure, the process followed should be gentle so that the DNA is not affected by any means and preservative removed. Quantification process proves better when high pH and high temperature are provided. Proper protocols should be followed for extracting of DNA. Little negligence can lead to loss of integrity of DNA samples.

Polymerase chain reaction technique can also be used for analysis. Use of FTA cards for collecting AM samples and then applying PCR is a commendable technique. It is less laborious because there is no need of extraction procedure and hence saves time and labor [6, 10, 18].

8.11 DNA Profiling

8.11.1 STR (Short Tandem Repeats)

In human identification, fluorescent PCR multiplex is a standard method. It is expedient that both antemortem and postmortem samples are analyzed for the same STR loci.

The analyst must record the set of markers and kits used for DNA profiling, and one should not discard the sample if it does not match with the questioned sample during profiling.

A number of kits like CODIS and European and Interpol standard sets of markers are employed and are available for analysis.

8.11.2 SNPs

SNPs are useful for contaminated degraded sample because the DNA fragments are short. SNPs are an imperative tool for DVI and kinship analysis. One STR marker is similar to 15 SNPs. For kinship analysis, set of 52 SNPs are developed and tested.

The only limitation is that the amount of DNA for SNP analysis is not adequate because of degradation. SNPs used for DVI have been proved to be beneficial.

8.11.3 Mt. DNA

Mt. DNA is the gentle material which is transferred from maternal side to the offspring. This DNA is more resistant to contamination because of circular structure. Mt. DNA is more abundant as compared to single-copy STR. This DNA is useful for kinship analysis. Mt. DNA is not much informative as compared to nuclear autosomal STR markers.

8.11.4 Y-Chromosome DNA

Y-chromosome contains both STR and SNP markers. These chromosomes help in establishing parental lineages.

Postmortem data of parents, samples collected, can be used for identifying offspring by Y-chromosome analysis.

8.12 Matching of DNA Profiles and Statistical Analysis

The findings or profiles are firstly cross referred with AM and PM records. Proper software has been made by the Interpol team to assemble all data collected. The data contains all dental records and physical, anthropological, and pathological details. Ideally, profiles are compared and calculated, and report is generated.

First choice of markers is STR analysis.
Software is made applicable to handle Y-chromosome.
SNP Data and Mt. DNA Data
Software handles three parts of the DNA process.

- Registering the data
- Laboratory routine
- Matching, calculation, and reporting

8.12.1 Challenges

- Fragmented remains are the main barrier which makes identification process difficult.
- In case of fire, contamination, or putrefaction cases, the DNA can't be used for DVI because of loss of integrity.
- Forensic identification focuses on direct identification and individualization using biologically stable molecule that is DNA. High temperature, microbial

infestation and putrefaction breakdowns the DNA, which makes identification impossible.
- DNA analysis is based on type of incident from which samples are collected. Recovered remains vary from intact bodies to complete fragment tissues or bones.
- "Mixed samples" are usually recovered from the crime scene which is also a limitation. [24]

8.12.2 New Outcomes

- Scientists are working on new primer sets which would amplify short DNA segments.
- Certain methodologies and technologies are going to be introduced which will fetch information from degraded DNA segments.
- Degraded sample profile would be compared with standard forensic STR profiles.

8.13 Case Study

- On August 29, 1996, DNA typing was first used for disaster victim identification. The "Spitsbergen aircraft disaster" found 141 passengers dead along with the crew onboard. DNA typing technique was followed for identification and re-association of highly fragmented bodies. STR (short tandem repeats) analysis was performed at 8 loci, of 257 body parts; 139 out of 141 individuals were identified. Reference DNA samples of two individuals were not available. DNA typing showed 100% success rate in identifying victims in aircraft disaster. Hence, DNA typing is employed as a means of identification [6].

DNA is considered as one of the essential methods of victim identification in cases of DVI. DNA typing is performed, and it starts with collection procedure. Collection of antemortem samples and postmortem samples is done, and then analysis and matching procedure follows [10, 18].

8.13.1 Fingerprints

After the external examination of nod, fingerprints are collected by specially trained officials employed for recovering fingerprints of deceased. In mass disaster cases, generally putrefied and skeletonized bodies are recovered which have crushed injuries and mutilated face and body. The fingerprint expert takes the fingerprint by moving the degraded finger of the deceased with a gloved finger. This is only possible because of slippery condition of the skin. Tri-Tech forensic spray is used for clarity of ridges of fingerprints. Use of this spray is recommended than to use the conventional ink. The spray dries easily, and antiseptic ridge builder helps in getting clear prints. Conventional methods are not being used because they lead to

smudging; black printing ink was applied on paper, and then fingerprints were rolled on the fingerprint card. Fingerprints are then photographed. Proper record is maintained regarding the tag number of the body. The recorded fingerprints are matched with the antemortem record. Glass technique and tape methods are also used for collecting fingerprints. The glass technique is similar to the ink technique, the only difference is change of substrate. Microscopic glass slide is used as a substrate to capture the impressions.

Tape technique is also adopted by using normal transparent cellophane tape. The finger is applied with adhesive surface containing the ink, leaving an imprint on the substrate. The tape was peeled off from the surface and placed on fingerprint card. Black powder and white adhesive lifters are used for recovering the fingerprint. The time span between black and white identification and confirmation is calculated [10, 13, 17].

8.13.2 Radiography

Culbert and Law first used radiology for human identification in 1927. In case of mass fatality incident, the first use of radiographs was held in 1949 in Canada. This was the first time x-rays were used for disaster victim identification [2].

Radiology has multiple functions in mass fatality incident. It helps in detection of extraneous matter that may be hazardous to on-site investigators. The role of radiology has increased in human identification and mass fatality examination. Forensic radiology has become an imperative tool in causality identification [1, 11].

Radiology is used to reveal the exact position and location of evidences which assists in victim identification. The use of mobile PMCT scanners was first reported in Japan. Mobile CT scanner was first used by Rutty and colleagues in small-scale mass fatality incident. A little advancement occurred, and then a new tool, namely, tele-radiology stem, for remote data reporting was introduced. This system evaluated and distributed PMCT scans globally and helped in identification of victims affected by disasters. This system was termed as FiMAG system. After using this system in varied disasters, many countries like Switzerland, Scandinavia, Japan, and Australia illustrated the benefits of mobile PMCT tool. It has the ability to collect information and aids in identification [2].

The International Society for Forensic Radiology and Imaging was established in 2012. Its main focus was to develop and strengthen the field of forensic radiology and imaging across the world. New advancements and techniques were launched in the field of forensic radiology. Six working groups were made for recommending development regarding certain areas including:

(a) Data acquisition
(b) Reading and reporting of images
(c) Education
(d) Certification and accreditation

(e) Networking
(f) Disaster victim identification

ISFRI suggested that radiographs should also be used as a modality in DVI. Radiographs include plain films, fluoroscopy, and digital or computed radiographs. Computed tomography is also a development in the field of radiology.

MRI was made part of postmortem imaging. MRI can be used in those cases only in which antemortem reports are available. The International Association of Forensic Radiographers proposed the use of radiographs for:

1. Identification of cause of death and factors related to it
2. Disaster victim identification
3. Identification of potential hazardous
4. Collecting evidences for legal procedures

Proper protocols and guidelines are made for the official who works with radiographs. Quality and standards are maintained for standard approach [1, 2, 11, 16, 19].

Fluoroscopy

Surface investigation through fluoroscopy usually takes 15 mm. In this, radiological images throughout the body are taken. The machine is moved along the length of the body to capture the images.

Plain Film

These radiograph stations require a separate staff. The radiographs are examined by radiographers or forensic pathologists.

Dental x-rays

Dental x-rays are generally undertaken by forensic odontologists. They work affectively according to the type of case. Earlier, conventional dental x-rays were employed, but now Nomad™ digital x-rays are available.

Mobile CT

By the introduction of postmortem computed tomography, the other methods are now not used widely. Mobile CT is a new practice and replaced all other modalities. This technique is useful for dealing with disaster cases involving chemical, biological, radiological, or nuclear materials [2].

8.13.3 Postmortem Computer Tomography

PMCT plays an imperative roll in forensic investigation of disaster victims.

PMCT scanner includes lorry-based mobile phone, and it can be used at the mortuary where examination of bodies is done. The scanner used for radiography should be integrated so that it can handle the body without disturbing it. This technique is done without opening the body; opening of body bag is done in cases where enhanced CT scan is required. Manipulation is required sometimes; opening of body bag and manipulation is done only if the lead investigator gives consent. All

the manipulations done to the body are recorded; entering the radiology room requires lots of precautions. Officials entering the room must follow the instructions provided to them. Radiation protection materials should be worn. The official performing the imaging should be a forensic radiologist or DVI-trained radiography specialist.

The entire body bag should be scanned first in one go, and then further scanning of specific organs or parts is done. Robotic biopsy technology is a newly introduced technology for obtaining biopsies and fluid sampling using CT image control.

The images produced after the radiography are either digital or in file format. After imaging, hard copy of scans are taken, and specific points which are useful are selected and recorded.

Proper provision for storing data should be made so that no evidence should be left. Interpretation of images is done by specialists of different forensic fields including pathologists, anthropologists, radiologists, and dentists. Antemortem images are usually collected from family members of deceased. These images are used for comparison and identification of deceased. Comparison is done with PM data [19].

8.14 Case Study

On December 2004, Sumatra-Andaman earthquake with a magnitude of 9.0 on the Richter scale along with a tsunami caused devastating effect to the people, properties, and natural resources. This disaster not only affected Thailand but also had massive impact on countries like India and Bengal and Andaman oceans.

Proper disaster victim identification or DVI team was set up for investigation and identification purposes. The DVI team consisted of three sub-teams:

- Antemortem team
- Postmortem team
- Logistics support team

A separate team, namely, “necrosearch” team, was made. This team was guided to collect victims to the autopsy site.

The Belgian DVI team comprised of:

- Policemen (having expertise in recovery, logistics, scientific laboratory, and photography)
- Medical examiner from forensic background
- Forensic odontologist
- Civil protection unit (for care and transport of body and machinery)
- Psychologist
- Medical doctor and his team (for medical needs of team members)

Postmortem team required forensic experts from different disciplines and a military personnel. Antemortem team consisted of five policemen, psychologists, and social assistants.

The Belgian DVI team got assistance from international DVI teams including France, China, New Zealand, Australia, etc. Several members of DVI teams were employed in database organization at Thai Tsunami Victim Identification Information Management Center in Phuket [20].

8.15 Methods Employed for Body Examination

Bodies from disaster sites were recovered by local men prior to arrival of the DVI team. After proper management of all the facilities like electricity, air conditioning, water supply, and proper ventilated autopsy room, PM identification team was divided into four sections:

- Fingerprinting
- Medical examination
- Odontological examination
- DNA sampling

First working area was the Fingerprint section. Fingerprints were taken by gloving and de-gloving method. Finger skin of the victim was carefully removed by de-gloving technique and was further gloved on the finger of the official. Powder was applied on the detached skin, and impressions were taken on the clean sheet.

A separate fingerprinting room was there which was very useful. After taking impressions, the body was sent to the autopsy room for external and internal analysis.

Clothing and personal objects were removed, cleaned, photographed, and recorded.

External body examination included:

- Height
- Description of scars, marks, moles, tattoos, and congenital abnormalities
- Description of physical characteristics (hair color, axillary, pubic and arm hair description)

The internal autopsy conducted by the experts was limited to abdominal cavity, gall bladder, and genital area only.

After the autopsy, the body was sent to third section where dental evidences were analyzed by forensic dental experts. Experts used dissecting technique for forensic dental analysis. This technique leads to easy access of both maxilla and mandibular dentition. Photography was done separately for the upper jaw, lower jaw, and teeth. The officials followed Interpol guidelines for investigation. Forensic odontologists

checked for oral anatomical abnormalities, attrition, and missing teeth. All the observations are recorded on F1/F2 forms provided by Interpol.

The recovered bodies were putrefied and fragmented; hence, for DNA analysis, femoral shaft sampling was performed. This sampling was performed by anthropologists. DNA sampling was carried out in a different area. Sterilized tools and equipment were used for this procedure to avoid contamination. Premolars and canines were also collected because they contain a sufficient pulp material for DNA analysis.

Further, the body was transported to the radiology area where the odontologist took dental radiographs of the deceased. Earlier, dental x-ray was used, but after sometime, the officials were provided with Nomad™ for facilitating dental analysis task.

Postmortem work was quite hectic, firstly, because of the number of victims and, secondly, each examination section was given 20 min per body examination. Officials faced lots of problems due to lack of time.

The findings stated that most of the victim died because of drowning and blunt trauma. The final desk performed duty as follows:

- Checking that all examinations are carried out properly
- Checking that tag number or labels were given to each body
- Checking the quality of radiographs after x-rays
- Quality assurance and quality control of all documents and reports.

After all the procedures, the body was stored in a refrigerated container, and its location and container number or body bag number is recorded by the body handler [10, 16, 20].

8.15.1 Identification Process

All the information from the PM teams was collected for further reconstructive identification. Dental, medical clues, fingerprints, DNA reports, and other physical attributes were collected for making reconstructed profile of an individual. The reconstructed profile was made in order to compare with AM data.

Interpol AM and PM forms were filled separately, and during reconstructive identification they were checked. AM data or information was collected from acquaintances, co-workers, friends, and even doctors of missing persons. It is imperative to collect photographs and fingerprints of the missing person. Fingerprints can be collected from old documents and other items which were used by the victim itself. These things may include shampoo bottles, perfume bottles, etc. Hair samples, toothbrush, and other personal stuff of the victim were also collected. These items were required for DNA analysis. DNA acts as a key tool in kinship analysis. Hence, to infer the relationship, blood or saliva samples of the relatives were also taken.

Antemortem teams were employed to collect certain details from the victim's family including:

- Medical reports
- Dental reports
- Morphological characteristics
- Photograph of victims
- Description of clothes, jewelry, and other personal stuff of the victim

All the collected data was then sent to the Identification section, and then proper data file was made in the computer. PlassData™ was used as a software which allowed research and automatic comparison of all recovered data.

The Interpol guidelines helped a lot in positive identification of multitudinous victims. For DNA identification, Interpol form contained DNA document in which methods to be followed and markers to be followed and markers to be used were enlisted. This was made for uniform comparative application all around the world. All the PM and AM data was uploaded in PlassData™ software. After that, daily automatic research for missing people was started. If the computer showed a match, then manual analysis is performed by the officials.

The final report was sent to the reconciliation board which comprised of forensic experts and investigative officials. Final decisions were taken by the board, and after confirmation, death certificates were issued, and bodies were released [5, 6, 8, 16, 18].

8.16 Modern Advancements

The concept of forensic identification is enhancing with the coming years. Many advancements can be seen in the identification tools in the past few decades. The Interpol has set certain guidelines for working at the disaster site. Most of the countries follow those protocols in case of emergency. There are other practices and protocols used by some other countries [10, 17, 21].

Johnson and Riemen introduced modern fingerprint technology. This technology focuses on capturing digital fingerprints for disaster victim identification. New advancement in fingerprint technology has brought a new revolution in the field of ridgeology. Digit fingerprints captured by the experts from mass disaster site are more accurate and efficient. This methodology saves time and labor. The gloving and de-gloving method usually employed for fingerprint collection is tedious and is not much accurate as the ridge impressions of fingers are not clear [4, 7, 14].

Genetic profiling is also a new methodology implemented for mass fatality investigation. It helps in importing different allelic data formats on different indexes. The data is imported according to the type of samples and the scenario of the disasters.

PATPCR-v 2.25 software is a lab-developed software. It is useful in kinship analysis. It can evaluate three types of parent-children relationship including:

- Single parental
- Conventional paternity trios
- Paternity and maternity cases

Gene Codes Corporation has developed a new bioinformatics tool called M-FISys for mass fatality identification. It is helpful in searching DNA profiles and tracking samples among the collaborating laboratories. Tillmar et al. introduced the massively parallel sequencing (MPS) for DNA identification of biological samples. By using this technique, a good amount of DNA can be obtained from the decomposed and degraded samples or remains. Research and advancements are still ongoing in this field, and positive comparative identification can be done using this technique.

CrimTrac provided a software known as NCIDD software. This software is used by all jurisdictions in Australia for direct matching. At the time of bushfires in Australia, CrimTrac was testing the CODIS 6.0 software for pedigree and relationship analysis. NCIDD software was employed for identification procedure and to detect the matches between antemortem DNA profiles and the profiles obtained from the postmortem DNA samples.

Virtual autopsy is growing quickly in forensic identification. It is used not only in craniofacial reconstruction but also in the entire autopsy process. Virtopsy project has different imaging methods [8]:

- 3-D photogrammetry-based optical surface scanning
- Multi-slice CT (MSCT)
- MRI (magnetic resonance imaging)

Virtopsy is a noninvasive approach; it helps in providing unambiguous, objective, and coherent documentation of forensic findings for testimony in court.

Craniofacial reconstruction (CFR) is used when the corpse face is unrecognizable due to mummification, calcination, or any other means. The main objective of CFR technique is to reconstruct the face of the unidentified body. This method is quite helpful in identification of victims. Currently 3-D manual methods are used. Use of clay or plasticine is done, but this method requires high skills and expertise. The improvement in imaging technology has introduced computer-based CFR methods.

Forensic odontology plays an imperative role in identification procedure in DVI. There is a need for antemortem records for comparison. Adequate antemortem records and proper skilled expertise in postmortem identification prove to be efficient and accurate for identification. Forest has introduced an imaging technology in odontological analysis for accurate identification [8, 13, 21].

Barone and Di Maggio prepared a ground-penetrating radar (GPR) which can be used for investigation procedure. This radar can locate the exhumed remains in the locations which cannot be documented easily. The location of such remains is very essential prior to identification.

8.17 Conclusion

Disaster victim identification is a major task for forensic investigators. These disasters can be natural or man-made. Each disaster has its own scenario, and the scenario defines its own methodologies for identification of the victims. Different forensic disciplines including anthropology, odontology, radiology, pathology, DNA, and fingerprint work together for identification of the unidentified bodies. Antemortem and postmortem data should be made accurately by following the DVI guidelines. The final results are made on the basis of these antemortem and postmortem records. This chapter consists of all the procedures and protocols followed by different forensic experts. There will undoubtedly be many more advancements made in this field which will make the identification process much easier and efficient.

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Review on Clinical Forensic Medicine

9

Gaurav Kumar Singh, Ankita, Muskan, Shubham Saini, and Ridamjeet Kaur

Abstract

Forensic medicines, clinical forensic medicine, and forensic pathology such terms are utilized across the globe. Clinical forensic medicine is also a branch of medicine which involves living individuals and working together with law, judiciary, and police personnel. In the more extensive sense, clinical forensic medicine implies the use of clinical information and abilities to living people relating to the uncommon necessities of the particular legitimate, legal, and police frameworks. It is significantly important that the forensic physicians should be trained, experienced, and qualified. Clinical forensic medicine has been challenged to contribute its role with the legal framework to expand the resources accessible to those patients associated with liability-related injuries, victims of a crime, or any perpetrator, suspect, and a criminal in police custody. Individuals suffering from trauma which is associated with a criminal activity often demand the enquiry of the injuries, and they are looking for well-trained, skilled, and experienced forensic pathologists. Specialists are capable enough to assess the surviving victims of sexual assault, domestic violence, related with liability

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injuries, pseudo victimization, self-harm, child abuse, etc. In this chapter, all the cases dealt in the clinical forensic medicine have been reviewed.

9.1 Introduction

Forensic medicine, clinical forensic medicine, and forensic pathology such terms are utilized across the globe [1]. Forensic medicine is defined as the branch of medicine which investigates individuals who have been either injured or murdered or suspected to have been injured or killed by external influence such as trauma, intoxication, etc. People with nonfatal wounds after purposefully self-incurred or accidental wounds are, then again, as a rule, taken care of solely inside the medical services framework. In numerous nations, forensic medicine addresses a clinical strength inside the general set of laws, not inside the medical services framework. "Forensic pathology is defined as the branch of Medicine applying the principles and facts of the medical sciences to the issues in the field of law." On the other hand, forensic pathology examines decreased individuals and finds out the cause of death [2, 3]. Clinical forensic medicine is also a branch of medicine which involves living individuals and working together with law, judiciary, and police personnel. This term is exploited since 1951 when the Association of Forensic Physicians (earlier known as Association of Police Surgeons; UK-based body) was launched and has become popular in the last two or three decades. And the practitioners involved in this field are known by several names, but the most accepted term is forensic physician. Practitioners in forensic pathology are called forensic pathologists dealing with decreased individuals. On the other hand, practitioners in the clinical forensic medicine are called forensic physicians which deal with living individuals. There are several doctors across the world which are involved in the field of clinical and pathological aspects of forensic medicine [1] (Fig. 9.1).

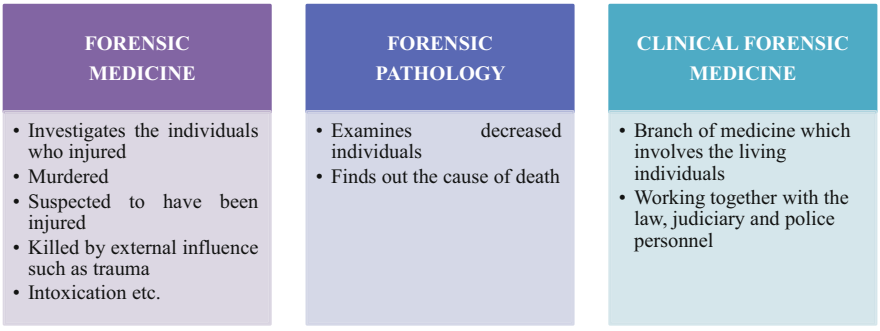

Fig. 9.1 Showing the basic description about forensic medicine, forensic pathology and clinical forensic medicine

9.1.1 Relationship Between Clinical Forensic Medicine and Forensic Medicine

The application of clinical knowledge and practices to living persons subsequent to the specific requirements of respective legal, judicial, and police systems is defined as clinical forensic medicine. On the other hand, forensic medicine is one of the significant branches of forensic science which is also known as "legal medicine," "medical jurisprudence," and "forensic pathology," applying the principles and skills in civil and criminal law. Both clinical and forensic medicine apply their knowledge and skills in the legal and judicial system and present in the court of law.

The most common of the cases among clinical forensic medicine and forensic pathology/forensic medicine are listed below:

1. Injuries
2. Trauma—sharp as well as blunt force
3. Sexual assaults
4. Road traffic accidents
5. Bomb blast cases
6. Strangulation

9.1.2 Clinical Forensic Medicine

In the more extensive sense, clinical forensic medicine implies the use of clinical information and abilities to living people relating to the uncommon necessities of the particular legitimate, legal, and police frameworks. The association of clinical legal work varies extensively from one place to another, part of the way relying upon the legal. Table 9.1 show all the categories of work and responsibilities which are dealt and handled in the clinical forensic medicine by several specialists of various disciplines such as legal medicine, pediatrics, gynecology, psychiatry, public health, emergency medicine, etc. [5].

Victims of a crime or suspected culprits sometimes have to be assessed with respect to the presence of injuries. The medical findings and the clinical report on the injuries are likely to have a significant impact in any resulting legal procedures. So, the actual assessment and the documentation of the applicable outcomes should be acted in an accurate manner. Portrayals and conclusions ought to be stated in wording which is additionally understandable to lay people. There should be an explanation of the reports if the scientific language is unavoidable [4].

The following are the things which need to be considered while dealing with living victims in clinical forensic medicine:

1. Documentation should be in an adequate and accurate manner.
2. Assessment of case if it requires sample collection in circumstances like alcohol/drug intake, genital swabs, hair samples, etc.

Table 9.1 Categories of duties in clinical forensic medicine [4]

S. No.	Category	Dealing with
1.	Assessment of live victims	• Body injury • Rape/sexual assault • Marital rape • Domestic violence • Child abuse • Abuse of elderly individuals • Torture
2.	Assessment of suspected perpetrators	
3.	Assessment of injuries	• Self-inflicted injuries • Accidental injuries
4.	Medical examination in traffic accidents	• Examination of driver and passengers • Examination of pedestrians
5.	Examination for fitness to drive	Examination of disability due to alcohol and/or drug intake Sample collection (blood)
6.	Assessment of effects	Alcohol and drugs
7.	Assessment of mental health	
8.	Assessment of fitness to be detained and interrogated	
9.	Assessment of physical capacity needed to perform work	
10.	Reports declaration and capability	• Documentation of all medical findings and observations in well and proper manner • Determination of wounds and injuries and other medical evidences
11.	Presenting the medical evidences in the court of law	• Witness • Professional witness • Expert witness
12.	Medical concern of convict	• Police custody • Prison custody
13.	Age determination	
14.	Healthcare of police officials	

3. Photography of medical observations, if necessary, for example, in the complex and patterned injuries.
4. Detailed recording of injury marks, etc. should be done because there can be self-inflicted injuries or absence of injuries in false allegations.
5. Medical reports must comprise all the basic details of the victims such as size, height/stature, body weight, etc.
6. Details of injuries and wound reporting the size, shape, and appearance with respect to the location, etc. of wound must be imprinted.
7. Diagrams and sketches of the body might be helpful.
8. Close assessment and accurate depiction of wounds and injuries should be done.
9. The condition of wounds/injuries must be assessed properly if there is any sign of repair or infection or it requires any surgical treatment.

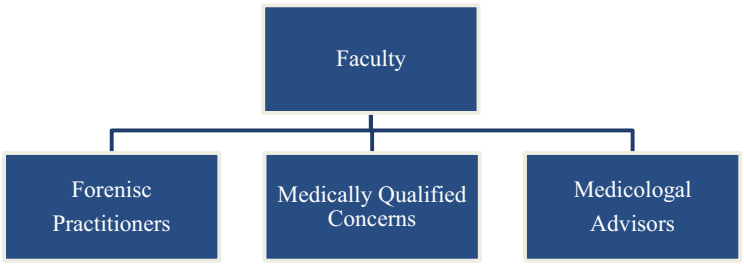

Fig. 9.2 Faculty professional groups [6]

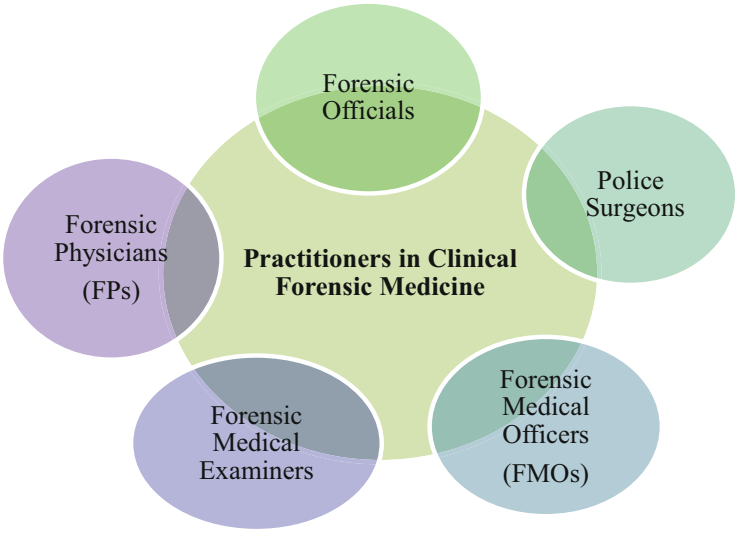

Fig. 9.3 Showing the different titles for the practitioners in clinical forensic medicine

10. Terms and description should be easily understandable to the lay people. If scientific language is not understood, then an explanation should be given [4] (Fig. 9.2).

9.1.3 Role of Forensic Physician (FP)

It is significantly important that the forensic physicians should be trained, experienced, and qualified. Forensic practitioners are doctors who are serving in the field of clinical forensic medicine and providing medical treatment and care to clients and assessment of victims in cases of sexual assault, rape/marital rape, domestic violence, and also assessment of detainees. They are also eligible for giving expert opinion in suspicious cases of death [6] (Fig. 9.3).

9.1.4 Clinical Forensic Medicine in the Emergency Department: Common Errors and Need

The techniques of forensic medicine which are usually applied on the dead are also utilized on the living in clinical forensic medicine. Medical findings are recorded, injuries are assessed, documentation in the emergency department is conducted, and all the essential evidentiary material is collected for presentation in the court of law. There can be some common forensic errors consisting of the inability to identify, collect, and preserve the evidentiary materials in the emergency department. Other common errors may include failure in the recognition of type of injuries, patterned injuries, ballistics examination, and sharp, blunt or any penetrating injuries. Such common errors further may cause difficulties to access significant information and evidence. Proper training should be provided in the emergency department of clinical forensic medicine to address the forensic requirements which is implemented in the emergency medicine residency program at University of Louisville and serving the desired forensic assessments and examinations. The progress and training in the field of clinical forensic medicine is unique at the international level [7].

9.2 Cases Dealt in the Clinical Forensic Medicine

9.2.1 Injuries: Assessment and Documentation

The assessment of living persons in the last few years has been recognized as "clinical forensic medicine" which is one of the classical fields of forensic medicine. Usually, forensic analysis of injuries follows the same morphological principle, facts, and knowledge whether in living (clinical forensic medicine) or dead (forensic medicine) persons. But there are some unique and specific features which are used to distinguish and differentiate between the homicidal, accidental, or self-inflicted origin. In the forensic medical context, the injury is defined as "damage to any part of the body caused by the intentionally or accidentally via using any mechanical or some other traumatic agent." The role of the forensic physician is to assess, document, and interpret the injuries appropriately and accurately to find out how that wound or injury was caused which might often found as an issue in the court of law [8, 9].

9.2.1.1 Injury Assessment

Appropriate assessment and interpretation of an injury lies upon the well and good physical examination, ascertaining a good history, and documenting and recording the medical findings clearly whether in the form of notes, body charts, computer records, etc. which will be evaluated by the doctors, legal authorities, and courts further. The consent of the individual who is being examined should be obtained for generating a medical report [8].

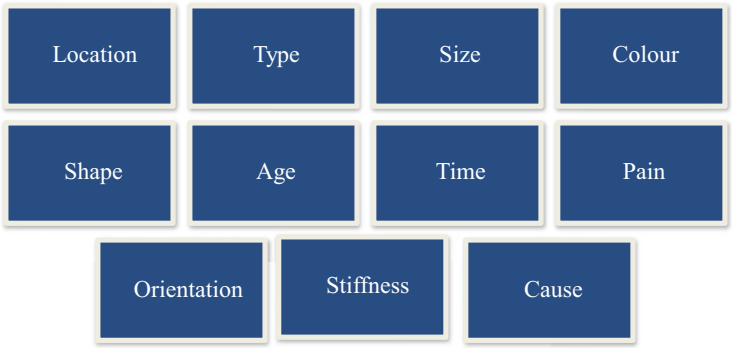

Fig. 9.4 Information required for injury assessment [8]

There are so many key factors associated in the successful assessment of injuries which are listed as below:

- How did the injury sustain?
- Any weapon used? If yes, then still available?
- What was the time when the injury sustained?
- Any protocols followed for the treatment of injury?
- Previous record, if there is any illness present such as any skin disease or allergy.
- Any medication used regularly.
- Alcohol and drug intoxication.
- Outfits worn.

There is also some other more of the information required for the assessment of injuries like (Fig. 9.4):

9.2.1.2 Documentation

Forensic injury or wound documentation serves an important role in clinical forensic medicine including many tasks such as source of interpretation, proofs, medicolegal reports, measurement records, and source which confines the area and dimensions of the wound/injury, matching the patterned injuries with the suspected causative weapon or instrument. These recordings of injuries which might get healed then are represented in the courtroom aiding the examiners, prosecution, and court in decision-making. Documentation of injuries can be done in various formats mainly comprising the handmade notes, pro forma diagrams, keeping the photographs, etc. Nowadays, records in the form of digital images have become the appropriate method for injury documentation which is followed by hand-drawn and handwritten notes, although digital photography is a usual technique for the documentation of injuries [9, 10].

9.2.1.3 Types of Injury

The practitioner who is assessing the injury should have a good knowledge and understanding of the terms, factors, and nature of injury describing clearly, using the medical context, injury classification. The classification of injuries which is appropriate and clear and mostly seen injuries has been represented in the following (Table 9.2).

9.2.2 Injuries Due to Arson/Burn

9.2.2.1 Introduction

Cases of homicides and suicides are on the rise across the globe. Approximately, one million people die because of suicides and homicides, and common methods are blunt and sharp trauma, hanging, strangulation, etc. The most uncommon method among all those practicing homicide and suicide is burns. Acids and hot liquids are most commonly used in our country for suicidal and homicidal purposes. Homicidal burning of young females is the most common in our country but stimulated as accidental incidence via their family members. Most frequent reasons given in history are explosion due to gas leakage, fire caught while in the kitchen, chimney falling on the victim due to explosion, etc., although most of these usual statements given by respondents are not found to be genuine during investigation by the forensic experts [11].

Burn injuries are underrated trauma which may affect anyone, anytime and anywhere. Reasons for such injuries have been listed in Fig. 9.5. The most common reason for burns in our society is accidental burns. Burn injuries have been considered as the most serious injury which makes human life miserable. In India, accidental burns are considered as the second major cause of death after vehicular accidental deaths [12].

Table 9.2 Types of mostly seen injuries [8]

Types of mostly seen injuries	
Erythema	Hematoma
Petechiae	Wheals
Abrasions (graze, scuff/brush)	Laceration
Stab wound	Bruises
Scratches	Incisions
Chop wounds	Slash
Bite marks	Firearm injury

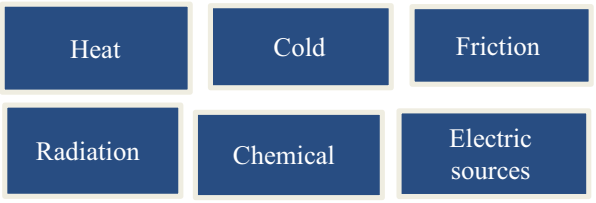

Fig. 9.5 Showing the causes of burn injuries

Appropriate treatment of burn injuries depends upon the determination of burn depth. On the basis of depth, burn injuries are classified into first degree, second degree, third degree, and fourth degree. Studies performed on burn injury assessment have found that there is about 50–80% of accuracy in the burn depth assessment at the clinical level. Therefore, there is a need to work more to understand burn tissues, so that detection of burns will be made easy [13].

The major cause behind burn injuries are heat, fire, and hot liquids. And the different physiological and pathophysiological reactions are associated for different causes [14]. Burn injuries have been considered as the most serious injury which makes human life miserable. In India, accidental burns are considered as the second major cause of death after vehicular accidental deaths [12].

9.2.2.2 Classification of Burn Injury

Burn injuries are categorized based on its size and depth. On the basis of depth, burn injuries are classified into four degrees as mentioned in Table 9.3 [15]. Each degree represents a certain type of depth of burn injury. These categories are superficial burns, degree 1; superficial partial thickness degree, 2A; deep partial thickness, 2B; full-thickness burns, degree 3; charring, degree 4 [16].

9.2.2.3 Burn Changes

In cutaneous injuries, the following three zones are observed:

1. Zone of coagulation
2. Zone of stasis
3. Zone of hyperemia

1. *Zone of coagulation* is identified by the irreversible injured tissues during the time of injury confining the region of necrosis.
2. *Zone of stasis* is recognized as adjoining to the zone of coagulation. In this zone, the area is exposed to a reasonable degree of damage related to vascular escape. As per the wound surroundings, this zone will either continue to exist or result in necrosis.
3. *Zone of hyperemia* is recognized by the increased supply of blood along with healthy tissues with no risk of death [17].

9.2.2.4 Burn Assessment

Findings of burn injuries comprise a broad spectrum ranging from local, minute, superficial, to consumption of bone and soft tissues. Both external and internal burn injury findings depend upon temperature exposure and time applied to the body. Also findings of burn injury depend upon the type of heat transmission to the body and some other prevailing circumstances [16].

1. Color assessment—vascularization, pigmentation
2. Metric variables—extent, height, volume

Table 9.3 Categories of burn injury based on depth [15]

Categories	First degree	Second degree 2A	Second degree 2B	Third degree	Fourth degree
Depth	Superficial	Superficial with partial thickness	Deep with partial thickness	Full thickness	Full thickness
Histology	Only the epidermis is affected	Both epidermis and papillary dermis are affected Skin appendages intact	Epidermis and reticular dermis Most of the skin cells are damaged	Epidermis and dermis All of skin appendages are damaged	Comprises the fascia and muscles and bone
Appearance	Erythema, blanching on pressure, no blisters initially	Blisters, moist red, blanches with pressure, erythema	Red, shiny, severe pain, blisters, wet, doesn't blanch with pressure	Dry, waxy white, moist, leathery skin	Dry, black, charred
Treatment	Not serious unless more or larger areas are involved; cleaning and cold compress	Cleaning and cold compress, disinfected dressing	Hospitalization is needed if 25% surface area affected; surgery or grafting	Immediate hospitalization	Surgical interference and grafting are required for the healing
Risk factors	Risk of skin cancer	Local infection	Skin grafting	Amputation, risk of developing hypertrophic scars	Gangrene, amputation, demise
Scarring	Nothing	Pigmentary changes	Severe	Very severe; hard and elastic eschar tissue	Hard and elastic eschar tissue
Healing period	3–6 days	7–20 days	>21 days	Never heals	Never heals
Pain condition	Painful	Painful	Sensitive of pressure	Deep pressure	Deep pressure

3. Biomechanical properties—elasticity, stiffness
4. Physiologic changes—hydration (Fig. 9.6)

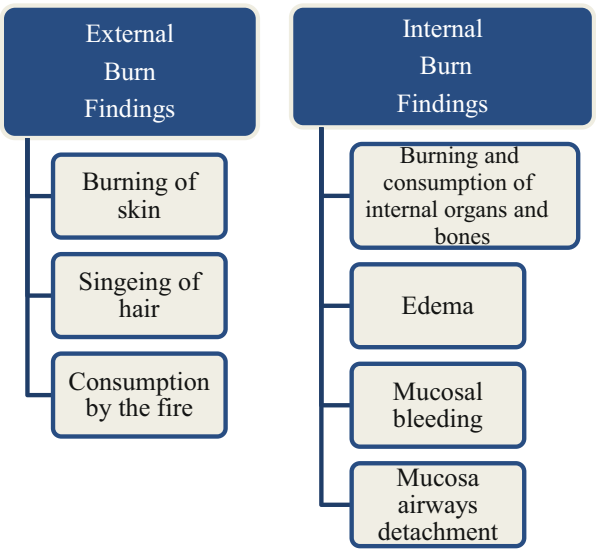

Fig. 9.6 External and internal findings of burn injury [16]

9.2.2.5 Burn Assessment Techniques

For the clinical assessment of burn injuries and their depth, the following techniques have been established [13]:

1. Biopsy and histology
2. Laser Doppler
3. Thermography
4. Vital dyes
5. Video microscopy
6. Orthogonal polarization spectral imaging (OPSI)
7. Reflectance confocal microscopy (RCM)
8. Multispectral imaging (MSI)
9. Optical coherence tomography (OCT)
10. Near infrared spectroscopy (NIRS)
11. Terahertz imaging
12. Ultrasound
13. Laser speckle imaging (LSI)
14. Spatial frequency domain imaging (SFDI)
15. Photoacoustic imaging

1. **Biopsy and histology—**this is often referred as the "gold standard" in assessment of burn depth which gives reference for the rest of the techniques. In this approach, tissue is first removed and then introduced into a reagent like formalin to avoid damage. The tissue is then cleaned, dried out, infiltrated, and cut into thin sections of size ~50 nm–4 μm based on the choice of microscope to be used. Later, the tissue is stained, placed under microscope and assessed. As biopsy and

histology is an excellent approach for analyzing burns, there is also requirement of more noninvasive approaches to be introduced for clinical purposes.

2. **Laser Doppler techniques**—there are two type of laser Doppler technique: one is laser Doppler flowmetry, and the other is laser Doppler imaging working on the same principle.
   (a) **Laser Doppler flowmetry**—this approach is the oldest of Doppler techniques assessing the microcirculation in burn injury with a direct contact giving high-accuracy burn depth results. Due to its direct contact with the injury, the use of this technique is limited which may spread the infection and lead to discomfort of patients.
   (b) **Laser Doppler Imaging**—this approach is more advanced and latest which provides non-contact scanning facility and can measure burn depth and the entire burn surface. LDI is the only approach for burn depth assessment which has been endorsed by the American Food and Drug Administration (FDA).

   This approach provides high accuracy and high efficiency. But accuracy is challenged when there is an extreme movement of patients. Along with it, LDI is quite expensive and demands long time for setup before clinical assessment.
3. **Thermography—**there are two types of thermography, i.e., active and static thermography.
   (a) **Active dynamic thermography—**temperature response is evaluated through thermal pulse excitation.
   (b) **Static thermography—**in this type of thermography, temperature is determined though the temperature difference between the burn affected area and unaffected area.

   As thermography is a quick and affordable approach, accuracy is challenged in some circumstances when granulation begins in burn wounds. Therefore, thermography is recommended to use within 3 days post burn for best possible outcomes.
4. **Vital dyes—**fluorescent and non-fluorescent dyes are introduced intravenously followed by optimal illumination sources to make the microvascular structure observable. ICG which is a fluorescent dye is considered as a nontoxic dye. ICG video angiography is associated with biopsy % histology, and the clinical results of this particular approach are nearly100%. But this approach is cost-effective, and there are some side effects also associated with it such as headache, diaphoresis, urticaria, etc. ICG video angiography is capable enough to identify the burn depth few hours post burn.
5. **Video microscopy—**this technique utilizes the transcutaneous microscopy along with fiber-optic light source to make the dermal papillary structure visible on the burnt surface and capable assessment of burn depth based on the integrity of structure visualized. This approach has advantages as it is quite simple and easy to operate when assessing burn depth within the 24 h post burn, while laser Doppler imaging takes 48 h. The disadvantage associated with video microscopy is that it also requires direct contact with wound which may spread infection, alleys discomfort among patients, and caters assessment only of a small burn area.

6. **Orthogonal polarization spectral imaging (OPSI)—**is a type of transcutaneous video microscopy which gives real-time imaging of capillary network via cross-polarization phenomenon. This approach provides good-resolution microvascular structure. There are disadvantages associated with OPSI, i.e., needs direct contact with wound which leads to discomfort and risk of infection and also time-consuming as it takes a long time while scanning the burn area.
7. **Reflectance confocal microscopy (RCM)—**this approach "optical biopsy" as the term indicating capable to study the tissue without physically dissecting with the help of a laser emitted from a source and skin contact device focused on the tissue and gathering the reflection via detector. There are many demerits of RCM. Firstly, it involves skin contact which raises infection risk and discomfort among patients. Time taken for sampling is quite long, and the technique is cost-effective. Also, there are no details available on the burn depth accuracy of RCM.
8. **Multispectral imaging—**this technique is noninvasive and demands no skin contact based on the principle of optical properties such as reflection, absorption, scattering, etc.
   The application of this approach is observed to have the capability to act as a clinical tool.
9. **Optical coherence tomography—**there are two kinds of OCT, i.e., polarization-sensitive OCT and spectroscopic OCT.
10. **Near infrared spectroscopy (NIRS)—**NIRS has the capability to distinguish between burn depths and alteration deeper into the skin. The accuracy of NIRS for clinical application of assessing burn depths is not reported yet.
11. **Terahertz imaging—** THZ imaging is used as burn assessment in both in vivo and ex vivo conditions. This particular approach has the capability to assess burn depth but lack the standardization of burn depth quantification. The accuracy of THZ imaging still needs to be reported.
12. **Ultrasound—**this technique is widely used in clinical applications. These approaches still need to be observed for burn assessment and also in combination with other methods like elastography to analyze tissue elasticity.
13. **Laser speckle imaging (LSI)—**LSI is capable of providing high-resolution images and is getting attention in recent years for burn assessment. This technique have shown advantages and satisfactory results over the laser Doppler imaging, and this LSI further needs more development for burn wound assessment.
14. **Spatial frequency domain imaging (SFDI)—**this is a wide-field optical and non-contact approach capable of assessing the tissue about 1–5 mm below the surface of the skin. SFDI is a recent, influential approach for scrutinizing the variation in burnt tissue properties. This approach has the potential for enumerating burn depth in the beginning stages, and its accuracy still needs to be reported [13] (Figs. 9.7 and 9.8).

9.2.2.6 Pain Management in Burnt Patients

It is critical to manage the pain in the rigorously burn patients or victims. In the early stage, during emergency, potent opioids are given intravenously to patients as per

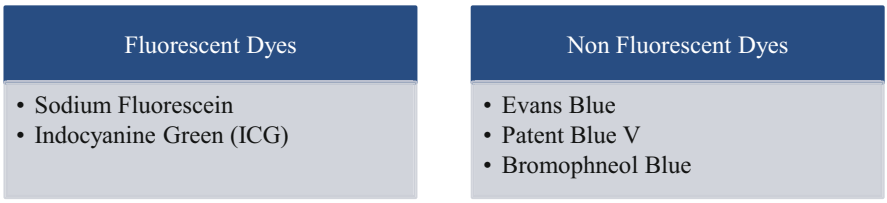

Fig. 9.7 Showing the types of vital dyes used

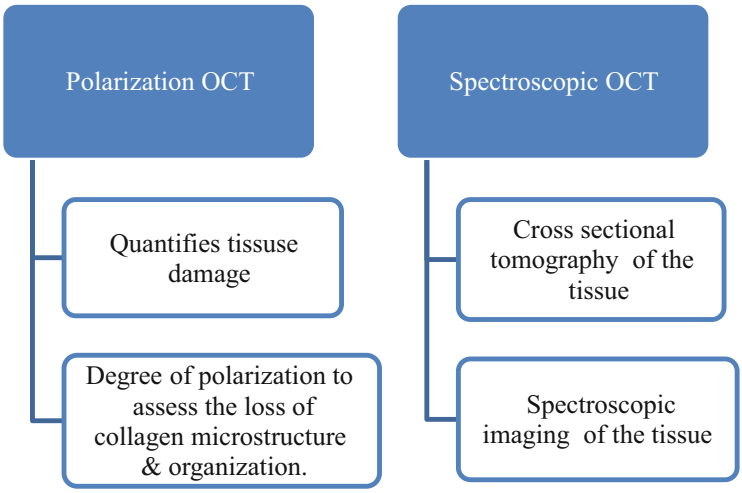

Fig. 9.8 Types of optical coherence tomography

their response such as fentanyl, morphine sulfate, hydromorphone, etc. For minor pain, acetaminophen is given. For moderate pain, an oral oxycodone or acetaminophen mixture is given. Those who are resistant to opioids, ketamine (0.1–0.2 mg/kg) can be given to them. Along with this, antidepressants, anti-anxiety, and anticonvulsants have been found effective in burn patients or victims [18].

9.2.2.7 Self-Inflicted Burn

Self-inflicted burns show a significant social and medical issue for society. Contrasts have been shown between patients who endeavor suicide and individuals who intentionally hurt themselves with no intention of committing suicide. These self-perpetrated injuries may take after wounds that are purposefully delivered by others and may require examination by defensive administrations. There is less information available on specific burn pattern injuries in psychiatric patients. There are rare cases of self-inflicted burn patterns. The factors responsible for the suicidal attempts might include loneliness, separation, hopelessness, stress, depression, or any associated mental health disorder. In self-inflicted suicidal burn attempts, accelerants have been used. The patients who are mutilating themselves have a pre-existing psychiatric

disorder. There is another term, i.e., para-suicidal behavior, which means intentionally causing self-injury which is not fatal but may raise the risk of tissue damage and death. Larger and deeper injuries are reported in patients who attempt suicide by burning. Studies have reported higher mortality rate for those patients who have previous record of suicidal attempts or self-harming. The management of such patients should involve the treatment of burn injury, psychological assessment, and regular monitoring by mental health experts [19].

9.2.3 Sexual Assault

9.2.3.1 Introduction

The cases of sexual assault are accruing in this new era. Sexual assault is a crime in which the assailant harms the modesty of a victim or touches the victim in sexual manner, and these activities occur without the victim's consent. Generally, the victim is forced to make sexual contact [20]. This activity is illegal in front of law. A man or a woman is said to commit sexual assault if he or she sexually touches a person or forces someone to make sexual contact with him or her in conditions like:

- Without his/her consent.
- With consent but the person is not capable of giving consent, e.g., intoxication or mental trauma, etc.

9.2.3.2 Examination of the Victim

1. The time, date, and location of assault should be noted.
2. The number of times the victim was assaulted should be noted.
3. The victim's statement and other people's statement who were associated with crime should be recorded by separating each one of them.
4. The victim should be asked for any kind of toxic agents like alcohol and drugs involved in the crime with all the details.
5. The victim should be looked for any kind of physical injuries.
6. All the chemical, physical, or biological evidences should be collected from the victim as well as from the scene of the crime.
7. All trace evidences should also be collected like hairs, mud, glass, buttons, etc.
8. The victim should be examined by a medical practitioner with the consent of victim. All injuries like nail scratches or other physical injuries should be recorded with proper proof like photographs for further use.
9. The victim's examination should be done in the presence of a third person. A female nurse is preferred for it; otherwise, any female relative of the victim can be present at the time of examination.
10. Proper treatment of injuries should be given to the victim after examination [21].

9.2.3.3 Examination of the Suspect

1. The suspect should be examined for any kind of physical injuries made by the victim in defense.

2. All the clothing of the suspect should be collected at the time of examination and looked out for DNA evidences.
3. The concerned body parts reported by the victim should be focused more, and all the possible evidences should be collected, e.g., if the victim was forced for oral sex, then the suspect's genitals will provide better evidence than the mouth of the victim.
4. All body injuries should be recorded with photographs [22].

9.2.4 Sexual Offences

Sexual offences are classified into two categories according to the manner of crime as follows:

- Natural sexual offences
  1. Rape
  2. Incest
- Unnatural sexual offences
  1. Sodomy
  2. Bestiality
  3. Tribadism
  4. Buccal coitus
- Sexual paraphilias
  1. Necrophilia
  2. Necrophagia
  3. Sadism
  4. Masochism
  5. Fetichism
  6. Frotteurism
  7. Undinism
  8. Transvestism
  9. Exhibitionism (Fig. 9.9)

9.2.4.1 Natural Sexual Offences

Rape

Rape is a serious crime which is increasing day by day [23]. This crime is not only happening with females, but the male population is also becoming a victim of it. A male is alleged to commit rape if he does any of these four things with a woman:

(a) If he penetrates his penis up to any extent, in the vagina, anus, urethra, or mouth of a female, or forces her to do the same thing with him or someone else.
(b) If he introduces any object or any body part excluding the penis up to any extent inside the vagina, anus, or urethra of a female or forces her to do the same thing with him or someone else.

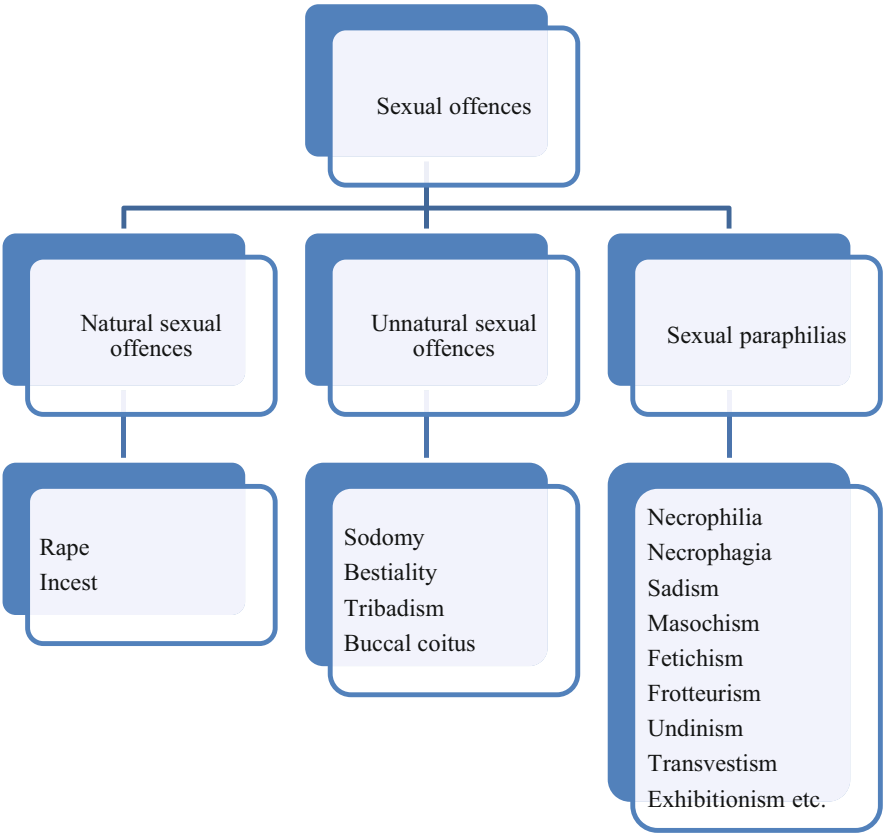

Fig. 9.9 Classification of sexual offences

(c) If he manipulates a woman's body parts to cause any kind of penetration in the vagina, anus, or urethra or any other body part or forces her to do the same thing with him or someone else.
(d) If he puts his mouth on a woman's vagina, anus, or urethra or forces her to do the same thing with him or someone else.

These are the seven circumstances which constitute above conditions of rape:

1. Without her will.
2. Beside her consent.
3. With her consent, but the consent was taken by putting her or someone close to her in threat.
4. By making false belief that they two are lawfully married.
5. With her consent, but the consent was taken when she was in the condition of unsound mind or intoxication.

6. When her age is less than 18 years, her consent is not valid.
7. When she is not able to communicate her consent [24].

Examination of the Victim

1. Firstly the consent for medical examination should be taken from the victim.
2. The examination should be done in front of a female family member or a nurse.
3. The victim needs to be examined only after requisition from the Magistrate or Investigating officer.
4. All information like name, age, gender, parents' name, and date and time of incident should be recorded.
5. The victim should be asked for her last consented sexual intercourse, and time interval from the incident should be recorded.
6. All the information about offence should be asked and recorded like exact relative position and number of assailant, number of sexual act, any drug or alcohol used, details about resistance and struggle, any weapon used, etc.
7. The examination should be done without any delay.
8. The victim should be asked if she took a bath or washed her genitals after rape.
9. The victim needs to be undressed completely, and examination should be done with the help of ultraviolet light for semen stain detection.
10. All injuries need to be recorded at the time of examination. The genitals and thighs should be checked properly for injuries [25].
11. Close-up photographs of injuries should be taken for record purposes.
12. The genitals should be checked for semen, and swabs should be taken for spermatozoa identification.
13. Clothes of the victim should be looked after for seminal or blood stains. Clothes can corroborate her story correctly. Undergarments should be examined properly as there can be semen stains due to drainage from the vagina.
14. The pubic hairs of the victim should be combed to collect foreign hairs. And 20–50 hairs should be plucked out from the victim's pubic area.
15. Nails of the victim should also be collected by cutting them as there can be skin tissues of assailant in nails due to scratching during struggle [26].

Examination of Suspect

1. The suspect examination is much similar to victim's examination. The suspect should be inspected for all the harms which the victim said that she has inflicted.
2. The consent of suspect should be taken, and he must be informed that the report will be submitted to police and courts, and it can be against him.
3. All information like name, age, gender, and personal identification marks should be recorded by investigating officer.
4. The examination of the suspect should be conducted as soon as possible as the marks of act may vanish after sometime.
5. Information like last consenting intercourse, last bath, last changing of clothes, etc. should be recorded.
6. His state of mind and general behavior should be observed and recorded.
7. It should be recorded if he seems under the influence of drugs or alcohol.

8. All the physical injuries should be noted including genital injuries; there can be bruises, abrasions, scratches, etc. Reddening of glans and patchy structure is more common.
9. The suspect's penis should be washed with saline, and the solution should be stained with Papanicolaou's stain.
10. The suspect's penis should be cleaned with filter paper, and the filter paper should be exposed to Lugol's iodine fumes. The filter paper will become brown if vaginal epithelial cells are present as they contain glycogen.
11. Specimens like swabs from the penis for infecting organism and blood, etc., blood samples, pubic hairs, head hairs, any loose hair found on body, and nail scrapings should be collected and preserved [27].

Incest

A sexual intercourse is called as incest when both the male and female are closely related to each other (e.g., sister, mother, granddaughter, stepdaughter, etc.). These are the cases which usually have psychological features [28].

9.2.4.2 Unnatural Sexual Offences

Sodomy

It is a sexual intercourse, when the male introduces his penis inside the anus of a male or a female. Sodomy was used to be practiced in Sodom town. Sodomy is also known as buggery [29].

Bestiality

When a human being performs sexual act with a lower animal, this sexual intercourse is known as bestiality. This includes animals such as dog, cat, cows, etc. [30].

Tribadism

When a female obtains sexual gratification by kissing, body contact, manipulation of genitals or breast, or rubbing external genital organs of another female, it is called tribadism [31].

Buccal Coitus

When the penis of a male is introduced inside the mouth of another male or female, it is called buccal coitus. This act was common in Gomorrah town according to the Bible. In this act, death may occur due to aspiration of semen in the hypopharynx [32].

9.2.4.3 Sexual Paraphilias

Necrophilia

This offence includes sexual intercourse with a dead body. In this condition, the desire of sexual intercourse with a dead body is there. This behavior is

sadomasochistic, and foul smell of decomposition and coldness act as stimulating agents, whereas murder for purpose of necrophilia is exceptional [33].

Necrophagia

When someone obtains sexual pleasure by sucking or licking wound, eating flesh, biting skin, or drinking blood of a dead body, that condition is called necrophagia. This act is an extreme degree of sadism [34].

Sadism

Sadism is active algolagnia in which algos means pain and lognia means lust or craving. When sexual gratification is obtained by infliction of pain or physical cruelty upon one's partner, it is called sadism [35].

Masochism

Masochism is completely opposite to sadism. In masochism, the suffering of pain leads to obtaining sexual pleasure. Masochism is passive algolagnia. People who are masochistic gets pleasure by being humiliated, tortured, abused, beaten, and enslaved by their partner. In a sadomasochistic relationship, one person is dominant over the other being sadistic [36].

Fetichism

When the person obtains sexual gratification by abnormal stimulus or objects, called as fetichism. Those objects or abnormal stimulus which are used to get sexual gratification are called fetish. Those objects can be anything which have sexual influence like underclothing, shoes, petticoat, etc. In this, the person obtains excitement leading to orgasm [37].

Frotteurism

Frotteurism is obtaining gratification sexually by rubbing his private parts on the female's body parts like the breast, buttocks, thighs, etc. in crowded places. People who are frotteuristic think that the victim will not be offended by being touched but instead will get pleasure [38].

Undinism

In undinism, to obtain sexual gratification, someone watches someone else urinating, either of the same sex or opposite sex. There are also some cases in which one gets sexual pleasure by being urinated upon by loved ones [39].

Transvestism

It is also called eonism. When the whole personality of a person is controlled by the desire of being recognized as member of the opposite sex, this condition is called transvestism. It is generally seen in males who use to obtain sexual gratification by wearing female dresses [40].

Exhibitionism

Some people expose their genitals to children or members of the opposite sex to fulfil their fantasies and obtain gratification sexually; this act is known as exhibitionism. This is generally practiced by males who expose their genitals to get attention and feel arousal [41].

9.2.5 Child Abuse

9.2.5.1 Introduction

Child abuse is defined as any harm to children by an adult either physically, mentally, or sexually. Child abuse is a social issue and is happening in the daily life of children. Some of them are aware of that and some are not. There are different kinds of abuse that are tolerated by children worldwide, and in most of the conditions, children are sometimes not capable of understanding the assault and sometimes not capable of complaining about what they are going through [42].

9.2.5.2 Types of Child Abuse

There are different types of child abuse:

- Physical abuse like beating, physical punishment, child labor, and child begging
- Mental abuse like torture and bullying
- Sexual abuse like rape, sexual assault, and harassment (Fig. 9.10)

1. **Physical abuse**: Physical abuse happens when physical injury is inflicted upon the child in different forms. Sometimes, the child is abused by their parents for labor; this mainly happens in cases of poor families. In some cases, the child is exposed to forced begging which is another form of physical abuse. Beating a child and physical punishment are also physical abuse [43].

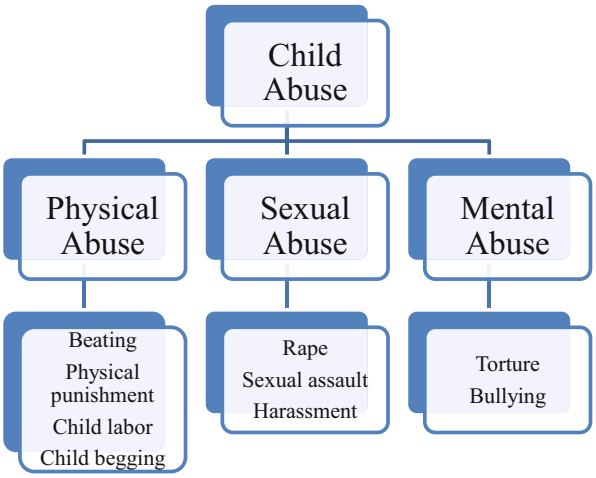

Fig. 9.10 Classification of child abuse

2. **Sexual abuse**: Sexual abuse happens when the child is used for sexual stimulation. There are so many cases of child rapes, sexual assaults, and harassment. It mainly happens because the child is not capable of rejecting or complaining about it.
3. **Mental abuse**: Mental or psychological abuse happens when a child is constantly tortured or bullied by someone. It is a form of abuse that is very hard to identify and has a very bad impact on the mental health of the child which in later life turns into a psychological problem for life.

9.2.5.3 Examination of Victims in Child Abuse

It is very hard to identify the victim in cases of child abuse. There are a few signs and symptoms we can look for [44]:

In Physical Abuse
- Cigarette burns may be present.
- Bruises.
- Fracture.
- Permanent damage to some parts of the body.
- Other body injuries.

In Sexual Abuse
- Physical examination
- Bruises around genitals
- Signs of rape
- Signs of force
- Bite marks

In Mental Abuse
- Fearful
- Hate toward parents
- Emotionally weak
- Difficulty in speech

9.2.5.4 Examination of Suspects in Child Sexual Abuse

In sexual abuse, the examination of suspects is done by a medical examiner for any type of sexual intercourse, and other samples are collected for forensic examination, which help prove the crime [45]. Samples that are collected for forensic examination are:

- Suspect's and victim's clothing
- Swabs
- Pubic hairs
- Nails scrapings
- Other materials like bed sheets, condoms, etc.

9.2.6 School Violence

9.2.6.1 Introduction

School violence is considered a serious issue across the world and more problematic when there is involvement of weapons. The term "school violence" is defined as a violent activity inside the school which involves bullying, physical as well as verbal abuse, harassment, physical violence, shooting, etc. between the students and also often towards the school staff members. The most commonly observed issues with the school violence are bullying and physical abuse [46].

9.2.6.2 Cause and Factors Responsible for School Violence

1. Family environment—exposure to violence over a longer period within the family, domestic violence, parental alcoholism.
2. Child abuse—physical and sexual abuse during childhood.
3. Physical punishment—leading to aggressiveness.
4. TV and video games—may act as triggers.
5. Neighborhood—surrounding environment with drug abuse and criminals is also linked with violent behavior of youths and children.
6. Poverty.
7. Lower academic performance.

9.2.6.3 Behavioral Distinction

The behavioral patterns associated with violence in school or among the youths have been represented in Figs. 9.11 and 9.12.

9.2.6.4 Preventive Measures of School Violence

The prevention of school violence should be done at four levels including:

1. **Family environment**—establishing a healthy environment at home
2. **Society**—reshaping the social norms and educational structures
3. **School community**—re-establishment or amendment in school characteristics, classroom management, supportive learning, and close supervision

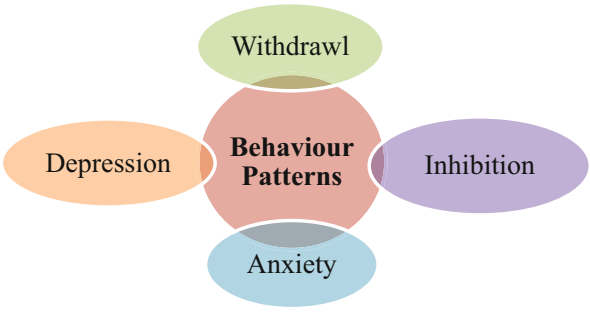

Fig. 9.11 Showing the behavioral pattern associated with school/youth violence

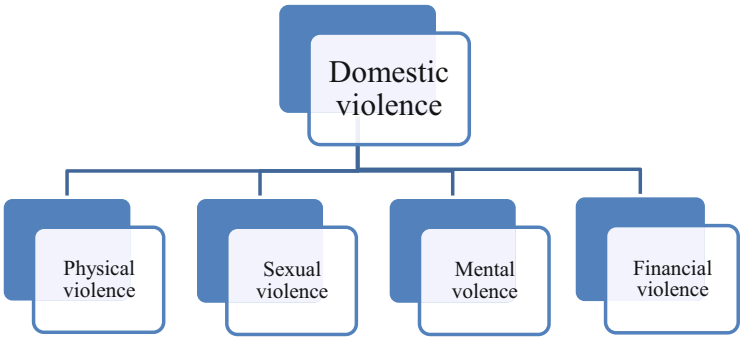

Fig. 9.12 Classification of domestic violence

4. **Individual level**—empathy, impulse control, reducing the level of physical aggressiveness and bullying in a modified curriculum with supporting strategies
5. **Anti-violence program [46]**

9.2.7 Domestic Violence

9.2.7.1 Introduction

Domestic violence is any abuse between partners living in the same place. Domestic violence includes physical, sexual, mental, financial, or any abuse either by a male on the female partner or by a female on the male partner. But in most of the time, the term domestic violence is used for male abuse upon their female partner; it may include any type of abuse [47].

9.2.7.2 Types of Domestic Violence

- Physical violence
- Sexual violence
- Mental violence
- Financial violence

1. **Physical violence**: In most cases, physical violence is done over the female partner. It includes physical assault, killing their partner, or grievous hurt to the partner. It is the most common type of domestic violence and happens in small, poor, uneducated families.
2. **Sexual violence**: Sexual violence is forcing own partner for sexual intercourse. Sexual violence includes marital rapes, unnatural sex, etc. This type of violence is not reported most of the time because of fear of losing a partner and financial support [48].
3. **Mental violence**: Mental or emotional violence is a type of violence in which one partner restricts doing normal things and resort to blaming or accusing the partner. It may include things like preventing from using the phone, using abusive words, etc.

4. **Financial violence**: Financial violence happens when one partner is dependent on others financially, and the victim is not getting enough resources and doesn't have access to money or credit cards.

It also includes demanding dowry from the partner.

9.2.7.3 Examination of Victims in Domestic Violence

Domestic violence victims mainly suffer from physical assault and abuse. The signs and symptoms we can look for are as follows [49]:

- Physical injuries
- Fractures
- Signs of assault
- Weakness
- Ill
- Psychological problems

9.2.7.4 Examination of Suspects in Domestic Violence

In domestic violence, the suspects are mainly one of the partners and maybe their family members.

In cases of sexual violence, the suspects are examined for any rape and unnatural intercourse; it is mainly done by medical examination and forensic examination [50].

In death-related cases, medical and forensic examination is done to examine the suspects.

9.2.8 Pseudo Victimization

There are many cases reported in recent years in which people harm themselves to put allegations on others. The cause of pseudo victimization can be revenge, jealousy, financial gain, or to harm someone's modesty. Pseudo-victimization cases are generally seen in assault cases, either through sexual assault or physical assault like false rape charges. Some plant products are being used like *Semecarpus anacardium* and *Calotropis gigantea* in these kinds of activities [24].

There are some chemicals also which are being used to produce artificial bruises on the body; however artificial and real bruises can be examined and can give accurate results. It can be a psychological disorder also as there is history of repetitive attempts by people. The numbers of cases of false charges are increasing due to interlink between the law and psychology. For example, there are strict rules for domestic violence in India, and the victim needs not to prove that, but the burden of proof lies upon the suspect, and this makes some psychologically ill people put false charges against in-laws. Other examples are false rape charges and general assault cases.

9.2.9 Self-Harm

9.2.9.1 Introduction

[Synonyms – "Self-mutilation," "Self-inflicted injury," "Self-injurious behavior"]

Self-harm reflects an extreme human behavior which defines the disturbance produced due to someone's personal or individual physical integrity and health without any suicidal intentions. Attempts such as biting and tearing out nails, pulling the hair, and making huge demands are outcomes of stress, diseases, or personal or financial needs [51].

9.2.9.2 Characteristics of Self-Harm

1. Mostly observed as cuts and scratches
2. Symmetrical, uniform, and same depth
3. Usually not severe
4. No defense wound/injuries
5. No damage to clothing
6. No injury on pain-sensitive areas

9.2.9.3 Categories of Self-Harm (Fig. 9.13, Table 9.4)

9.2.9.4 Diagnosis of Self-Harm

1. **Whole body examination**—whole body examination is included in the forensic medical physical examination.
2. **Documentation**—documentation of injuries to be done in detail; exact description as possible.
3. **Diagnostic differentiation**—lesion count and types, severity, intensity, structure, size, shape, etc. [51].

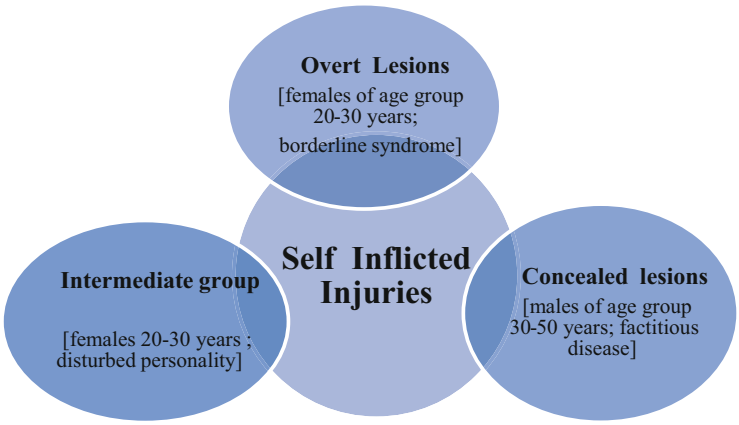

Fig. 9.13 Categories of people showing self-inflicted injuries based on psychological criteria

Table 9.4 Categories of self-harm

General relevance with clinical forensic medicine	More—relevance with clinical forensic medicine
Heredity	Simulation
Ornamental scars/self-injuries	Penitentiary system
Body modification/bod-mod	Military service
Psychological disorders	Insurance fraud
Genital self injuries	Fake criminal offences
Self-burning	Personality disorders

9.3 Conclusion

Forensic pathology, clinical medicine, and forensic pathology such terms are utilized across the world. Clinical forensic medicine is also a branch of medicine which involves living individuals and working together with law, judiciary, and police personnel. In the more extensive sense, clinical forensic medicine implies the use of clinical information and abilities to living people relating to the uncommon necessities of the particular legitimate, legal, and police frameworks. It is significantly important that forensic physicians should be trained, experienced, and qualified. There are several ways of accreditation of forensic physicians. But in some countries, there is not any accreditation system found. There must be introductory training programs for beginners under a qualified supervisor, setting up the standards of quality and service, professional development, etc. When acting as expert witness, they are supposed to present the reports in an adequate and accurate manner without adding any information intentionally which might be harmful for patients; otherwise, they can be charged for their negligence. Trauma and violence both are found as a serious issue to health throughout the globe. New emerging tools and techniques and advancements in the classification and assessment of burn injury and wounds may provide an opportunity for the treatment of burn patients more accurately. The techniques further need more practices and work for clinical applications and accuracy to be tested. The cases of sexual assault are accruing in this new era. Cases of sexual assaults are increasing day by day which are not only happening with females, but the male population is also becoming a victim of it. There are different kinds of abuse that are tolerated by children worldwide, and in most of the conditions, children are sometimes not capable of understanding the assault and sometimes not capable of complaining about what they are going through. There are also many cases reported in recent years in which people harm themselves to put allegations on others. The cause of pseudo victimization can be revenge, jealousy, financial gain, or to harm someone's modesty. Pseudo-victimization cases are generally seen in assault cases, either sexual assault or physical assault like false rape charges. Clinical forensic medicine signifies a new era of medical practice. The new role of forensic experts has been identified as applying the principles, knowledge, and standards in injuries assessment, trauma care, extreme human behavior,

violence, child abuse and its related offences, sexual offences, pseudo victimization or self-inflicted injury, etc.

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Firearms and Ammunitions: A Sentient Approach to Criminal Investigation

10

Sudhanshu Sawhney, Akashra Johri, and Priyanka Chhabra

Abstract

Firearms and ammunition have been in use since 100 AD in China. Since then, their approach and advancement have been taking place with passage of time. With guns, ammo has additionally been created to improve its force and utilization. The most prompt weapons conveyed in Europe during the fourteenth century were firearms and hand guns: clear chambers shut down toward one side except for a little touch—opening drilled into the breech end of the drag. The following century saw the advancement of serpentine (matchlock) weaponry which permitted the mechanical bringing down of a seething nitrated rope or breaker into a container of powder neighboring the touch opening. The sixteenth century saw the advancement of wheel-lock arms which used a serrated iron wheel that was caused to pivot by perfect timing against a piece of iron pyrites to deliver a shower of sparkles coordinated toward the blaze container. These headways sometimes constrained the utilization of these guns and ammo in criminal cases and connected this hardware with forensic examination. In this chapter, various sorts of guns and ammo have been depicted. A legal perspective has been depicted for examination purposes with various impacts on the victim and the suspect. This chapter summarizes the history, development, and future prospective of firearms and ammunition while linking it to forensic aspects.

Keywords

Firearm · Ammunition · Examination · Advancement · Forensic investigation

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10.1 Introduction

Guns, rifles, shotguns, and furthermore automatic weapons and other military gunnery are gadgets which hold a shot or shots that can be dispatched or projected with an incredible power. These firearms are also encountered in some very uncommon violent criminal activities like murder, burglary, police encounters, terrorist activities, homicides, and suicides. These are guns that can be carried easily. Ammunition is any material that is fired or detonated. It can be fired by any weapon. Ammunition can be classified into either exploding type (bomb, missiles, grenades, land mines) or a component of firearm (bullet and warheads) [1]. Guns are utilized in the desperate shooting, usually found in Western nations and in India as well; of late, covert transfer of complex guns has utilized guns in a wide range of cases. Therefore, the number of cases has increased in ballistic division. Ballistics deals with the examination of firearms and ammunition used in committing crimes for investigation and identification. Firearms evidences are very important in criminal investigations and trials. Identification of firearms is pertained through ammunition fired by the particular firearm. It is like finger impression acknowledgment; the imprints made on the cartridge or on the slug by the gun used to shoot the shot are perceived all around. Declarations made by specialists are correspondingly acknowledged as the statements by specialists for fingerprints are excepted. The given affirmations express that no two guns even of a similar make, model, or group made comparable imprints on the discharged ammo like no fingerprints are comparative left by an individual's two distinct fingers. The shot is discharged by the power applied which makes an extension of gases which brings about the copying of the powder charge. A barrel, activity, stock, terminating pin or striker, breech power, chamber, extractor, and ejector are the pieces of gun gathering. The gun has a barrel, an activity, and a stock. The barrel or chamber gives development of gases. Activity is the system of stacking, extraction, and discharge of the cartridges, magazines, and other well-being gadgets. Stock keeps parts in position and provides support for firing purpose. Older firearms usually used black powder as a propellant but modern firearms use smokeless powders. Comparison of firearms and ammunition using comparative techniques like microscopic analysis is very helpful in connecting crime with the individuals. The evidences often found on the crime scene provide aid to recreate the crime scene with the help of firearm discharges and leftover ammunition casings. Also, the firearms left behind may contain some fingerprints which are also considered and recovered for investigations. Gunshot residue (GSR) is also one of the considered factors for investigation of crimes. Wounds formed by firearm can relate the particular firearms. Crime branch laboratories and police agencies with Integrated Ballistic Identification System (IBIS) are very significant in performing automated search for matches for ballistic evidences. But still manual confirmation using microscopic comparison is trusted for firearm examination. These ballistic evidences are most trusted physical evidences to the crimes related to them.

10.2 History of Firearms

The concept of firearm started from the tenth century with Chinese fire lances. It was not a car but an addition to the soldier's spear [2]. A barrel of paper or bamboo was filled by gunpowder within it that could be lit once to fire projectile at the enemy. Later, the barrel was made of metal and capacity of holding more gunpowder. Another predecessor of firearms was hand cannon that was also loaded with gunpowder. It had fuse in place of rear which was lit causing to ignite the gunpowder and led the cannonball to propel. Hand cannons were replaced by lighter carriage-mounted artillery pieces and ultimately by the harquebus. During 1420s gunpowder was used to propel missiles from handheld tubes during the Hussite revolt. Musket, a muzzle loading, was among the first firearms to develop. The musket was loaded through muzzle with gunpowder or wadding and then a bullet. Muzzle loaders have to be manually reloaded after each shot. Most of the early firearms were muzzle loading. It had slow rate of firing and slow reloading. The loading was done through muzzle and the weapon had to be pointed upright to pour the powder through muzzle. As an effective method of sealing the breech was developed. The weathered proof, self-contained metallic cartridges, and muzzle loaders were replaced by single-shot breech loaders. Further repeater-type weapons replaced single-shot weapons. Internal magazines were used in firearms made in 1950s during the nineteenth century to load cartridges into the chamber. Spencer and Henry repeating rifles were most revolutionary weapons evolved during the A.S.C.I.I. war. These used fixed tubular magazines. It was placed under the barrel but before it was at the buttstock of the firearm. Fixed magazines allow the use of larger cartridges. They were inserted using stripper clip which was used to transfer cartridges into magazines. Mosin-Nagant, the Mauser Kar 98k, the Springfield M1903, the M1 Grand, and the SKS were the first notable mostly used weapons. Internally magazine firearms, such as the Mauser C96 handgun, were usually but not invariably rifles. Detachable magazines were employed in lateral guns. Lateral firearms used detachable magazines. They were removable from the weapon without disassembling the weapon by pushing it to release from the weapon. After more advancement, ammunition belt feed was used to feed firearms. Composition of belt was formed of canvas or cloth with pockets evenly spaced which allowed belt to feed firearms mechanically. These were later on prone to malfunctioning due to the effect of oil and other contaminants. Designs were improved to permanently connect metal that was made tolerant to exposure to solvents and oils. The M240 and the M134 Minigun were few of them (Fig. 10.1).

10.3 Historic Examination of Firearms and Ammunition

In the sixteenth century the invent of rifling in firearms created an idea of the ability of ammunition to be compared. This was not the thought which focused while rifling but only the purpose of rifling was to create accuracy of bullet with respect to target while travelling from the barrel. This gradually created an idea of being utilized for

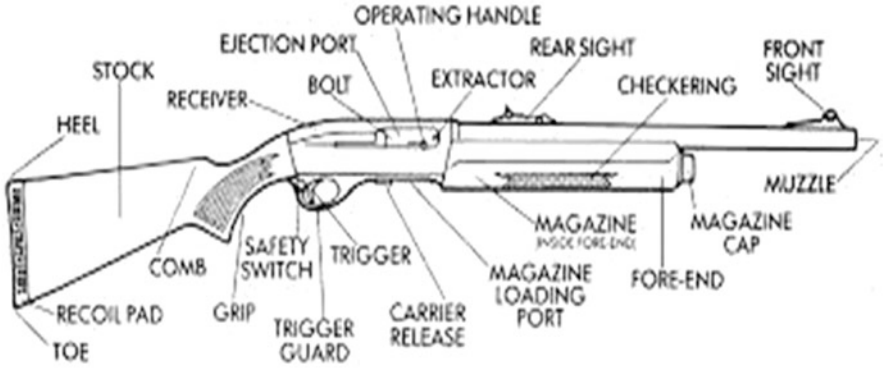

Fig. 10.1 Different parts of rifle

the purpose of comparison. It would help in relating firearm through which the ammunition had been hurled. The marks created by rifling on the bullet or ammunition indicate the particular barrel of firearm. Gunsmiths used to make unique barrel and bullets which were hand made. In 1835, the first firearm examination took place with successful documentation. It was first documented by a person who was a member of Bow Street Runners in London. He matched a recovered bullet from a victim of murder case, in a mold to confirm the making of a bullet, and found that the person who made the bullet was a perpetrator and he was convicted. With the advancement in manufacturing and automation, the comparison was made difficult. But examination experts postulated microscopic manufacturing differences in barrels which results in different individual identification marks on the fired bullet. This was used for investigation and matching a particular bullet to a barrel through which it was fired. In 1915, Calvin Godard and Phillip O. Gravelle modernized the firearm examination by invention of comparison microscope. It made it easy by simultaneous comparison of two different objects at the same time for the striations present on the bullet and to conclude whether they are matched or not. The implication of this technique resulted in solving the case of Valentine's massacre in 1925, when Godard was asked to collect and examine the evidences to discriminate the killings by the North-side gang or by the police. The examination of the evidences resolved the case, by new ballistic-forensic technique using test fire examination. After this successful testing, Godard was solidified as the father of the firearms examination.

10.4 Ballistics and Its Parts

Ballistics can be further divided into three different parts depending upon the projectile movement, target, and its motion.

(a) Internal ballistics

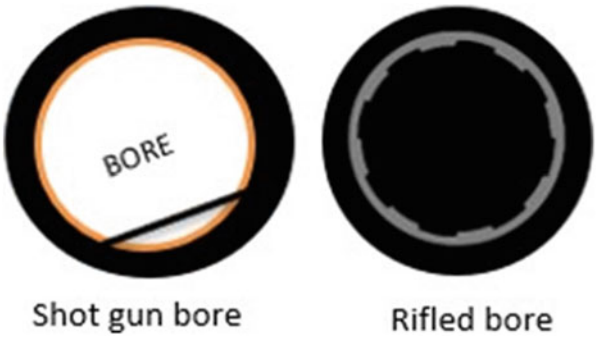

Fig. 10.2 Internal characteristic of shotgun and rifle

Internal ballistics is identified as the study of movement of the shot from the time the trigger system is actuated till it leaves the muzzle end of the weapon. Internal ballistics overall studies the burning of propellent and nature of the projectile after the gas pressure is formed in the barrel (as shown in Fig. 10.2).

(a) External ballistics
External ballistics manages the shot movement from the muzzle end till it contacts the person in question. External ballistics manages movement, soundness, air opposition, and gravitational draw impacts on the shot [3].

The exact trajectory of the projectile can be known by the below points:

1. Shape of the bullet
2. Velocity of muzzle
3. Gravitational effect
4. Barrel-handling angle

(a) Terminal ballistics
Terminal ballistics deals with the impact caused on the surface of contact. Terminal ballistics is also known as wound ballistics.

There are three ideas by and large held by most regarding the impact of a shot striking an individual. The first is that the projectile "drills" its way through leaving a little section and a similarly little leave opening. The second is that the projectile leaves a little passage opening and a huge leave opening. The third is that when somebody is shot by something besides an air rifle, the effect is sufficient to lift the individual off his or her feet and send him or her flying through the air. Essentially, each of the three ideas are mistaken somehow.

At the point when a shot goes through a living tissue it makes two kinds of wounds: temporary injury and permanent injury. The energy bestowed by the projectile discards the tissue and these tissues' structure briefs injuries which are reestablished because of their flexible nature. Temporary injuries shaped are bigger in distance across than the slug size (as shown in Fig. 10.3). Though the perpetual injuries are shaped by the actual projectile, these injuries are framed on shot track which it covers in the body.

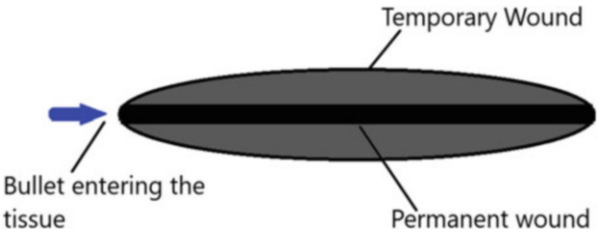

Fig. 10.3 Diagram of temporary and permanent wounds

10.5 Examination of Firearms

When firearm is found at the crime scene it makes it easy to carry out different examinations to recover different evidences. Mostly the specific evidence includes recovery of serial numbers of weapon and fingerprints on firearms. These are very helpful in connecting weapon with criminal. Fingerprints are already known for their individuality and universal identification characteristics. So, in firearms examination it serves as one of the tools. It can relate the criminal to the crime with minimum percentage of error. The method generally used superglue, which is a cyanoacrylate fuming on the surface of the firearm. The firearms are placed in the fume hood where the superglue adheres to the fingerprints in the form of fumes. These are evenly distributed as a result of which it turns the prints white. Later on, these white prints can be treated with fingerprint powders for the enhancement. Sometimes it is difficult to recover prints from the surface of the firearms because of the textured grip and condition in which it is found. Various parts of the firearm can also be examined for the touch. Different firearms have unique and different serial numbers. It indicates the manufacturing and model of the weapon. If the serial number of the weapon is altered or destroyed, it can be recovered. The methods used are magnetic particle inspection and chemical restoration. The magnetic particle inspection uses deformation of the irregularities on the weapon formed due to magnetic resonance. Then the ferrous sulfate binds to the deformed magnetic area and further fluorescent particles can be added for enhancement under UV light. Chemical restoration uses chemical milling. The desired shape could be created. It is a process of removing the small amount of metal corresponding to the serial number that is visible. But it is only applicable to superficial obliterations. Then examiners usually choose an acid that will be used to bring the numbers back slowly. For magnetic materials, Fry's reagent is used and for the non-magnetic material acidic ferric chloride. Magnetic particle inspection was used due to its nondestructive nature, but in case it fails chemical restoration is used for restoration of the serial number. The benefit of recovering the serial number is that it is very useful in tracking the history of the weapon and determination of the owner of the weapon.

10.5.1 Examination of Ammunition

Other than whole weapon, bullets and cartridges are most commonly found physical evidences. They can be examined for the comparison and **fingerprint** recovery. Cartridges are used for the examination of unique tool marks like firing pin and ejector marks. These are used for the comparison to know whether the same weapon is used for firing or not. It is done using comparison microscope. Questioned cartridges are compared with the exemplar cartridges for finding if there are any similar marks left during the process of firing. The cartridges are also examined for the recovery of fingerprint left during loading the ammunition into the magazine or chamber. Cartridges can also be swabbed for the DNA left. Bullet is found penetrated into the wall, furniture, or floor at the crime scene or it can be extracted from the body of the victim. It is generally found in damaged condition but if it is handled carefully, lots of information can be extracted out of it. It is best to compare with class and individual characteristics. Class characteristics are same on the bullet fired from same make and model: the number, width, depth, direction and pitch of lands and grooves of the barrel, caliber of the bullet, and rifling twist. These characteristics can be used to link the firearm by which the bullet is fired. Lands and grooves are created when the rifling is made, and direction of the striations is the marks or twist of rifling left in the barrel. The diameter of the barrel is the caliber. More finer details on the bullet contribute to the individual characteristics. They are different and can be never same. It determines the weapon that was used for firing a bullet. The bullet and cartridges found at the crime scene are the required exemplar to be compared. Integrated Ballistic Identification Network (IBIN) and Ballistics Intelligence Service (BIS) record the databases of the compared marks like striation patterns (Fig. 10.4).

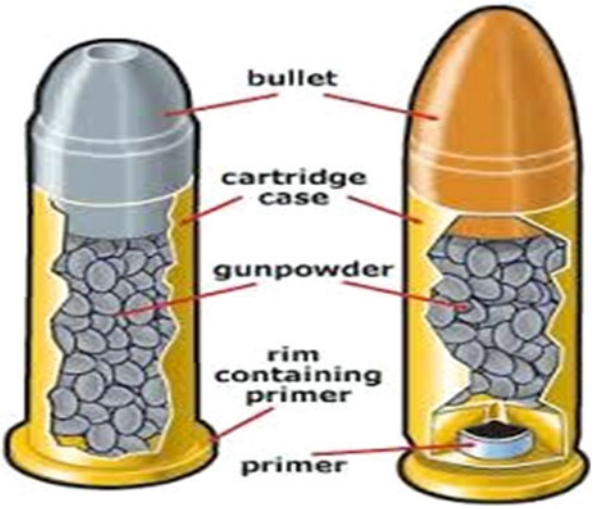

Fig. 10.4 Different parts of cartridges

10.6 Primers and Propellants

10.6.1 Primers

In guns and big guns there is the synthetic as well as gadget liable for starting the force burning that will push the shots out of the weapon barrel. In more modest weapons, the groundwork is generally of the main kind and coordinated into the foundation of a cartridge. Primers are the chemical substances which are responsible for creating combustion and igniting the propellant so as to push the projectile out from the barrel.

In 1807 a Scottish clergyman, James Forsythe, found the stun delicate dangerous called mercury blast, HG (ONC). This sort of touchy will explode on the off chance that it is struck or stunned. A sparkle will likewise set if off. By 1850 cartridges were being made that contained mercury explode inside the top of the cartridge as the groundwork. Toward the start, the groundwork was embedded inside the edge of the cartridge. A little pin projected from the rear of the edge. At the point when this pin was struck by the mallet, it struck the preliminary, exploding it. The explosion made the powder inside the cartridge touch off. By 1850, this framework was supplanted by a less complex one wherein the preliminary was embedded into a small cup inside the focal point of the cartridge head. The terminating pin was mounted on the finish of the mallet. At the point when it struck the cup of groundwork, it packed the preliminary and exploded it. The fire created by the explosion got away through an opening in the cup and ignited the fuel. After some time, the structure of preliminary changed, first by potassium chlorate (KCIO) and today by a combination of lead styphnate, antimony sulfide, barium nitrate, and tetracene. At the point when shot buildup is investigated from the hands of a shooter, the analyst searches for particles of antimony, lead, and barium from the groundwork.

10.6.2 Cartridge Types

There are three different types of cartridges depending upon the primer arrangement:

10.6.3 Pinfire Cartridge

A pinfire cartridge is an old kind of metallic gun cartridge where the preparing compound is touched off by striking a little pin which projects radially from simply over the foundation of the cartridge.

10.6.4 Rimfire Cartridge

Rimfire ammo is a kind of gun metallic cartridge whose preliminary is situated inside an empty circumferential edge distending from the foundation of its packaging. At

the point when discharged, the weapon's shooting pin will strike and squash the edge against the edge of the barrel breech, starting the groundwork compound inside the edge, and thusly light the fuel inside the case. The edge of such cartridge is basically an extended and smoothed end part of the case, and the preparing compound is filled from inside into the box hole inside the edge. The case is then loaded up with force powder and closed by the shot. Rimfire cartridges are restricted to low pressing factor types since they require a slim case with the goal that the terminating pin can pulverize the edge and touch off the preliminary (as shown in Fig. 10.3).

10.6.5 Centerfire Cartridge

A centerfire cartridge is a gun metallic cartridge whose preliminary is situated at the focal point of the foundation of its packaging. Not at all like rimfire cartridges, the centerfire groundwork is regularly a different part situated into a recessed pit for the situation head and is replaceable by reloading.

Centerfire cartridges have displaced the rimfire assortment in everything except the littlest cartridge sizes. Most of the present handguns, rifles, and shotguns use centerfire ammo.

There are two types of centerfire cartridges:

- Berdan
- Boxer

The two preliminary sorts are practically difficult to recognize by taking a gander at the stacked cartridge; however the (at least two) streak openings can be seen inside a terminated Berdan case and the bigger single opening seen or felt inside a terminated Boxer case (as shown in Fig. 10.5).

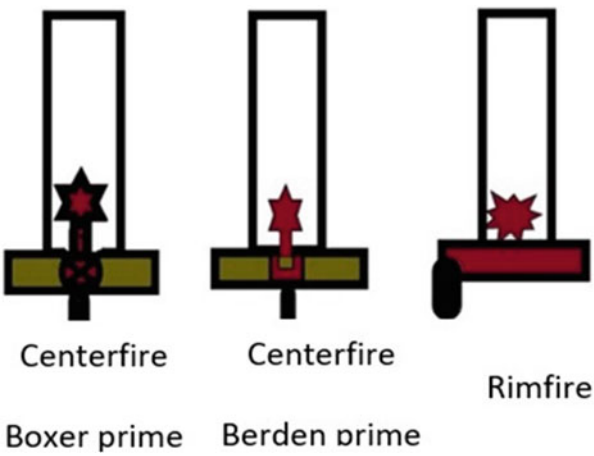

Fig. 10.5 Comparison of centerfire and rimfire

10.6.6 Ricochet

When a bullet is fired from a firearm, it either penetrates the target or changes the direction after an impact. Therefore, ricochet is the change of angle of the projectile after an impact.

10.6.6.1 Impacts of Ricochet

Aside from the reality of adjusting the course of movement of the shot the frequency additionally goes through a changing point of angle of incidence. The direction of slug gets changed after the ricochet and the rocket will lose a lot of its energy which be anything dependent upon 33% of its all-out energy. It will lose its gyroscopic steadiness and tumble in the wake of ricocheting. The reach will diminish which is in opposition to mainstream thinking that a ricocheting projectile will convey farther than one terminated at the rise for greatest reach [4]. The shot may convey some material from the outside of the objective after ricochet. Wounds brought about by ricocheted slugs are curious and of surprising shapes because of various places of striking projectiles subsequent to ricocheting which can be nose forward, side ahead base forward, or some other position.

10.7 Classification of Firearms

Firearms are used for different purposes rather than criminal uses on the basis of different characterizations like their size, handling, uses, rifling, and the ways they are loaded. This classification makes it easy to understand what type of firearms are used according to the requirement. Rifled bore is the gun with lands and grooves made in the barrel. The rifling is responsible for the stability of the bullets when fired from the barrel. This increases the range and accuracy of the aim. The canal shaped grooves are made with the number of twists. The depth and width, number of twists, and angle of twist vary with manufacturing. The lands are the raised portion between the grooves corresponding to the number of grooves. The projectile when passed through the barrel rifling provides it with the class characteristics and more finer details are counted as individual characteristics. It includes pistols, revolvers, sporting rifles, service rifles, and machine guns. Smoothbore includes the weapons like signal pistols muzzle-loading guns, the musket, it additionally incorporates the greater part of the improvised firearms. The barrel does not have grooves and lands. Because of the smooth cross section of the barrel, the projectiles used are generally pallets and shots or it uses rifled balls and slugs. Very few shotguns are known with the rifling with two shallow grooves, which are rare. The shots fired cover a wide area as they spread when fired. The diameter of the shotgun defines it. The size of the balls is correlated for the measurement of the bore. If the lead ball weighs one-twelfth of the pound the gun is 12 bore shotguns (Fig. 10.6).

Firearms can also be classified on the basis of their uses. Except for the criminal purpose the firearms are used in sports like shooting sports, which generally involve shotguns. Other than sports, they have great significance in military. Machine guns

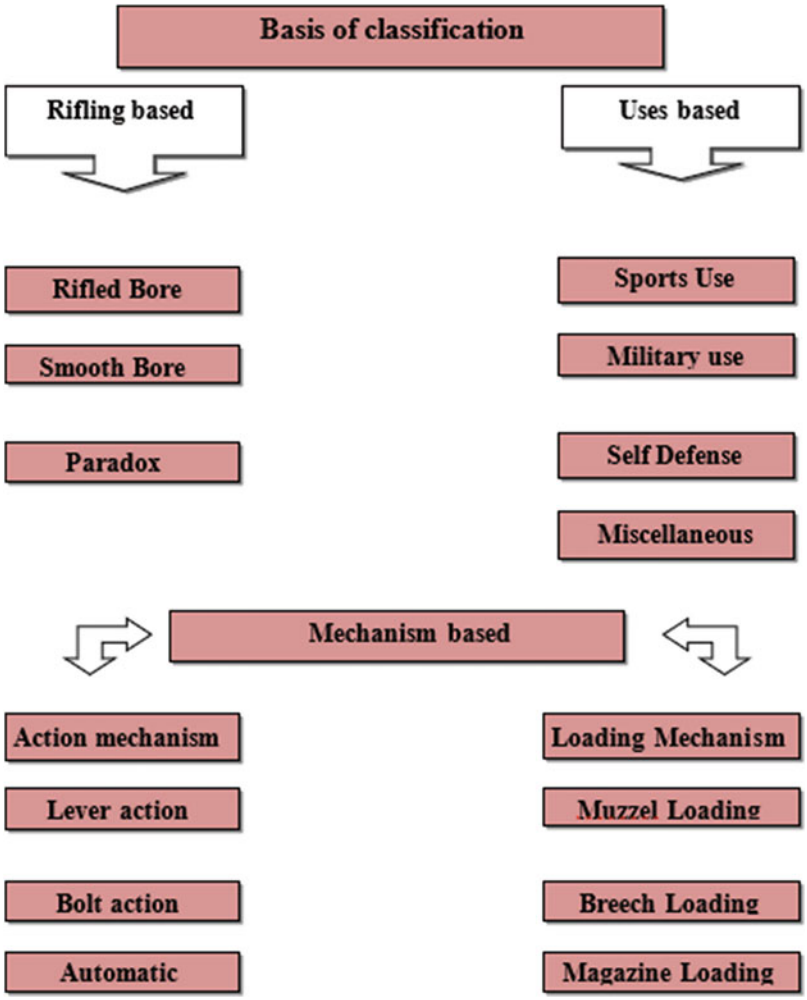

Fig. 10.6 Classification of firearms

and many other automatic and semiautomatic firearms are used in defense system. They have quick applications and accurate aiming. Self-defense arms like air guns and revolvers are used. The firearms have good recognition specially either in defense or in criminal activities. The mostly used arms in criminal activities include improvised firearms or generally known as country-made firearms. They are also known as zip guns and pipe guns. Their manufacturing does not involve any standard materials and are made by ordinary blacksmith. Most of these are extremely dangerous. The readily available materials are used for their manufacturing. In India, these improvised firearms are at high demand, including in states like Bihar, Uttar Pradesh, Chhattisgarh, and many others. The demand of these firearms is regulated

by many localized criminals as they cannot afford costly manufactured standardized firearms. Repeaters utilizes launch, extraction and further reloading is done in single manual operations. The arms that belong to this class are some shotguns and old bolt action service rifles like 303 rifle. Loading methods vary in different firearms. In case of self-loaders or semiautomatic firearms like pistols and modern service rifles, when fired, the fresh cartridges from the magazine are automatically loaded and the used cartridge is ejected automatically and ready for the second shot. The ejection, extraction, and reloading are repeated until the magazine is exhausted. Submachine guns, machine guns, and some pistols are fully automatic and have a quality of self-loading as well as it goes on firing the first shot as long as the trigger is kept pressed till the magazine is exhausted. Assault rifles can be both automatic and semiautomatic. They are the most misused weapons by terrorists around the world. AK 47 and AK 74 rifles are from this class. According to their loading ability some of the firearms are also breech loading, muzzle loading, and magazine loading. Breech loading and muzzle loading firearms are rarely used, due to advanced loading methods.

10.8 Shotguns

A fired firearm is a smooth bore weapon. Width is measured utilizing a bundle of unadulterated lead which finds a way into it precisely and gauges 1/12 of a pound. It is known as a 12-bore fired firearm. Barrel length is little when contrasted with that of a rifle and there might be two barrels next to each other, or one over other. Scope of shot is more modest when contrasted with rifled one.

10.9 Rifled Weapons

Aside from smooth bore fired firearms there are a few weapons which do not have smooth bores however: rifled barrels like rifles, guns, and different assault rifles. All rifled guns shoot a solitary projectile at a time. As their barrels are longer they can fire relatively powerful ammo. The rifled barrels are scored. The depressions are cut as twisting from inside the drag. The winding score is gotten by steadily and consistently turning the cutter during the way toward cutting the depressions. This turning is called bend and point of turning is called pitch.

10.10 Pistols

The magazine of a gun is encased in its hold. Guns are accessible in various types going from around 5 to 12 mm. Both Colt-type and Smith & Wesson-type guns are accessible. For the most part, guns are self-loader or self-loader types in which pulling of the trigger will bring about the discharging of a cartridge. Launch of the terminated cartridge case just as reloading a new live round is consequently prepared

to fire again on pulling the trigger a second time by the usage of a release. 7.65 mm and 9 mm are instances of automatic guns in which one draw of the trigger will make the gun magazine void consequently as one discharging is trailed by the other inasmuch as cartridges are accessible.

10.11 Revolver

Revolvers have a spinning chamber so they are known as gun pistols. Notwithstanding, guns have numerous seminaries just as contrasts with guns. Guns having barrels with six terrains and six scores and left-hand rifling turn are known as Colt type and that one which has five grounds and sections and right-hand contort is known as Smith & Wesson type. There is some space between the barrel and the rotating chamber which is liable for spillage of gases and powder deposits there by lessening its viability. Some of the powder released while firing may be found on the suspect weapon. Pistols are single activity just as twofold activity as single action revolver: In single-activity revolvers, the hammer is positioned manually, which rotates the chamber to bring one of the chambers in accordance with the barrel. The squeezing of the trigger is to deliver the mallet for terminating.

10.12 Crime and Investigation

Physical evidences whether visible or invisible play a very important role in criminal investigation. Finding such evidences like ammunition and firearms at the crime scene adds to an aid in crime investigation. Shotguns, rifles, pistols, revolvers, and country-made firearms are preferably used for committing a crime. National Crime Records Bureau (NCRB) of India published statics in 2013 according to which total 7179 people were murdered using licensed and unlicensed firearms [5]. The rate of committing crime is increasing simultaneously. The crimes committed can be a result of psychological effects, intentionally committed or accidental crimes. The firearms and ammunition recovered as an evidence can answer many questions regarding crime [6, 7]:

- Who committed the crime?
- Why it was committed and what were the reasons?
- How was it committed?
- What was the tendency of crime?
- Was the person directly involved?
- What was the psychological condition of the criminal and victim?
- How many people were involved?
- And most specifically what helps to determine the make and model of the weapon?

The sources that carry firearms evidences are the victim, the culprit, the scene of crime, the firearm, and the ammunition.

Projectiles, bullet holes in the body and clothing, deposits and imprints on cloths and skin due to hot gases or fired residues are all examples of firearm injuries in the victim body.

The culprit may carry primer and powder residues. The deposits and residues help in the establishment of culprit with the firearms.

The scene of occurrence carries each and every evidence including the firearms and the fired projectile. The most significant evidences related to firearms cases can be extracted from the scene of occurrence [8–10].

The firearms and the ammunition are the most important evidences which directly relate to the crime. It is not left behind until the case is to be shown in favor of the criminal. If the firearm is left behind it shows that the crime was planned to show the case as suicidal or accidental [11]. They carry fingerprint and other identification marks. Ammunition provides solid material to be tested. It is generally recovered from the house of accused or from the dealer from where the ammunition was purchased [12]. Fired bullet and cartridges can be recovered from the victim or scene. The thumbprints present on the ammunition provide link to criminal.

10.13 Types of Crime

The firearms are used in fatal and nonfatal crimes. The frequently occurring crimes that use firearms are:

- Homicides
- Suicides
- Accidental cases
- Rape cases
- Robberies
- Dacoit cases
- Illicit trafficking of firearms and ammunition
- Revenge and conspiracy

Cases of the homicides, suicides, and accidents are the most commonly happening activities. There are different reasons which give rise to these cases [13]. The criminal tendency of a person depends upon the psychological or physical conditions [14]. The physical conditions refer to the economy, physical health, and state of one's relation and psychological conditions refer to the general mental health and the mental tendency of the person to take revenge [15]. Rape cases, robberies, and dacoit cases are committed usually on the gun point. Illicit firearms and ammunition trafficking is vulnerable among many Gulf countries and other countries. It involves the trafficking of mostly small arms used in guerilla warfare. In India, states of Punjab and Jammu and Kashmir are particularly vulnerable to arms trafficking [16]. Under 1959 Arms Act, this illicit trafficking is punishable. According to the Firearm

Act 1959, it is also not permitted to keep any arms without license except low-powerful air gun [17].

10.14 Ballistic Examination of Firearm Evidences

Arms and ammunition can be recovered live and fires can be examined for different purposes. These play a significant role in extracting crime-related information and also convincing the court. It is essential to know how and where the evidences can be recovered, how they can be examined, and what these evidences indicate. The ballistics is divided into specific areas of external ballistics, which deals with all the evidences found at the crime scene, and internal ballistics or wound ballistics, which deals with the firearm injuries and also the trajectory of the firearm projectile [18, 19].

Live or fired ammunition like cartridges, bullets, shots, slugs, and pallets are examined for their class and individual characteristics. This examination can link the ammunition to the firearm they are related to. The firearm evidences may also include the penetrated target like glass panels, furniture, and cloths [20]. They can be examined for the patterns formed by fired projectiles and also for the gunshot residues (GSR) [21]. Damaged cartridge cases, damaged bullets, burnt bullets, cartridge cases, smooth barrel marks, buck shots, and pallets are some of the odd identifications that can be used for the identification of the kind of firearms used for firing them [22]. Gunshot residues (GSR) are produced during the firing of a cartridge. It is formed of barrel scrapping, propellants, and primer mixtures. It can be found on the hands of the person who fired a gun. It is found on the target around the projectile holes, including cloths and exposed or affected skin. GSR is found on the inner and outer surface of the firearm and the fired cartridge cases and the projectile involved, and on intermediate targets and articles around the target and the shooter. This can be detected using soft X-ray radiography, dermal nitrate test, Walker's test, Harrison and Gilroy's test, Price's spot test, and scanning electron microscopy [1]. Firing range is one of the important aspects of the forensic ballistics. For the determination of range of fire muzzle pattern, scorching (Fig. 10.7), blackening, tattooing, powder residues, ward distribution, pallet pattern, metal particles, and direction of wound are considered [1, 23]. Firearm injuries are examined by medical doctors and evaluated by firearm experts. Correct evaluation of firearm injury is very effective in the reconstruction of crime scene [24]. It has three parts: entrance wound, exit wound, and internal wound. The shape of wounds may differ; they can be oval, circular, key shaped, ragged, or explosive. They can be of smaller diameter than the diameter of projectile or sometimes have equal diameter or even larger diameter. Exit wounds are often irregular or sometimes circular but dimension is larger than that of entrance wounds. Internal wounds are zigzag due to deflection of projectile or may be multi-channeled due to fragmentation of projectile. The wounding effect of projectile depends upon the target, the velocity, the constructional features, and the range [25, 26]. The threshold velocity of projectile in the skin is 40–50 meters per second and 60 meter per second for the bone penetration.

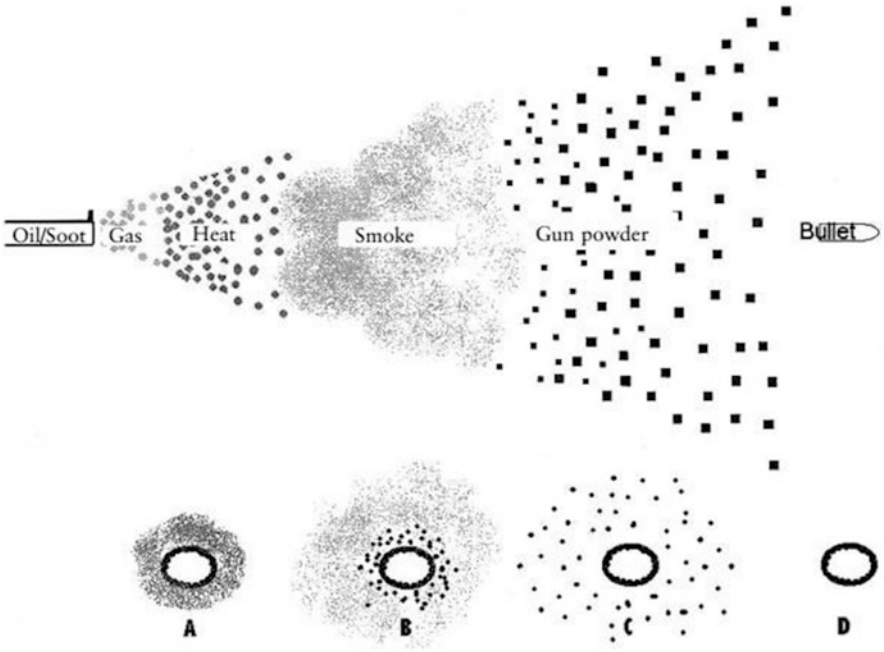

Fig. 10.7 Particles from firearm

Firearms can be identified either from the extraneous deposits or from the characteristic damage produced by shot. Antemortem and postmortem injuries are examined to strengthen the evidences. Moreover, in recent years with more advancements it has been discovered that the DNA can be obtained from the ammunition that remain unfired or casings that remain after the firing process. DNA can be obtained by swabbing and tape-lifting and can be treated with phenol:chloroform for the extraction, silica-based method, and PCR (Fig. 10.8) [27–29].

10.14.1 Range of Fire

Range of fire is the absolute distance between the muzzle end of the firearm and the victim or the target. Determination of range of fire is an important aspect in the field of forensic ballistic investigation. It can be very much helpful in determining the height of the culprit and distance of fire, and gives a relevant connection with the plea of self-defense.

The range of fire can only be estimated if the ammunition or suspected firearm is present, or by observing the type of the wounds, or with the use of crime scene photographs.

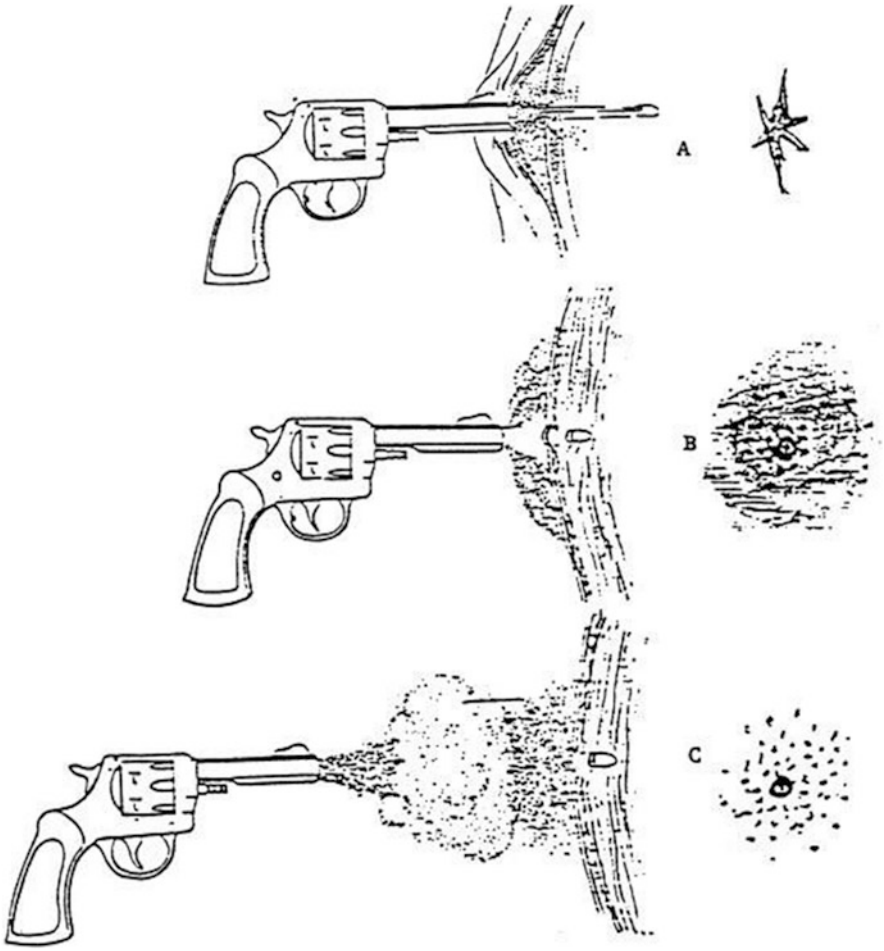

Fig. 10.8 Gunshot impacts

There are many items which can be found at a crime scene or victims' body that can be further used for the determination of range of firearm and some of them are:

- Muzzle pattern/contact firing
- Powder patterns
- WAD distribution
- Pellet patterns
- Details of entry and exit wounds

10.14.2 Muzzle Pattern/Contact Firing

When the muzzle end of the weapon is pressed against the human or animal skin and fired then it leaves a print on the skin. This impression is known as muzzle pattern. The arrangement of muzzle pattern is because of the gases that do not get adequate room to grow inside the objective, and consequently they come out and tear the skin making an opening and framing a star-formed injury with unpleasant edges. When range of firing is zero then none of the pattern is formed on the contact skin [30].

In this case of muzzle pattern, it can be drawn that it could be a case of homicide or when a sleeping person is killed and the ammunition is kept nearby the victim at an adequate distance to deceive the investigating agency [31].

10.14.3 Powder Patterns

Whenever a firearm is fired it releases unburnt, burnt, partially burnt, or black powder from the muzzle end. These particles can be found on the victim's body or nearby area and on the body of a victim it forms majorly three types of patterns (as shown in Fig. 10.2):

(a) Burning
(b) Blackening
(c) Tattooing

10.14.4 Burning

Burning is caused due to the hot flame gases produced when projectile leaves the muzzle end. Therefore, burning denotes the close range of fire. When hot flames are released from the muzzle, it consists of some gases which come in contact with the air, resulting in scorching of skin.

The range of burning depends upon the length of the barrel as well as the gases produced. In hand revolvers the range is for about some centimeters and similarly in case of rifles it is for some decimeters.

10.14.5 Blackening

The deposition of smoke particles in close reach between the entry wound is present in all kinds of powders. That intensity of blackening is maximum in the case of gun powder, less in semi-smokeless powder, and least in smokeless powders with equal quantity and same range. Since smoke particles are light, they lose their speed and are deposited in the material on the way. The range is nearly 2 times the range of burning. Blackening can be separated from burning as it is possible to erase blackening by wipe but this is not the case for burning. In different powders, the

shade of blackening is not the same. For gun powder it is black but for smokeless powders, it is greyish black.

10.14.6 Tattooing

Tattooing is the embedding of burnt, semi-burnt, or unburnt particles in the surface of the skin, hence making a tattoo-like print on the skin. These particles are heavier in weight and that is why they cover more distance. Each force of tattooing can be dictated by the nature of the powder charge (as shown in Fig. 10.9).

Tattooing is also known as peppering or stippling.

10.14.7 Wad Distribution

Various wads are utilized in a fired weapon ammo to keep the fuel and shot charge in position. In the cartridge implied for guns and rifles conventionally no wads are found as cartridges do not have any wads in them [32]. Wads play out the capacity of fixing the barrel to forestall break of gases yet they are projected alongside the other charge. The wads enter the objective up to 3 meters in the event that they are not impeded, and they dissipate up to 5 meters or more. Distance between the person in question and wad when known and the scope of terminating can be discovered. Assessment of scope of shooting should be possible better by test discharging with the speculated weapons. The climate and wind may change places of wads. Henceforth test terminating technique can give all the more precisely the terminating range.

10.14.8 Pellet Patterns

Pellet patterns are majorly seen for the shot guns; the area covered by the spread of pellets is directly proportional to the distance between the muzzle end of the shot gun and the target or the victim.

The spread of pellets depends upon three major factors:

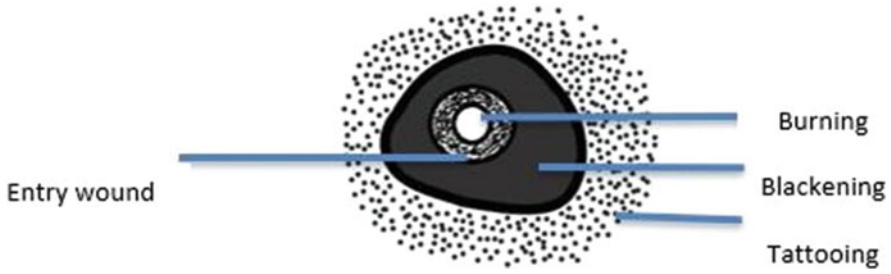

Fig. 10.9 Powder patterns

- Length of the barrel
- Condition of the firearms

10.14.9 Gunshot Residue (GSR)

GSR also known as gunfire residue (GFR) is a residue deposited on the person who handles the firearms; it majorly consists of various discharges such as unburnt particles, burnt particles of propellent or a primer, and cartridge case fragments.

GSR can be majorly found at spots if we see through the forensic aspects:

- At the crime scene
- On the body of the suspect
- On the cloth of the victim

Gunshot residue can act as a game-changer evidence, as it links the suspect, victim, and crime scene. When a person handling the firearm fires the projectile due to the recoiling of the gun the propellent powder or the primer blows from the gun toward the handler. Therefore, traces of GSR can be found on the hands of the suspect and even on the clothes [33].

But if see the victim's area, on which the projectile is fired, the powder can be found on the clothes and majorly antimony, lead, and copper can be detected for establishing the crime.

The GSR can be divided into two parts depending upon its composition:

- Organic GSR components
- Inorganic GSR components

These buildups can be used to distinguish the presence of GSR on the skin and garments of the shooter. A few guns can create bigger measures of GSR and these particles can store deposits on individuals or items in nearness to the underlying release.

Organic GSR components	Inorganic GSR components
• Nitroglycerin • N-nitrosodiphenylamine • 4-Nitrodiphenylamine • Diphenylamine • Ethyl centralite	• Lead • Antimony • Barium • Other heavy metals

10.15 GSR Analysis and Examination

As discussed above GSR can play a major role in connecting the crime with victim and suspect. So, its analysis and examination are also an important task, which can be accomplished with the help of various chemical tests mentioned below:

10.15.1 Walker's Test

- This test distinguishes nitrite in the deposits utilizing desensitized photograph bromide paper. The leftover part of this space might be cleaned utilizing another dampened cotton fleece. Both swabs might be set in plastic sacks marked right/left hand (individual's name and address and other description).
- The pack ought to be fixed to make it hermetically sealed. Appropriate inspecting is finished by cleaning around for 30 s with each swab and revolution of the swab to use all surfaces of the cotton tip.
- The swab ought to be scoured against the surface. The unique finger impression design region ought not to be cleaned. Inspecting then again might be done and kept up similarly in order to recognize one hand from the other. This may likewise fill in as a control test.

10.15.2 Swabbing Technique

The assortment of buildup particles from the hands of a suspect to have discharged a shotgun can be done by both the strategies of cleaning and washing hands.

The inspecting hardware should comprise a pack with cotton-tipped application swabs saturated with 5% HNO; during examining methods, there ought not to be any immediate contact between the hands of the subject and the sampler.

New elastic gloves ought to be put on prior to taking care of materials. Completely swab a bit of the thumb, pointer, and interfacing space of the correct hand as demonstrated by the concealed part of the past figure.

The swab ought to be eliminated from the bundle, and the cotton tip ought to be soaked with a couple of drops of 5% nitric or hydrochloric acid dropped over it with the assistance of a dropper.

10.15.3 Paraffin Test

The test is designed to detect the existence of nitrate buildups, which may be retained on the shooter's hand due to the backblast of gases that escape after the release.

These substances are buildups from smokeless powder, the fuel utilized in present-day cartridges.

The term "paraffin test" is obtained from the paraffin projected strategy, which is utilized to eliminate the buildups from the hands. After expulsion, the cast is tried

with a reagent, either diphenylamine or diphenyl benzidine. A shading response "dull blue spots" shows the presence of nitrate deposits. The chief issue with the paraffin test is its nonspecificity. Countless substances other than explosive deposits contain nitrates and, along these lines, additionally produce a positive response.

10.15.4 Dermal Nitrate Test

This test is called one of the basic tests which were first utilized in 1950 and are still being used as a primary test. A cast produced using a dainty layer of wax spread over the hand of the shooter will get GSR. The obtained deposits are treated with diphenylamine broke up in solid sulfuric corrosive. Spots of blue shading appearance would demonstrate the presence of nitrates, showing the presence of GSR. The strategy has lost its dependability as a few materials not containing nitrates give a positive reaction to this test. Walker test has supplanted it, on account of its being more explicit, basic, and advantageous. The test has effectively been clarified.

10.15.5 Harrison and Gilroy's Test

It is utilized for the location of metallic components to be specific lead, antimony, and barium. In the instances of shooting by pistols, great outcomes are obtained; however in cases including different guns, the test is temperamental as it regularly comes up short. The outcomes are just subjective and progressed instrumental procedures are to be used for greater dependability and quantitative examination. The subtleties of the test are as follows. A piece of perfect and dry material is treated with one drop of triphenylmethyl arsonium iodide alcoholic arrangement (10%). Antimony if present will be shown by the presence of an orange ring in around 2 min. The test material ought to be dried prior to putting two drops of sodium rhodizonate arrangement in the ring on the test fabric. Red tone, which it creates, will demonstrate lead and barium or both. The fabric needs drying once more. A drop of weakened hydrochloric corrosive (1:20) is put on the red spot on the dried test piece of fabric. No adjustment of shading means that it is barium. The test may be useful in identifying a shooter or a shot opening, but it is disliked by criminological researchers who are aware of the flimsiness of shadings and the interference of three components with one another, and thus use of instrumental techniques for investigation for better and more solid discoveries.

10.15.6 Griess Test

The test is utilized to identify nitrite particles in the GSR gathered from various sources or on the surfaces. The test is explicit for GSR since nitrites are not habitually found in everyday life and are extraordinary. Thusly, its location becomes critical and the test gets explicit. The region/surface suspected to convey GSR is

showered with 10% hydroxide arrangement. Whatman channel is dunked in Griess reagent which comprises 3% sulfanilamide, 0.3% N (-naphthyl) ethylene diamide dihydrochloride, both broken down in 5% sulfuric corrosive. The sodden paper is put over the GSR design and gently pressed for about a large portion of a moment. The presence of rose shading spots demonstrates the presence of nitrite particles. The test is well known and generally utilized on the grounds that it is explicit for nitrites [34, 35].

10.16 Significance of Firearms Evidences in Criminal Investigation

- Firearms evidences can be used for the establishment of the relationship between the used arm and the user.
- It can be used for the identification of the kind of firearms that were used in committing crime.
- The fingerprints and DNA analysis extracted from the obtained firearm evidences can be used for the identification of the convict.
- There are different classes and individual characteristics of the ammunition that can be examined for the firearm from which they were fired.
- Firearms with different manufacturing have different serial numbers. If they are destroyed for the purpose of misguiding, restoration of the serial number is possible using chemical and mechanical methods to achieve the identification of owner and history of firearm.
- GSR is very useful in the identification of the firearm owner.
- Wound ballistics deals with the examination of firearm injuries that can be used for the determination of velocity, range, and time of the projectile.
- Size and shape of the wound help in the determination of the size of the projectile, distance, and angle at which the projectile is fired.
- These firearm evidences are used for the reconstruction of scene [36].

10.17 Conclusion

The examination of firearms and ammunition has been introduced before the tenth century. It was concluded that the firearm evidences play a vital role in criminal investigation. They can establish the relationship between the criminal and the weapon used. With advancement in centuries there has been an increase in the variety of cases which not only involve murder and robbery case but also involve illicit trafficking of the firearms and ammunition. The firearm evidences including ammunition can be identified for their manufacturing and owner. These evidences can be used for the criminal and the type of firearm used in crime. These evidences also help in the reconstruction of scenario. Rand, distance, velocity, and time can also be evaluated with the help of external and internal ballistics.

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Digital and Cyber Forensics: A Contemporary Evolution in Forensic Sciences

11

Sameer Saharan and Bhuvnesh Yadav

Abstract

Digitalization has a revolutionary impact on human society as it has provided easy access to information and increased interpersonal connectivity, ease of data storage, and businesses. Most of the personal, financial, and official transactions are now conducted digitally. The security of the online/offline data becomes a necessity as these data are now more prone to cyberattacks and breach of information. Criminals are taking advantages of any loophole in the security of these data, and that has resulted in skyrocketed cybercrimes. Both preventive and responsive measures are required to nullify the cyber or digital crimes. The conventional forensic investigations are not sufficient to investigate such crimes, and therefore, new contemporary branch of forensic evolved, that is, utilizing principles of forensic investigation for the digital data. This chapter will explain the development and role of digital and cyber forensics in cybercrime investigations. The chapter also focuses on the processes and techniques involved in prevention and investigation of cybercrimes.

Keywords

Digital forensics · Cyber forensics · Cybercrimes · Data acquisition

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11.1 Introduction

With advancement of the digital technology, computers have become ubiquitous as a processing and controlling device. The information that was processed manually and preserved as record on paper has been gradually shifted to the digital processing. Personal profile, business transactions, processes, and technical details are now stored in computers. Easy access to high- speed Internet, easy operation, and worldwide connectivity have shown efflorescence in the digital processing of data. The information which was initially stored and authenticated by the signatures and thumbprints on the security documents is now identified and authenticated by digital signatures. As the moon has a dark side, so is with the digital world. The information which is being created, stored, and transferred by digital means have security threat. The breach of cyber privacy can lead to significant loss of valuable information that can be misused by the hackers, resulting in significant financial and professional setback to the industries and persons. The misuse of digital platform increased manifolds in the last decade, resulting in cybercrimes like leakage of personal information, ransomwares, defamation, riots, and threats to national security. In 2020, nearly 445 million cyberattacks were reported which was double of the previous year. It shows the necessity of tracing cyber criminals [1]. Cybercrime cannot be prevented by traditional methods of crime investigations; therefore, new approach, i.e., cyber forensics, came into existence developed which works on the digital footprint of web search history, messages, emails, documents, etc. Cyber forensics is based on the retrieval of the entire information by following these digital footprints [2]. Locard's exchange principle also applies in digital and cyber forensics. Just as criminals leave fingerprints, blood, hair, etc. on the crime scene, here, also, criminals leave their traces in the form of registry keys and log files, which can be tracked with the help of some tools in digital and cyber forensics [3]. Digital and cyber forensics has gradually attained its position as that of other branches of forensic science like fingerprint, toxicology, biology, etc.

11.1.1 Digital Forensics and Cyber Forensics

Until the late 1990s, digital forensics was referred to as "computer forensics." The terms "digital forensics" and "cyber forensics" are used interchangeably; however, there is a minor difference between these two terms. Digital forensics deals with all digital electronic devices, e.g., cell phone, digital networks, hard drives, flash drives, digital cameras, electronic files such as image file, and email [4]. Technically, digital forensics is the science of identifying, extracting, analyzing, and presenting digital evidence stored in digital devices, whereas cyber forensics is the process of gathering and documenting evidence from a computer to the computing device in a manner that can be shown to a court using investigation and analysis technique. Digital and cyber forensics have the same goal, i.e., to determine whether a tool was used for illegal purposes (illegal data breach by hacking computers) or not and, if yes, then to trace the culprit. Digital forensics is not only confined to retrieval of data

but also playing highly significant role in data breach analysis. The following are the subgroups of digital forensics that deal with different digital devices [5]:

(a) Computer forensics (deals with computers, its embedded systems, and static memory).
(b) Mobile device forensics (deals with data recovery from smartphones/mobile devices).
(c) Forensic data analysis (deals with tracking online transactions).
(d) Network forensics (deals with monitoring and analysis of network traffic for collection of evidence).

11.1.2 History of Digital Forensics

The demand for computer forensics came into existence with the beginning of the information age/digital era, which was characterized by greater information production, transmission, consumption, and reliance. In the mid-twentieth century, modern computers were largely owned and operated by huge firms, such as universities and government agencies. Theft of computers and computer components was the major focus of standard computer crime investigations. Such crimes were easily solved by the conventional forensic strategies of trace evidence collection and analysis. With introduction of personal computers in the mid-1970s, old crimes with new tactics arose, and computer crimes were no longer limited to computer or component theft. Newer methods of computer crimes were evolved, particularly for financial crimes (fraud, embezzlement), majority of which were done by persons who have access to private information about the corporation's operations [6].

By the late 1970s, computer crimes became a major concern for national and international firms, and issue was raised in the conference held in France (1976) entitled "Council of Europe Conference on Criminological Aspects of Economic Crime." Many types of cybercrimes were described during that conference. The Federal Computer Systems Protection Act of 1977 was introduced as the first federal cybercrime law in the United States. Though the act was not accepted, it was recognized for drawing attention to the importance of cybercrime law [7].

The digital revolution initiated in the 1980s, when IBM offered PCs for the general population. These systems were quite powerful, but they only had a few programs. The FBI Magnetic Media Program (1984), which later known as Computer Analysis Response Team (CART), was the first known effort to tackle cybercrime. Subsequently, Electronic Crimes Special Agent Program (ECSAP), Seized Computer Evidence Recovery Specialists (SCERS), and Defense Computer Forensic Laboratory (DCFL) came into existence. Access Data was founded in 1987 and is widely regarded as a pioneer in the field of cyber forensics. FBI hosted the International Conference on Computer Evidence (IOCE) in 1993 and 1995, which was attended by delegates from 26 countries, and a jointly decision was taken about sharing experiences and aid regarding cybercrime. The G8 countries assigned the

task of creating international guidelines, protocols, and procedures for digital evidence to IOCE in 1998 [6, 8].

The Scientific Working Group on Digital Evidence (SWGDE) was constituted with law enforcement officers, forensic laboratory scientists, and employees from private companies who collaborated to produce cross-disciplinary digital evidence guidelines which were published as "Best Practices for Computer Forensics" in 2002. At the Budapest Convention on Cybercrime (2004), an international treaty was formed that recognized crimes perpetrated on computer systems and networks via the Internet, such as copyright infringement, child pornography, and fraud [9].

In 2005, ISO released the General Guidelines for Testing and Calibration Laboratories (ISO 17025). Cyber forensic tools quickly gained attraction; EnCase by Guidance Software and FTK by Access Data led the commercial tools category, achieving significant success and legal acceptability [8].

In 2006, US Courts approved the new Rules of Civil Procedure that classified digital information as a new type of evidence and established a mandatory method for dealing with digital evidence, known as electronic discovery or "eDiscovery." In 2007, the FBI stated in Congressional testimony that its Computer Analysis and Response Team (CART) analyzed over 2.5 petabytes of evidence. The Forensic Science Education Programs Accreditation Commission (FEPAC) and American Society of Testing Materials (ASTM) are stepping toward accrediting US academic programs in digital forensics [6].

After all those efforts, digital forensics is growing with the advancement of technology. There are new and advanced tools in the market which make tasks very easy.

11.1.3 Digital Forensics Standards and Guidelines

The following are the most common organizations which play an important role in digital forensics.

11.1.3.1 National Institute of Standards and Technology (NIST)

NIST was established in 1901 and is working under the Department of Commerce (USA). NIST provides measures to support the simplest to the most complex human-made products. NIST's technology, measurements, and standards are used in a wide range of products and services throughout the world. There are three major digital forensic projects at the NIST which are supported by the US Department of Justice, law enforcement, and other sponsoring organizations [10]. These projects are:

(a) National Software Reference Library (NSRL).
(b) Computer Forensic Tool Testing (CFTT).
(c) Computer Forensic Reference Data Sets (CFRDS).

11.1.3.2 National Institute of Justice (NIJ)

This institute is working as the research, development, and evaluation wing of the US Department of Justice. Its goal is to use science to improve our understanding of crime and justice concerns [11].

11.1.3.3 International Organization on Computer Evidence (IOCE)

The major task of IOCE is to develop international standards for the exchange and recovery of electronic evidence. IOCE has established working groups in Canada, Europe, the United Kingdom, and the United States in response to the G-8 Communique and Action Plans of 1997. IOCE presented the following five main principles at the International Hi-Tech Crime and Forensics Conference (1999) [9]:

(a) Any actions made after seizing digital evidence should not change the evidence.
(b) When accessing original digital evidence, a person must be forensically qualified.
(c) All activities involving the seizure, access, storage, or transfer of digital evidence must be adequately documented, preserved, and available for review.
(d) During the time that digital evidence is in their possession, an individual is liable for any activities performed with regard to it.
(e) These standards must be followed by every agency in charge of seizing, accessing, storing, or transferring digital evidence.

11.1.3.4 American Society of Crime Laboratory Directors (ASCLD)

It is a nonprofit organization of forensic science managers and crime laboratory directors. The organization's goal is to promote professional interests while also assisting in the development of laboratory management ideas and procedures. It is the only accrediting body dedicated solely to laboratories that conduct criminal justice testing [12].

11.1.3.5 ISO SC 27 CS1

This contains generic security and privacy approaches, techniques, and guidelines, such as [13]:

(a) Methodology for gathering security requirements.
(b) Information and ICT security management, including information security management systems, security procedures, and security controls and services.
(c) Cryptographic and other security measures, such as those that secure information accountability, availability, integrity, and secrecy.
(d) Support documents for security administration, including terminology, standards, and methods for registering security components.
(e) Identity management, biometrics, and privacy security aspects.
(f) In the field of information security management systems, there are standards for conformance assessment, accreditation, and auditing.
(g) Criteria and methodology for evaluating security.

11.1.4 Glossary Used in Digital and Cyber Forensics

To get familiar with the concepts of digital forensics, the following terminologies are frequently used [14]:

- Acquisition: In digital forensics, data acquisition refers to the techniques for collecting digital information, including cloning and copying evidence from any electronic source. It means developing a forensic image from digital devices that can store electronic data, such as servers, tablets, CD-ROMs, gaming consoles, portable hard drives, hard drives, thumb drives, and other computer technologies.
- ACPO guidelines: ACPO (Association of Chief Police Officers) has developed a set of computer-based evidence guidelines. It provides a set of four key principles:
  - There must be no actions taken that alter data stored on a digital device that may later be used as evidence in court.
  - If accessing original stored data on a digital device is required, you must be both capable and able to justify your acts, as well as the effect they may have on any digital evidence used in court.
  - Both steps taken and applied to the digital proof must be recorded and maintained safely and securely. If another forensic expert reviews the procedures, the conclusion must be the same.
  - The investigation's lead investigator is ultimately responsible for ensuring that these guidelines are followed.
- Active data: The data we can actually see is known as active data. Data files, applications, and operating system files are included under this category.
- Ambient data: The information on a device that is not viewed or used as part of routine operations is referred to as ambient data. It is created accidentally, as a by-product of other tasks, and serves no particular purpose.
- Archival data: Archival data is often compressed and stored on another medium, such as tape or CD. Such information is normally not readily accessible to the user and must be recovered from archival media before it can be accessed.
- ASCII: ASCII (American Standard Code for Information Interchange) is a character encoding standard for electronic communication. Computers, telecommunications equipment, and other devices all use ASCII codes to represent text.
- Audit trail: An audit trail is a security-relevant historical record, collection of records, and/or destination and source of records that provide documentary evidence of the chronology of activities that have affected a particular action, process, occurrence, or system at any given time.
- Backdoor: A backdoor is method by which any users (authorized/unauthorized) can bypass usual security protocols and gain high-level user access to a computer, network, or computer program.
- Backdoor Trojan: Backdoor Trojans are malicious software programs that allow unauthorized access to a computer in order to set up a remote attack. Remote

attackers may use a compromised computer to submit commands or gain complete control.

- Backup: Backup is the copy of computer data stored on a hard drive to protect against accidental loss or corruption.
- Backup server: A backup server is a server that allows you to back up your data, files, applications, and databases. It can be locally based or a remote backup server, and it offers both hardware and software features for managing and recovering your backups.
- Backup media: Backup media refers to the storage media used for backup electronic data, such as discs, disc drives, and tapes.
- Bit: A bit, or binary digit, is a basic unit of information or the smallest unit of data in computers and digital communications. Each bit is represented by a 1 or a 0.
- Cache: Cache is a temporary storage area that helps web pages, browsers, and apps load faster.
- Compressed file: Any file containing one or more files or directories that are smaller than their original file size is referred to as a compressed file. These files allow for faster downloading and more data to be stored on a removable device.
- Cookies: Cookies are text files that include small amounts of data, such as a login and password, and are used to identify your computer when you access the network. HTTP cookies are used to identify and improve the web browsing experience of users.
- Corrupt file: A file that has been corrupted. When a problem occurs during the saving process, a file becomes corrupted.
- Cyberattack: The theft, alteration, or destruction of a specific target using digital devices, computer networks, or technology-dependent companies. Attacks are carried out by distributing malicious software, creating bogus websites, or gaining unauthorized access, and they frequently result in extensive destruction.
- Dark web: The dark web is a decentralized network of websites that tries to keep users as anonymous as possible by routing all of their communications through several servers and encrypting them at every stage.
- Deep web: Parts of the Internet that are not fully accessible through typical search engines like Google, Yahoo, and Bing are referred to as the deep web.
- Deduplication: Deduplication is a method of reducing storage capacity requirements by eliminating redundant copies of data.
- Digital forensics: The use of investigative and analytical procedures to identify, preserve, extract, and document digital evidence in a way that is suitable for court presentation.
- Directory: A list of files stored on a hard disc or other media that is organized hierarchically is called directory. The root directory is the topmost directory.
- Disk mirroring: Disk mirroring is a method of protecting a computer system from data loss and other potential losses caused by disc failures. The data is replicated using this method by writing it to two or more identical hard drives, all of which are attached to a single disc controller board. If one of the mirrored hard drives fails, the data can be recovered from the others.

- Encryption: Encryption is the process of converting data into a secret code that hides the true meaning of the data.
- File carving: The technique of retrieving files that have been deleted but not totally erased from a digital device. It operates by scanning and reassembling the raw bytes of a hard disk.
- File server: In a computer network, a file server is a central server instance that allows linked clients to utilize the server's storage capacities.
- File signature: A file signature is information that is used to identify or validate a file's contents.
- File slack: File slack is the unallocated space on a hard drive where a file is kept. Because each cluster on a drive has a storage threshold and files are of varying sizes, this space remains empty or unused.
- Forensic image: A forensic image is a type of copy of original evidence that has all of the data found in the original but wrapped in a forensic file format that prevents tampering.
- Hash value: A hash value is the result of a calculation (hash algorithm) on a string of text, an electronic file, or the contents of an entire hard drive.
- Hash match: The hash match operator performs a variety of logical operations, all of which employ an in-memory hash table to locate matching data. Hash match can be used with a single or two inputs due to its versatility. The *build input* is the initial input, which is shown on top of a graphical execution plan. The *probe input* is a second input that is optional.
- Insider threat: An insider threat to an organization's security comes from former or present employee, contractor, or third party. Sabotage, theft, fraud, access rights abuse, and espionage are all examples of common insider threats.
- Imaging tools: A storage medium can be imaged using either forensic software or hardware. There are both free and commercial solutions that can help with the procedure. The most significant characteristics to check for when selecting a tool are the speed with which it can conduct an image and its reliability.
- ISO 9001 certification: An organization that meets the certification requirements regularly offers products or services that fulfil customer and regulatory standards.
- Keylogger: Keyloggers are a sort of monitoring software that records a user's keystrokes. These keystroke loggers, one of the oldest types of cyber threat, record the information you type into a website or application and send it to a third party.
- Keyword match: A keyword match is a typical approach used in computer forensics and electronic discovery to find and identify every instance of a particular word or phrase on a computer or other media, even if the word or phrase appears in unallocated space or deleted files.
- Live analysis: The process of analyzing digital media in real time rather than turning it off and sending it to a lab. This strategy frequently proves to be the most effective means of capturing evidence since it decreases the chance of information being tampered with and allows for more retrieval of volatile data.
- LNK files: LNK files (also known as labels or Windows shortcut files) are files that are created automatically by Windows operating system whenever a user

opens a file. The operating system uses these files to ensure quick access to a certain file. Furthermore, some of these files can be written by users to help them with their tasks.

- Log file: A log file is a computer-generated data file that contains information about usage patterns, activities, and operations within an operating system, application, server, or another device.
- Master file table (MFT): One of the most significant files in the NTFS (New Technology File System) file system is the Master File Table (MFT). It keeps a record of all files on a volume, including their location in the directory, physical location on the drive, and file metadata.
- Metadata: The data embedded within a file that describes the document's characteristics. Although some metadata may be seen by the user, such as modification dates and file sizes, other hidden or embedded information requires the assistance of a technical expert to locate.
- Network: A network is a collection of computers, servers, mainframes, network devices, peripherals, and other devices that are connected to allow data to be shared.
- New Technology File System (NTFS): The Windows NT operating system uses the New Technology File System to efficiently store, organize, and find files on a hard disk.
- Outsider threat: A threat to an organization's security that originates from the outside, such as a cybercriminal, hacktivist, or competition-sponsored attacker. Economic gain, corporate espionage, and social or political change are all common reasons for an outside attack.
- Program: A program is a computer software that can be executed. It's similar to a script, but it's usually much bigger and doesn't need a scripting engine to run. A program, on the other hand, is made up of compiled code that can be run straight from the operating system of a computer.
- Registry hives: A hive is a logical group of keys, subkeys, and values in the registry that is accompanied by a collection of supporting files loaded into memory, when the operating system is started or a user logs in. When a new user connects to a computer, a new hive with a distinct file for the user profile is created. This is called the user profile hive.
- Shadow volume: Shadow volume is a feature of the Microsoft Windows operating system. It enables Windows users to create manual and automated backup copies of their computer data and volumes. Even if such files or volumes are in use, this feature is available.
- Software: Software is a collection of programs that are designed to execute a specific task. A program is a group of instructions designed to address a specific problem.
- Steganography: Steganography is the art of concealing a secret message within a non-secret object. Many forms of steganography nowadays involve hiding a secret piece of text within a photograph. Alternatively, you may hide a secret message or script inside a Word or Excel document.

- System integrity: Methods for ensuring that data on a computer is genuine, correct, and protected from unauthorized user alteration.
- TOR (The Onion Router): An open-source privacy network that allows users to surf the Internet anonymously.
- Window registry: A swap file is a section of the hard drive dedicated to temporary data storage. The swap file is used by Windows to increase performance. A computer's primary memory, or RAM, is used to store data for present activities, but the swap file acts as additional memory that can be utilized to store additional data.
- Write blocker: A write blocker is a device that allows data to be read from a hard disc without changing the data on the disc. On the hard drive, the device allows a read command but not a write command to be executed. The examiner can use a built-in write blocker in most imaging tools while imaging a hard disc. While software tools or changes to the Windows registry can achieve write blocking, hardware methods will be favored in digital forensic laboratories.

11.1.5 Electronic Evidence

Digital forensics focuses on collection of data from electronic evidence, its transformation into useful information, and delivering the findings to the court. The IT Act and its amendments are based on the model law on electronic commerce established by the United Nations Commission on International Trade Law. The Information Technology Act of 2000, related amendments to the Evidence Act of 1872, and the Indian Penal Code of 1860 established the concept of electronic evidence. According to Section 2(1)(t) of the Information Technology Act, 2000, "electronic record" means data, record or data generated, image or sound stored, received or sent in an electronic form or microfilm or computer-generated micro fiche [15–17].

So, electronic evidence is the data that is manipulated, stored, or communicated by any man-made device, computer, or computer system, or transmitted through a communication system, and that has the ability to make either party's factual account more or less reasonable than it would be without the evidence. In short, electronic evidence is the data or information that exists in digital format and utilized in a court of law to "prove" or "expose the truth" about a crime.

11.1.5.1 Challenges with Electronic Evidence

Unlike other evidence (DNA, fingerprint, blood, etc.), the process is very challenging in case of digital evidence due to the fact that [18]:

- The data may be dispersed across numerous physical locations, even countries.
- Data can be easily and very quickly transferred across jurisdictional borders.
- The data is highly volatile, meaning it can be readily changed, rewritten, damaged, or destroyed with a single keystroke.
- It is possible to copy the data without causing it to degrade.

- Electronic evidence has a short lifespan before it is considered useless, unlike any other type of forensic evidence.

11.1.5.2 Guidelines for Electronic Evidence

The following guidelines must be followed when dealing with electronic evidence [19]:

(a) It is necessary to gather electronic evidence in a lawful manner.
(b) Before handling electronic evidence, the staff involved must undergo the relevant training program.
(c) Any changes made to the electronic evidence must not affect the data. If access to the original data or changes to the system settings are required, only authorized personnel should be able to do so, and those personnel must be able to justify their actions.
(d) If possible, any activity that requires accessing or changing the original data should be recorded and witnessed by another practitioner.
(e) All acts made while dealing with electronic evidence must be documented and kept on file so that they can be audited. Those actions should be repeatable by an independent third party with the same effects.

11.1.6 Forensic Investigation Process

The purpose of a digital forensic investigation is to learn everything there is to know about an incident. It entails locating and assessing digital evidence relevant to the case. The basic processes of investigation are followed by digital forensic experts; the complexities of these processes vary depending on the model of the organization in charge of the inquiry [2]. In the digital forensic laboratory, electronic evidence analysis usually involves four phases (Fig. 11.1).

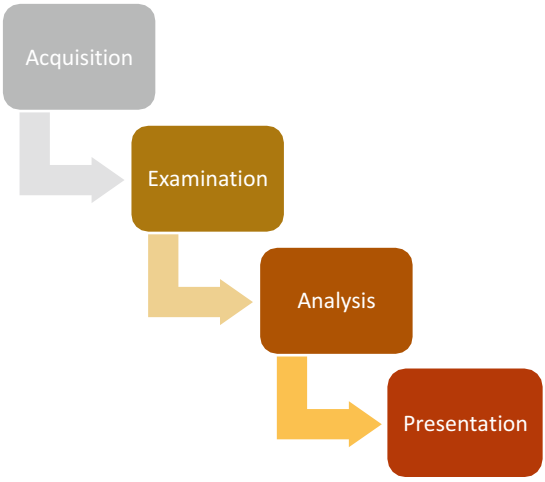

Fig. 11.1 Processing of digital evidence during digital forensics investigation

The chain of custody of the evidence must be updated anytime it changes hands throughout the procedure, and its integrity must be always maintained. The examination and analysis steps might be repeated until the work meets the requirements of the case. Although it is widely assumed that conducting digital forensics work in the laboratory requires these four phases, not all cases will require all four. In some circumstances, the acquisition step can be bypassed entirely to undertake triage during the examination step. For example, when there are enormous quantities of data, conducting acquisition on each evidence item may not be possible. The detail explanation of these four phases is as follows:

11.1.6.1 Acquisition

The process of generating a forensic copy of electronic evidence (exhibit) such as a hard drive, USB drive, or server in the form of an image file/s is known as acquisition or data acquisition. The image file/s will then be used to analyze the evidence in the next stage of the process. The acquisition is conducted to protect the electronic evidence's integrity. It is to make an exact copy of the data without altering the electronic evidence's content in any manner. The acquisition of electronic evidence must be done in a forensically suitable way. Typically, data is obtained by capturing volatile data from a running computer during a search or by obtaining a storage device from a seized computer, or at any other point during an inquiry. Data and information saved in electronic form are intangible, making them easier to manipulate and alter than traditional forms of proof. As a result, having a defined and tested acquisition procedure is critical [2, 5, 20].

Both the exhibit's hash value and the image file must be recorded once an image file has been created. The image file is hashed to ensure that the content of the exhibit is identical. In digital forensics, hashing algorithms like Sha-256 are employed. The hash-generating capability is available in most forensic software and hardware.

Unless circumstances prevent examiners from doing so, examination and analysis must be done on a forensic copy of the original evidence. This is critical to protect the evidence's integrity. The forensic copy of the electronic evidence must be preserved elsewhere, not on the evidence itself. To avoid being confused up with the original evidence or forensic copies from other instances, the forensic copy must be carefully labelled. As a result, before receiving cases, the digital forensic laboratory must prepare certain storage medium.

This chapter demonstrates how to perform a digital forensic investigation and analysis on computer.

Levels of Data Acquisition

There are two levels of data acquisition [2, 21]:

- **Physical data acquisition:** Physical data acquisition includes all raw data. At this level of acquisition, all data on the disc will be copied, including the partition scheme, partitioned area, and un-partitioned space. Because it includes deleted files and unallocated clusters, the Examiner frequently chooses this level of data acquisition of the entire drive.

- **Logical data acquisition:** Logical data acquisition only includes a subset of raw data. On the disc level, logical data acquisition copies only a logical partitioned area. When dealing with encryption, logical data acquisition of unlocked data is recommended over physical data acquisition of encrypted data. The examiner must first decide on the exhibit's state before making a copy.

Types of Acquisition

- **Live acquisition:** Live acquisition is performed on a live system. A live system is one that is up and running and in which data is constantly being processed, allowing information to be changed. Switching a live system off may result in the loss of volatile data, such as data saved in the cloud, encrypted data, ongoing processes, network linked, and mounted file systems, due to the substantial evidentiary value that can be discovered in a live system. The level of volatility in a system's data varies. If the machine is turned off or rebooted, these data will be lost. When collecting live data, the examiner should start with the most volatile data. The order of typical levels of volatility, from most to least volatile, is as follows: Memory, Swap File, Network Processes, System Processes, and File System Information. Live acquisition is also used in two situations: (i) when a system is business critical and cannot be shut down and (ii) when volatile data is more significant than erased data.
  - **Dead acquisition:** Dead acquisition is carried out on a dead system. A dead system is one that is not in use; it is switched off and has no power. Volatile data in temporary storage regions on a computer, such as RAM memory, ongoing processes, cache, or current application dialogues, will no longer be accessible after the machine is dead. Dead acquisition is a simple procedure that is usually carried out automatically using forensic equipment. If feasible, remove the hard disc from the computer before connecting it to the equipment. In some circumstances, dead acquisition cannot be used to recover netbook PCs or devices with soldered solid-state drive storage. In such circumstances, other extraction methods, such as starting the system with a live CD/USB, should be considered. When erased data is more important than volatile data, a dead acquisition is performed.

    After then, the examiner must decide whether to clone the exhibit or generate an image. The data is copied bit by bit from one storage medium to another by the clone. The image, on the other hand, moves data from one storage media to another, bit by bit, producing an image file. After that, the file can be saved on another medium. The latter method is more typically employed because the image file can then be read by most forensic software and processed for forensic analysis. Cloning is frequently utilized in simulations.

Imaging Formats

Raw and E01 (EnCase Evidence File) are two of the most prevalent image file formats. In a raw file, these formats store all data from the original medium. E01

contains physical bitstream copy stored in a single/multiple files enriched with metadata. Expert Witness Format (EWF) and Advanced Forensic Format (AFF) are two other formats that are used for analysis. They have characteristics such as:

- Compression of data.
- Encryption of data.
- Error checks.
- Case metadata.
- Hash sums.
- Splitting the image in chunks.

In addition, several forensic software systems have their own proprietary picture formats with similar features. It is recommended to choose an image format that is supported by most of the forensic software. A few digital forensic laboratories utilize distinct forensic software; thus, if the examiner selects a unique image file format, the image file may not be opened.

Process of Acquisition

The common process for conducting data acquisition can be divided in the following four steps [21]:

(a) **Identify storage media:** Before handing over, the examiner must provide a compatible storage media with sufficient data size. The examiner may need to prepare many storage media to store the image file if the exhibit is substantial.
(b) **Imaging the exhibit:** Before imaging the exhibit, make sure it is connected to a write blocker for protecting its integrity. This function is available in most forensic software. The image file is subsequently saved to the storage media that has been prepared. Use a standard labelling format to protect the storage media and the image file by hashing all the evidence with SHA256. The examiner should be aware that utilizing a write-blocking approach does not prevent data modifications on a solid-state drive or flash media with a controller chip. The controller will begin reorganizing data on the flash chips as soon as it is connected to a power supply. Even when connected to a write-blocking device, the controller performs tasks such as wear-leveling, write-amplification, and trash collection. At present time, the only technique to generate a proper forensic copy of the flash media is to use a lot of resources. This is accomplished by unsoldering the chip(s) from the circuit board and, if possible, reassembling the data in the proper order.
(c) **Verify exhibit and image file:** After creating the image file, the examiner must verify that it can be opened with forensic software and that the hash values of the exhibit and the image file match.
(d) **Documentation:** In the case notes, the final stage in examining and analyzing the computer is to document the procedure, including the tools used, hash values, date and time, and the examiner's initials. The flow chart of the process of acquisition is shown in Fig. 11.2.

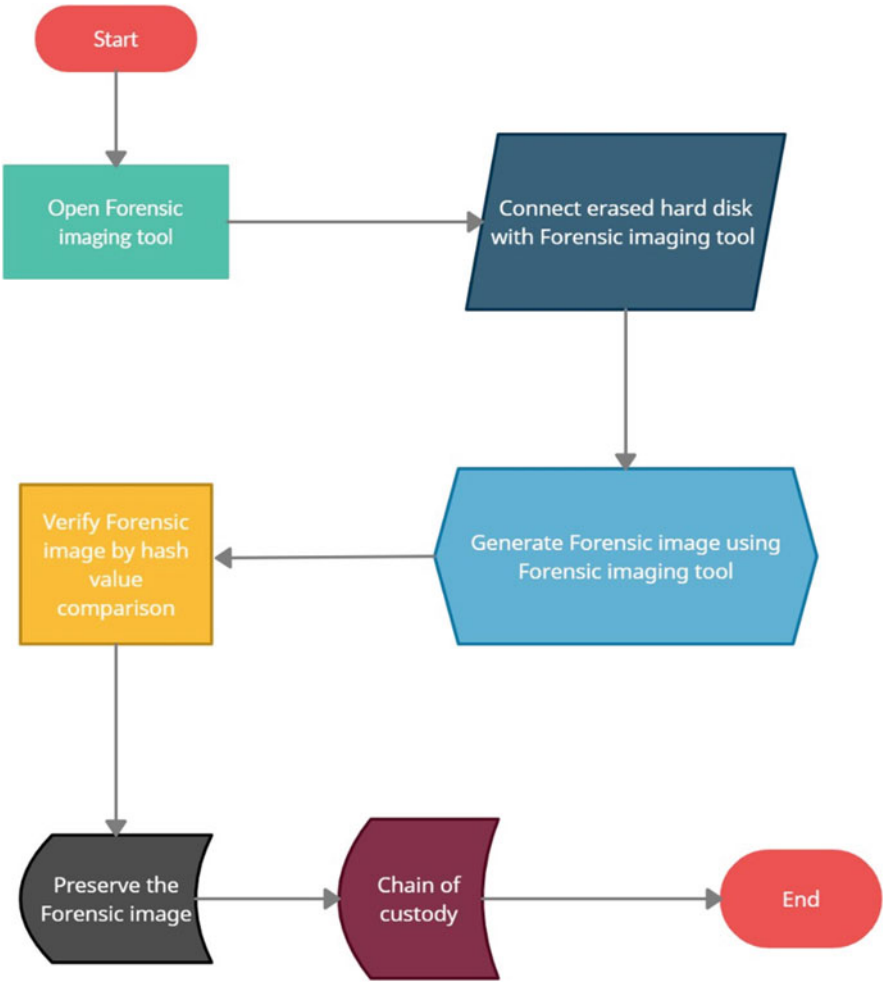

Fig. 11.2 Process of acquisition

11.1.6.2 Examination

Wherever feasible, original evidence should not be examined. The examiner must always work on the evidence's forensic copy (image file). If this is necessary, data access must be restricted using a write blocker. Examiners may need to perform the examination in an isolated or pre-set environment in some cases, for example, using a database system or game software to run the simulation. Examiners can accomplish this by encapsulating the case in a functional container using virtualization technology. When the examination is over, the examiner can use a recognized image, or a feature provided by the operating system to restore the workstation to its prior condition [2, 21].

Triage

The process of prioritizing cases, exhibits, or data for analytic procedures based on their relevance to the case is known as triage. Cases, exhibits, or data will be analyzed in order of importance, from most important to least important, based on the results of triage. It's likely that some won't be examined at all since they're irrelevant to the matter under investigation. Triage is carried out to deal with problems such as:

- There are a large number of exhibits or large amounts of data that must be analyzed in a short period of time.
- Exhibits can no longer be stored owing to legal difficulties.
- It is a high-priority case with imminent outcomes, such as when physical damage or death is a possibility.

Though the triaging is beneficial, it cannot be used for a comprehensive exam. Automated processing, such as that provided by forensic software or the application of self-written programs to exhibits or data, is used to conduct triage. Triage remains a viable option for dealing with a situation that cannot be resolved in any other way. There is a lot of software available that performs triage functions, some of which is commercial and some of which is open source. Triage can be performed while the exhibit is still operational or by booting the exhibit utilizing forensic bootable media. The examiner then enters keywords and runs the system before picking and storing pertinent files on removable storage media. There is a chance that this automated data analysis will just look at subsets of data, leaving out some crucial information. This drawback must be conveyed to the investigator, prosecutor, and court, who are the deciding authorities to proceed with the triaging procedure or not.

Methods for Computer Examinations

A computer can be examined using a variety of methodologies and procedures. Some tasks require a high level of expertise, while others, such as running an automated process, need only a basic level of expertise. The examiner can employ a variety of forensic software. Some software can recover passwords, correlate data among electronic evidence, and execute keyword searches, depending on the software capacity.

(a) **Examination on Dead System**
The following information must be considered while examining a dead system:
- Active files, deleted files, file slack, partition slack, disk slack, and shadow files.
- Device artefacts—operating system files, file registry, file metadata, encrypted files, log files, and database files.
- Browsing history, e-mail, social media, and peer-to-peer file sharing.

(b) **Examination on Live System**
The following information should be considered when examining a live system:

- Random access memory (RAM).
- Running processes.
- Network connections.
- System settings.
- Storage media.
- Cloud services.

  Examination of a live system on any of the above data may be undertaken depending on the case request.

(c) **Automated Processing**

Automated processing is frequently carried out employing forensic software's readily available features. The examiner normally determines the scope of automated processing which can be applied to other similar investigations. Running a hash comparison on images in a child pornography case is an example. The following are typical automated processing actions and sequences:

(i) Extraction of data from the operating system and users.
(ii) Mount containers, for example, ZIP, RAR, and encrypted containers.
(iii) Extract and analyze objects like emails and web history.
(iv) Analysis of signatures.
(v) Recover files and folders that have been erased.
(vi) Carve specific file types.
(vii) Recover deleted partitions.
(viii) These analytical approaches may be utilized depending on the case request:
  - Optical Character Recognition (OCR) of PDF files.
  - Create thumbnails for simple viewing.
  - Extract photos from videos.
  - Skin-tone detection for films.
  - Hash comparison.
(ix) Logs from the operating system are examined.

Data Recovery

Data recovery is the process of recovering data that has been deleted, corrupted, hidden, or lost from storage medium. The storage medium may be damaged, corrupted, or formatted, rendering data inaccessible. As a result, data recovery also entails the process of repairing the storage media so that data can be recovered. Data recovery can be divided into two categories: logical recovery and physical recovery. When storage media is accessible but the data is formatted, corrupted, buried, or lost, logical recovery is used. Typically, forensic software is used in the recovery procedure. When the storage media are inaccessible due to mechanical or technological breakdown, physical recovery is performed. The recovery process is time-consuming and requires specialized knowledge. Physical recovery in some situations requires the use of a special room. When repairing a cable in a USB thumb drive, for example, a soldering equipment is required. Because the cost is

significant and a highly experienced examiner is necessary to do the activities, not many digital forensic laboratories can afford to create a physical rehab facility.

Filtering

Applying filters to an image file before analyzing it can assist the examiner by limiting the quantity of data he or she must look at and analyze. Hash sets are commonly used in filtering strategies to either filter out known operating systems or application files (whitelisting) or to explicitly search for hash matches inside databases of known illicit content (blacklisting). When only specific types of findings are relevant to the case, filtering can be used. Signature analysis can be used to filter files based on size, date, owner, and a variety of other attributes found in the metadata. Most commercial forensic software includes this filtering feature. The process of examination is shown in Fig. 11.3.

11.1.6.3 Analysis

Categories of Digital Traces

A criminal who commits a crime by computer will leave traces at a "digital crime scene," just as he or she would leave physical traces at a crime scene. Some of these traces can be configured to be discovered by the examiner, while others can be made to be hidden [2, 21]. There are two types of traces:

(a) **Discoverable:** Artefacts that are automatically saved on the computer. Even if a suspect tries to hide his/her tracks, the chances of uncovering such traces are high. Some of the discoverable traces are:
- Slack space.
- Unallocated space.
- MFT entries.
- RAM.

(b) **Non-discoverable**: Artefacts that can be set up such that they aren't saved on the computer. For example, a web browser that allows the user to disable or wipe download history. Some of the undiscoverable traces:
- Thumb caches.
- Most recently used lists.
- Log files.
- Browser histories.
- Browser caches.
- Most used programs.
- Form data.
- Pagefile.sys.
- Hiberfil.sys.
- Volume shadow copies.
- Download history.

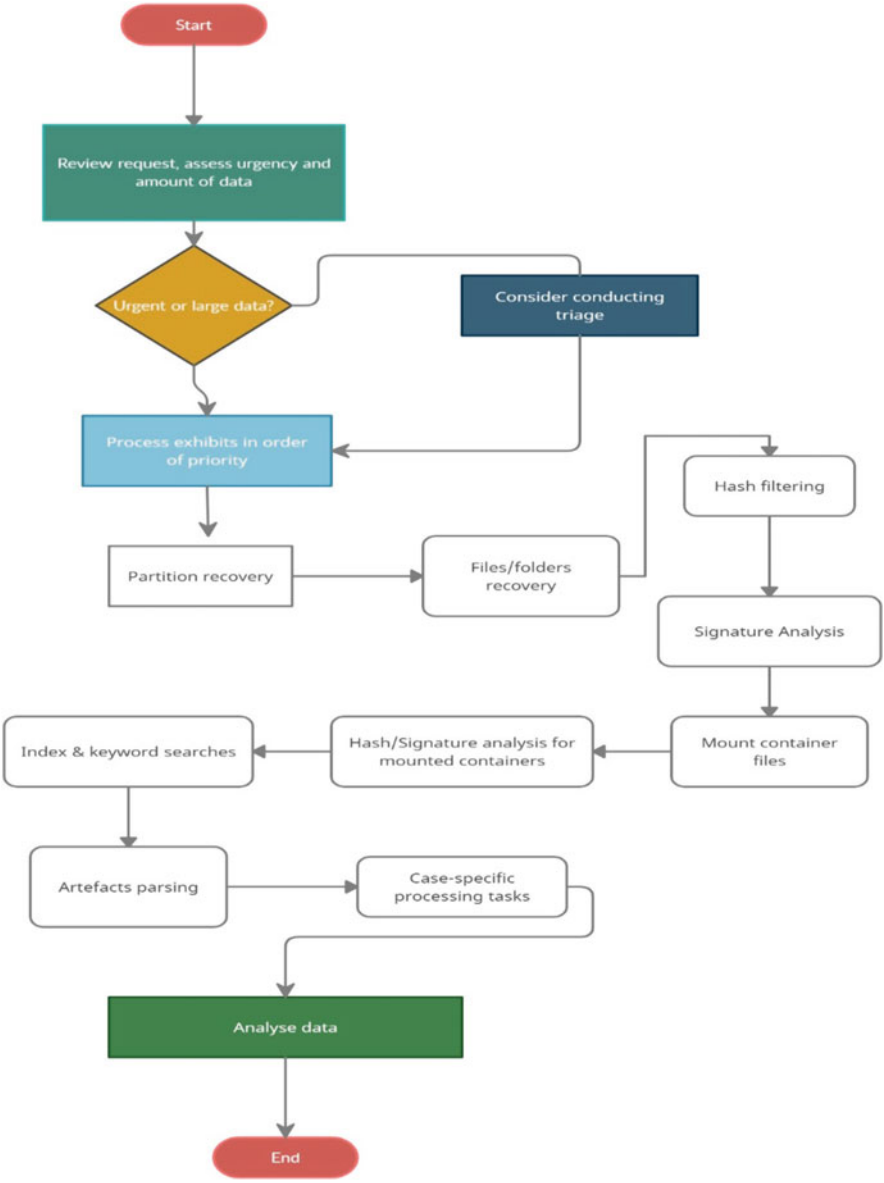

Fig. 11.3 Process of examination [21]

Procedure for Different Traces

The sort of data and information that must be taken from a computer is determined by the case. For example, data/information taken from a computer in a fraud case is often in the form of spreadsheets, emails, and office documents. Pictures, movies,

and communication messages are examples of possibly connected data/information in a child-abuse case.

The following sections explain in detail the types of data that can be extracted from a computer.

(a) emails: email analysis, which usually involves mail applications like Outlook, Thunderbird, and Mail, as well as webmail accounts. Different forms of artefacts will be produced by different mail clients. Personal folder files, such as PST, OST, and PAB, are used by Outlook to hold evidence. Inbox files are used by Thunderbird to store messages. Although forensic software usually need these files, it does not always retrieve all messages. As some forensic tools are unable to recover deleted communications from personal folder files, a data recovery approach may be required.
(b) Office documents: Filtering the files of interest is usually done after file signature analysis. To ensure that the file header and extension are the same, file signature analysis compares the two. If they don't match, the document header or extension may have been changed to disguise the information. Using a keyword search to filter files is necessary. Both processes are usually performed automatically by most forensic software. It is recommended for the examiner to refer the requester for content analysis after similar documents have been located. This is to ensure that the document being extracted is relevant to the case under investigation. When the requester confirms the documents, the examiner can go deeper into the document, its metadata, and who created it, as well as determine whether it was delivered or received on the computer.
(c) Pictures and videos: To undertake image and video analysis, the examiner must first have a clear understanding of what the requester is looking for. If searching for identical photographs is required, the requester must provide the examiner with the required photos. If the files have known hashes, the requester may need to provide the hashes to the examiner, or the examiner may be able to use a list of hashes from established databases. If it involves a specific segment of a video, the requester must provide its distinguishing characteristics. One example is extracting all motorcycle-related images from a video. The most common starting point for image analysis is signature analysis. The examiner can then use the thumbnail view to filter through the images in the gallery. A hash comparison can be used to find a set of known photographs in a case, such as a child abuse case or stolen blueprints. Some forensic software includes a capability that detects comparable pictures. The retrieved images can then be displayed in a gallery format. The examiner should consider extracting the metadata of photos and video files if the location or production details of those media are essential. Metadata are collections of data that describe and provide information about other data, such as GPS coordinates, creation date and time, and the equipment used to acquire the image. It is impossible for the examiner to go through thousands of photos and videos to find one specific video or picture file in some exhibits. The most efficient method is to extract all images and then send them to the requester. After the examiner has found the

relevant photos/videos, he/she can undertake further analysis to extract further useful information, such as GPS coordinates and creation or modification data.

(d) Internet browser: Internet browsers are of evidential value in many cases. They typically contain the following artefacts:
- Website visit history.
- Local cache/temporary Internet files.
- Bookmarks/favorites.
- Session's information.
- Cookies.
- Saved usernames and passwords.
- Entries from form fields.
- Internet keyword searches.

Analyzing browser artefacts can be useful for determining purpose or intent; for example, search engine phrases can be used to prove intent. Google Chrome, Microsoft Internet Explorer, Mozilla Firefox, and Apple Safari are all popular browsers. They all save data in the user's home directory. All other browsers use SQLite databases to store the artefacts stated above, except for Microsoft browsers. Browser analysis is available in most online forensic applications. However, due to rapidly growing technology, some forensic software may take some time to refresh its database due to frequent updates to particular browsers. As most browsers nowadays deal with SQLite databases, the examiner can manually analyze the artefact using free SQLite database browsers.

(e) Software: When a software needs to be examined, it almost always requires the extraction and comprehension of its artefacts. Communication software (e.g., WhatsApp and Skype), steganography software (e.g., OpenStego), password safes (e.g., KeePass), file sharing software (e.g., uTorrent), and cryptocurrency software (e.g., cryptocurrency wallets) are all examples of such software. Although there are no standard techniques for analyzing all software artefacts due to their diversity, it is generally done by conducting information gathering on the software artefacts from credible and reputable sources. A simulation can then be used to confirm the findings.

(f) User activity: The computer operating system tracks user activity at many different places. For example:
- Power on and shutdown.
- Software settings.
- Most recently used files lists.
- Device use.
- User logins.
- Wi-Fi connections.
- Preferred programs.
- Setup of user environment.
- Frequently accessed files.

This user activity can be analyzed to have a better knowledge of the user's behavior and even to prove evidential behaviors. The relics are stored in

various locations depending on the operating system. Most artefacts in Microsoft Windows are saved in the Registry, Event Logs, and Jump Lists. The artefacts are saved in the Library and log folders on OS X systems, whereas the majority of the data is stored in the user home folder, or the "/etc" or "/var" directories on Linux systems.

(g) Log file: Analyzing log files is critical, especially in times of system attacks. The examiner should not only retrieve allocated log files but also traces of deleted/unallocated log files. For log file analysis, specialized software are available. The premise of such an analysis is to look for specific phrases, unusual patterns, or logs that fall within a specific time window.

(h) Encryption: The majority of recent operating systems have built-in encryption capabilities. The user can easily enable full disc encryption for a system drive. Before the exhibit is transferred to a digital forensic laboratory, it is recommended that the passwords or encryption keys be acquired at the crime scene using live data forensics. Other passwords can also be extracted from the disc if possible. These passwords and their variants can be used to build a dictionary that can be used to launch a password cracking assault. Traditional law enforcement activities, such as obtaining physical evidence, such as written passcodes, keys, or recovery strings, should also be carried out to locate passcodes.

(i) Computer memory (RAM): The memory dump can be analyzed in the digital forensic laboratory if the computer memory was captured while the seized computer was still functioning. Understanding the memory architectures of various operating systems to analyze the memory dump necessitates a high level of technical knowledge and specialized software. Volatility and Rekall are two examples of this, both of which are freely available on the Internet. The following are examples of artefacts that can be retrieved from memory dumps:
- Running processes, including their memory.
- Process information (e.g., handles).
- Encryption keys.
- Opened files.
- Usernames, passwords.
- Unsaved documents.

Virtualization

When it comes to virtualization, a picture is worth a thousand words. The examiner can see the operating system environment of an exhibit in the same way that the suspect has viewed it. Finding evidence inside a virtual computer can be quicker and more expressive than reassembling data traces from an image file. Viewing pirated gaming software is one example. When mounting an image, use write-protected or read-only parameters with a write cache so that the virtual operating system can write log files without compromising the image's integrity.

Process of Handling Mass Data

Some instances involve many machines and a large amount of data. To complete the task quickly, separation of forensic analysis from the content analysis is required. Examiners focus on forensic analysis activities such as exhibit recovery, parsing, mounting, and processing, while investigators with case-specific knowledge perform content analysis. To ensure smooth functioning, the examiners and investigators may need to create and apply correct methods for handling and examining extracted files.

11.1.6.4 Presentation

The presentation phase means compiling findings and presenting them to stakeholders in a clear and intelligible manner. The examiner must compile the findings and results into a forensic report once the analysis step is complete. Judges, prosecutors, and all parties involved should be able to grasp the examiner's explanations and translations of sophisticated technical circumstances. They may also be asked to analyze the data and provide an opinion on their significance. When a significant number of exhibits are analyzed, the examiner may find it difficult to convey the findings to the investigative team. It is suggested that analytic tools be used to make it easier to link digital evidence with other data from the inquiry [2, 21].

Admissibility of Electronic Evidence

The requirements for electronic evidence admissibility may range from one jurisdiction to the next. When examining electronic evidence for trial, the examiner should examine the following criteria:

(i) Authenticity: The evidence must establish facts in a form that cannot be contested and must be indicative of the original situation.
(ii) Completeness: Any examination of the facts, or any opinion based on it, must tell the complete scenario, and not be skewed to fit a more favorable or desired viewpoint.
(iii) Reliability: Nothing about the collection and subsequent handling of the material should raise any doubts about its legitimacy or validity.
(iv) Convincing: The evidence must be convincing in terms of the facts it depicts, and it must be able to persuade the stakeholder in court of the truth.
(v) Proportionality: The procedures employed to acquire evidence must be fair and proportionate to the purposes of justice: the prejudice caused to any party's rights must not outweigh the evidence's probative value.

Report Writing

A forensic report must be written in simple, straightforward language. The outcome must be correctly summarized, as well as provide a clear response to the requester's case request. All technical details should be put in the appendix section rather than being included in the main content. This is to make it easier for laymen to grasp the report. The examiner must also avoid making any statements that cannot be supported by evidence. In the statement "The suspect tampered with File A," for

example, “File A located in Computer B has been changed” would be an appropriate sentence.

It can be difficult for the examiner to communicate the conclusions in the report due to the complexity of the case. Visual aids and visual representations, such as animation, slides, images, and live demonstrations, are effective ways to improve comprehension.

Expert Witness

In some jurisdictions, submitting a forensic report in place of the examiner attending the court session is adequate in court. In other jurisdictions, however, the examiner is required to appear in court and offer his or her expert testimony in connection with the case. An expert witness is a person who, via education, training, talent, or experience, possesses specialized knowledge and expertise that goes beyond that of the average individual. Others may rely on the witness’ specialized (scientific, technical, or other) opinion concerning evidence or a fact within the field of his or her skill, referred to as the expert opinion, because his or her knowledge is sufficient. In some countries, the trial judge determines expert status in each case, and the person is only an expert in that case. In some jurisdictions, the legal institution appoints an expert, who is then responsible for any case that falls within his or her area of knowledge. The rights and responsibilities of an expert witness vary by country. Examiners must be well versed in their jurisdiction’s legislation, court procedures, and role, as well as their rights and responsibilities.

11.1.7 Digital Forensic Tools

With gradual development of cyber forensic, various digital forensics tools have been developed for countering and analyzing cyber threats. Few top digital forensic tools are listed below.

11.1.7.1 Forensic Tool Kit (FTK)

A comprehensive computer forensics tool which is compatible with Windows, Linux, and macOS. It brings together all the most common forensic tools in one spot for investigators. It has features for email analysis, file decryption, data carving, data visualization, web viewer, Cerberus, and optical character recognition [22].

11.1.7.2 Autopsy Kit

It is an open-source forensic tool which is compatible with Windows, Linux, and macOS. Autopsy examines disc images, local discs, or a local file folder. Autopsy examines disc images, local discs, or a local file folder. It supports raw or E01 formats and can work on features like Keyword search, graphical interface, extraction of camera and geolocation from a .jpeg file, registry analysis, email analysis, hash set filtering, strings from unallocated space and unknown file types, Android support, and web artifacts [23].

11.1.7.3 Volatility

The Sleuth Kit focuses on the hard disc, but it's not the only place on a computer where forensic data and artifacts might be kept. Important forensic data might be kept in RAM, which must be acquired swiftly and carefully in order to be forensically valid and helpful [24].

It is also open-source tool and compatible with Windows, Linux, and macOS. It can examine raw dumps, crash dumps, VMware dumps (.vmem), virtual box dumps, and a variety of other types of dumps.

11.1.7.4 Registry Recon

The Windows registry serves as a configuration store for the Windows operating system and the apps that run on it. The registry is one of the most popular places where malware deploys persistence methods, and these applications can store several different data in it. Registry Recon is a commercial application for reconstructing Windows registries from forensic images. It also has the capacity to reconstruct deleted parts of the registry using unallocated memory space analysis [25].

11.1.7.5 Cellebrite UFED

With the growing relevance of mobile forensics, acquiring a mobile-focused forensics solution could be beneficial. Cellebrite UFED is recognized as the most advanced commercial mobile forensics tool available. It works on a variety of platforms (not only mobile devices) and has exclusive mobile device analysis methodologies and tools. It provides for physical as well as advanced logical acquisition and passwords for devices. It enables the examiner to open a password-protected encrypted raw disc image file [26].

11.1.7.6 Wireshark

Although many forensics tools concentrate on the endpoint, it is not the only source of information in a forensics investigation. The majority of cyberattacks take place over the network, and network traffic captures can aid in the detection of malware as well as provide access to data that has been deleted or overwritten on the endpoint [27].

Wireshark is the most popular and commonly used tool for network traffic analysis. Wireshark is a free and open-source network traffic analyzer that includes dissectors for a variety of network traffic types, a simple and easy-to-use GUI for traffic analysis, and a lot of capability behind the hood. It can either capture live traffic or ingest network capture files for analysis.

11.1.8 Applications of Digital and Cyber Forensics

There are many challenges fronting digital and cyber forensics in the present scenario. These challenges are because of easy availability of hacking tools and significant use of the Internet. The investigation is complicated by the large amount

of stored data. There are many advantages of cyber and digital forensics, and a few are enlisted as follows:

- It helps in protecting the integrity of computer system.
- It aids in the development and presentation of evidence in court, which may result in the criminal's punishment.
- It assists businesses in retrieving critical data if their computer systems or networks are attacked.
- It locates cyber criminals from all around the world with ease.
- It aids in the protection of the funds and time of an organization.
- It allows to extract, evaluate, and analyze factual evidence to show cyber criminal behavior in court.

However, the digital evidence analyzed by cyber forensic experts have limitations also which are listed below:

- In court, it must be proven that that no tampering has occurred to the digital evidence.
- The expense of creating and storing digital data is exceedingly high.
- Judges and lawyers must have a broad understanding of computers.
- If the digital forensic tool utilized does not meet the required criteria, the evidence may be rejected by the court of law.
- Due to the investigating officer's lack of technical understanding, the desired outcome may not be achieved.

11.2 Conclusion

The digital forensics have emerged as a contemporary science in the past few years. As the exponential growth in Internet use has increased cybercrimes manyfold, it is essential to develop new investigative tools to counter the cyberattacks. Hackers have their own ways to breach the data which can be threat to privacy, personal data, financial, and security. Both preventive and responsive measures are required to counter such attacks. Therefore, advanced technological development of cyber forensics is essential. It is the fastest growing field of forensic science as newer techniques are being introduced. However, the validation and authentication of these techniques are essential for their admissibility in courts. Therefore, digital forensic guidelines and technology should be so strong that digital evidence can be relied upon and courts cannot dismiss digital evidence in the absence of any other evidence.

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Fire Investigation: Arson or Accidental

12

Ekampreet Kaur, Jaskaran Singh, and Supriya Awasthi

Abstract

Fire investigation is conducted to decipher the cause and origin of fire, that is, whether the fire is accidental or deliberately caused. Fire investigation is a demanding area of expertise because there is uncertainty of evidences. The analytical techniques used must have a high degree of reliability and sensitivity and be subjected to quality control and quality assurance. This chapter consists of the protocols followed while investigating the fire scene, ignitable liquid classification, sample collection and analytical techniques used for performing analysis.

12.1 Introduction

Forensic science is the study which focuses on providing scientific evidences and analysis of the recovered samples and making final report for legal purposes. The report prepared by forensic expert signifies the evidence to the court of law [1].

Fire investigation is one such discipline in forensic science which involves application of other disciplines including material science, chemistry, physics, criminalistics and forensic analytical methods. Investigation in a fire case is very complicated because almost all of the evidences are either burnt or destroyed by the

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action of fire. Experts investigating the fire scene must have knowledge regarding fire science which includes:

- Chemistry of combustion.
- Ignition of substances.
- Mechanism of heat transfer.
- Development of fire.
- Fire extinction.

The fire investigator visits the scene, identifies the route of fire and reconstructs the crime scene for designing the phenomenon of fire spread [1–4].

Origin and cause determination is an integral part for fire investigation. Fire investigators must have knowledge and expertise in identifying faulty or improperly designed and installed products which might cause fire or played a role in fire [3]. The investigators focus on determining fire cause, cause of property damage, cause of injuries of deceased and loss of life of civilians and firefighters. Fire investigation is a two-stage approach:

- Detailed examination of the scene affected with fire.
- Analysis and interpretation of evidences collected from crime site.

Fire usually spreads at a very high rate and produces heat as well as toxic gases. It is imperative to find out the cause of fire breakout to allocate responsibility to the alleged and to make important legal documents in criminal law. Arson case in fire investigation is regarded as a criminal behaviour in which the culprit deliberately damages property [4].

12.2 Fire Investigation

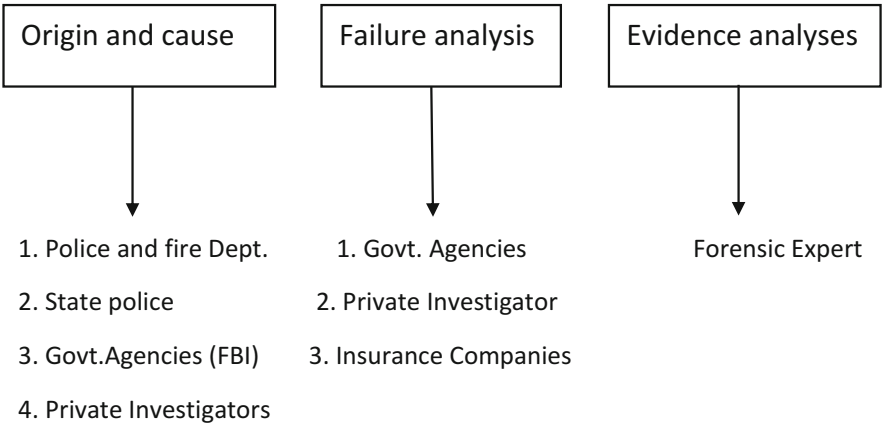
Fire investigation is done on a basis of different disciplines:

- Origin and cause determination.
- Arson investigation.
- Failure analysis.
- Reconstruction analysis.
  1. Origin and cause of fire includes the investigation for finding the cause and origin of fire, that is, from where and why the fire has caused.
  2. Investigation of fires which are caused due to criminal intent and to prosecute the culprit for his/her crime.
  3. Investigating whether there was any fault or failure in some manner or finding out the root cause for any failure.
  4. Investigating all the events, information from the site, observations for purpose of reconstructing or recreating the crime act. Various modelling techniques and computer technology are used for this purpose [5–7].

12.3 Conducting Fire Investigation

Fire investigation is conducted by different govt. agencies like:

- Police and fire departments.
- Private investigator.
- Federal Bureau of Investigation (FBI).
- Forensic analysts.
- State Police.



Fire investigation is done by different officials for different reasons. Fire investigation is independent on agency [identification] employed for investigation and type of the incident [7, 8].

Incidents included in fire investigation are:

- Suspicious fires or suspected fires which are deliberately caused.
- Injury fires.
- Fires causing large property loss.
- Fires caused due to failure of fire protection.
- In cases where there are liability issues pertaining to cause and property lost.

Fire investigation is performed for three primary reasons:

- Criminal suspicion.
- Financial recovery.
- Education.

12.3.1 Criminal Suspicion

Fires which are suspected to be caused due to criminal intent are investigated in order to fix out criminal responsibility. The investigators play a role in identifying and arresting the culprits responsible for committing the crime. Arson cases are usually handled by legal authorities. Motives behind arson fire are profit, revenge, insurance fraud and concealment of other crimes.

12.3.2 Financial Recovery

Fire investigation is done to fix financial responsibility which is similar to filing of civil charges against any manufacturer, contractor or building owner or may be any person for losses occurred because of fire. Investigators try to find out the root cause or reason what failed, why and the person or thing responsible for that.

12.3.3 Education

Through fire investigation, one can learn about the science behind the fire including phenomenon of fire, combustion, human reactions to fire, etc. By investigating fire, one can acquire knowledge and skills regarding the cause of the fire. Building material can be improved, and fire safety education can be provided. Architects and engineers would make use of better material to avoid fire-related issues and for greater safety [6, 7, 29].

12.4 Fire Science

Fire is defined as the oxidation process which leads to evolution of light and heat in various intensities. Fire science study involves different aspects like heat transfer, ignition, growth, toxicity, smoke production, fire modelling, etc. These aspects are studied for reconstructing fire scenarios [3, 9].

12.4.1 Nature

Elements like air, water and earth change into each other by nature; on the flip side, fire controls its own generation and destruction. Fire is the only element which depends on the substrate for existence [10].

12.4.2 Behaviour

Fire behaviour is defined as manner in which fuel ignites, flame develops and fire spreads. The behaviour is influenced by accelerator or fuels, weather and topography. The fire keeps on burning in certain conditions where heat, oxygen and fuel are present. These three components together make the "fire triangle" [10, 11].

12.4.3 Chemical Nature

Combustion is the process which results in fire. At a certain point of combustion, there is ignition point. At this point, flames are produced. Flames produced usually consist of carbon dioxide, water vapour, oxygen and nitrogen. Fire emits heat and light. The chemical reaction of fire is exothermic in nature. In order to make combustion to occur, three things must be present, fuel, oxygen and energy in the form of heat. The reaction starts with energy, and it continues until fuel and oxygen are available. Basic principles of heat transfer include heat transfer, fluid flow, flame spread theory and ignition [5, 12, 13].

Importance of fire scene in forensic scenario is majorly in reconstruction procedure. Fire is analysed during reconstruction of events [1, 14]. The parameters that are imperative and need to be followed include:

- Ignition of fire.
- Growth and development of fire.
- Simulation of fire production.
- Toxicity and fire propagation.
- Fire dynamics.

12.5 Investigating the Fire Scene

The first official who visits the crime site should check the location, condition of the victim, site and witness. Vehicles leaving the scene along with all unusual activities are noted. Also, the flame condition or the smoke present at the site is noted. Volume, colour, height, location of flames and the direction in which flames are moving are recorded. The type of building, that is, whether an apartment, etc., fire through roof, windows location and whether they are opened or closed are also recorded by the officials. Weather condition of the location and any unusual activity around the scene, for example, any container, fire accelerants, external fire or unusual odour, are checked properly. All these observations provide the starting point for collection of evidences and aid in further investigation. All these information should be recorded by the first officer visiting the scene. The record should be made by making written notes, voice clips or video recording [2, 4, 7].

Investigators must take care of themselves when visiting the scene. They should evaluate the site for security hazards like chemicals, smoke or electricity or even

biological hazards. Officials must communicate these hazards to other officials and investigators visiting the crime scene. Proper tools and protective equipment are used by investigators during their visit to the scene and for performing operation. There is a need to reassess the scene to check for safety hazard because of fire conditions and suppressing efforts [15–17].

12.6 Preserving the Scene

In arson cases, the evidences present at the site are not permanent, that means they are transient and that's why they will disappear easily. The investigators visiting the site must take presumptive steps for preserving such evidences, record them and discuss them with other officials visiting the site. These evidences include:

- Fire patterns.
- Identity card.
- Injuries and burns of victims.
- Ignitable liquids, accelerants, unusual things.
- Igniting devices (matchsticks, lighters, aerosol spray).
- Shoeprints.
- Debris and broken stuff like glass.
- Windows and doors.
- Tools and tool marks.
- Trace evidences including hair, fibre, blood, fingerprint, etc.

Along with these, other unnecessary stuff need to be preserved like containers which are not used routinely, as well as clothing and other evidences which are not related to arson investigation like drugs, bodies and weapons [17, 18].

Every culprit tries his/her best to remove the evidences, so investigators must check such evidences. These include:

- Fire-suppressing activities (e.g. use of water or any diluent). These activities are usually done at the point of origin. So, investigators must check for such evidences.
- Rescue activities are usually done by the culprits to remove the physical evidences from the site.
- Use of any weapon or device to cause destruction to evidences.
- Electrical appliances.
- Weather conditions including wind, temperature, rain, etc. can also harm the evidences especially transient ones.
- Removal or movement or corpse.
- Act of removing or distributing belongings of victim or deceased (e.g. clothing).
- Vehicles at the site (use of fuel might be done to destroy evidences).

It is imperative to protect the crime scene to avoid any contamination or destruction of evidences by any means. To protect the scene and evidences, investigators must follow these guidelines:

- Block the entry of witness or other people by using barricades.
- Limiting excessive fire suppression and rescue.
- Officials should make sure that there is not much destruction of property.
- Leaving the corpse undisturbed.
- Marking of evidences and documenting them.
- The evidences must be covered or preserved in containers in which they will not get contaminated.
- Preserving transient evidences and documenting them.
- Protecting the evidences which are at risk of catching fire so that their evidentiary value isn't lost.

Preliminary scene assessment is conducted by the first officer who visits the site. This assessment is done to determine the extent of damage and note the areas which are least damaged and highly damaged. Officials should not disturb the evidences. The areas with little or no damage are also analysed and checked for other evidences. On the basis of this preliminary analysis, investigators identify the origin of fire and the cause of fire. If the origin and cause are not identified in the preliminary assessment, then the scene is examined with National Fire Protection Association (NFPA) 921 guidelines [7, 9, 19].

The National Fire Protection Association (NFPA) 921 has listed standard equipment which are used for fire investigation. These include:

- Sterile containers for collecting evidences.
- Compass.
- Decontamination equipment.
- Labels, tapes for marking evidences.
- Gloves and tools (hammers, screwdrivers).
- Lights.
- Masks and other personal protective equipment.
- Measuring tape.
- Documentation equipment (cameras, writing pads, pen, markers, voice recorders).

The crowd and the fire in progress are photographed. All doors, windows, walls and exterior and interior of the fire scene are photographed systematically without leaving any clue. The origin of fire, ignition sources and ignited materials are also photographed. Photography plays a vital role in reconstruction of events. Chain of custody is maintained. The officials working in the crime scene must mention their name and findings in the logbook. There are certain forms for different officials in which they give their personal details and their findings.

Aerial photography, photogrammetry and all possible photographs are taken for better analysis [8].

The investigator must make a sketch with representation of the scene. The sketch must include the dimensions, locations of doors and windows, least affected area to highly affected area, ceiling height, combustible contents, electrical appliances or floor plans. A written documentation is also mandatory as it is advantageous for investigation, scientific analysis and legal proceedings. All the measurements are also noted down. Written notes and sketches both are correlated during investigation [8, 9, 19].

12.7 Sketching System

Sketches are graphical information of the scene. The key role of sketches is for orientation which shows the position of objects that were collected as evidence. Overall view of the scene is not available in photographs, so sketching is a relevant technique.

After preliminary search of the scene, a rough sketch is made by the officials. The scene is not disturbed by the officials until the sketch is complete. The sketch must contain all the information including the person making sketch, date, case number, smoke damage and dimension of the area. While drawing the sketches, location of the body is very important. The diagrams are made in connection with photographs [8].

Sketching types:

- Two-dimentional (bird's eye view).
- Three-dimentional.

12.8 Processing of Evidences at the Scene

During the recovery and evaluation of the evaluation of the evidences, the officials must follow these guidelines:

- Precautions must be taken to prevent contamination.
- Documentation must be done side by side. It must include the location, type of evidence, evidence tag, container label, etc.
- Evidences from the origin area must be collected carefully.
- The locations where fire is not accidental are also documented with great care, and other precautions are taken when evidences are collected from that point.
- It is commendatory to label the containers and place evidences safely for transportation.
- Evidences like ignitable liquids must be placed in sterile containers which are vapour-tight; after that, they are sealed properly.

- Each container is labelled uniquely, and the name of investigator, date and time of collection, case number, sample number, location of sample recovery and description should be mentioned.
- Comparison samples should be collected side by side.
- Other physical evidences like body fluids, fingerprints, shoe prints, trace evidences and tool marks are preserved properly and recovered only by experts.

It is mandatory to avoid cross-contamination of the samples. Fire debris collected from site have high evidentiary value, so they need to be stored separately. All the liquid samples are also collected in sealed containers and transported separately. Before transporting, investigators must communicate with laboratory officials, so that they can follow any specific laboratory requests, for an instance, submitting any unused container or absorbent medium for detecting any containment. Evidences must be packaged according to the laboratory policies and procedures [3, 9, 16].

The evidences collected from the crime site are firstly documented. It is obligatory to maintain the integrity of the evidence collected from the scene. The investigators must overlook that all the evidences are properly collected, documented, labelled and preserved. Proper chain of custody is maintained, and the evidences are sent to laboratory for analysis [27, 28].

The chain of custody must be maintained right from initial assessment to the final disposition. This ensures the integrity of the evidences [10, 15, 20].

The lead investigator must ensure that all tasks are completed before leaving the scene. They must:

- Make sure that all the evidences are inventoried and chain of custody is maintained.
- Discuss the preliminary analysis and post scene issues with all the experts including forensic officials, insurance companies and other officials.
- Assign responsibilities of post scene to other officials and law enforcement agencies.
- Address legal consideration.
- Verify that all the evidences are documented properly.
- Remove all the equipment used for investigation.
- Note the time and condition at the time of release. Photography should be done before leaving the site.

The investigator must collect information from all the officials, integrate it and deposit it for the legal proceedings. Proper data is maintained for identification of fire trend and further proceedings.

Fire can consume evidences due to its destructive nature, giving challenging task for investigators; conclusions are made on the basis of several parameters including:

- Analysis of fire debris through physical evidence.
- Chemical testing.
- Photographs.

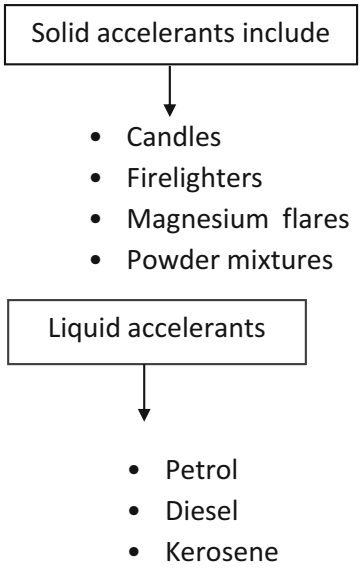
- Medical records of victim.
- Testimonies from victims and eye witness.

In other disciplines of forensic science, the scientists match the evidences collected with a unique source, and conclusions are made, but in fire or arson cases, the experts interpret whether the fire is accidental or deliberately set.

The investigation of fire is done by using protocols from published manuals, books and guides. Fire engineers work to control fire growth. New advancements like computer modelling are introduced which helps in new findings. Unlike other disciplines of forensic science, investigators collect and analyse the burn pattern and debris [8, 11, 21].

12.9 Accelerants

Accelerants are substances that are ignitable fluids that are deliberately introduced to the scene for facilitating the fire spread.



The most commonly used ignitable liquid have almost similar chemical properties. The only difference is in their boiling point. These liquids are very difficult to identify because of their varying components and, hence, are stated as complex substances. The analysis is affected by presence of plastics and carpets [1, 4, 22].

12.10 Petrol

Petrol is the most commonly encountered accelerant in arson cases. It contains lighter hydrocarbons as compared to kerosene or diesel.

- It has great volatility and causes great damage upon ignition.
- Unlike other accelerants, petrol has characteristic chromatographic patterns.

Studies conducted revealed that with the use of tetra-alkyl lead content or by profile analysis, we can obtain "fingerprints" for petrol from different sources. This is helpful in establishing the link between suspect and the arson scene where presence of petrol was detected.

Soft independent modelling of class analogies (SIMCA) was used to identify ignitable liquid of any class. Using this model, petrol was detected at the arson scene after 3 h. Petrol is exclusively used as motor fuel; therefore, everyone has access to it. Hence, it is commonly encountered accelerant [4].

12.11 Kerosene

Kerosene is also used as a major accelerant. Kerosene is less volatile, and hence, it is more difficult to ignite.

- Burns longer if appropriate ventilation is provided.
- Consists of large amount of lighter hydrocarbon content and has high boiling point.

Kerosene is permissibly found in lots of household items like paint thinners, charcoal lighters, etc. and, hence, is widely used as an accelerant. Kerosene is present in several household products, so it is not necessarily indicative of criminal act. Investigators must analyse and then make a final decision [4].

12.12 Diesel

- Like kerosene, diesel has low volatility and, hence, is difficult to ignite.
- It consists of heavier components of crude oil and has high boiling point.
- It has higher boiling components which makes it different from kerosene. Therefore, care must be taken during sampling. Sample collectors should make sure that high boiling components are not lost. If it happens, then the resultant chromatogram would resemble like kerosene.
- New libraries are established which can identify degraded ignitable liquids and helps in comparisons [5, 22]..

12.13 Accelerant Detection at the Scene

Accelerant is the key evidence which helps in determining whether the fire was accidental or deliberately caused. Floor burn-through and high localized burning are the revealing indicators regarding the presence of ignitable liquids [4, 22].

Colour of smoke and flames is also a parameter to determine the accelerant used. The type of material burnt can be analysed by the colour of smoke and fumes liberated by it. Complete combustion does not lead to much emission of smoke. In case emission occurs, it is quite minimal. The greater the amount of hydrocarbons present in smoke, the darker will be the fumes. Lack of sufficient oxygen causes flames to be of dark colour as compared to the flame produced when fuel is burnt in a well-ventilated area [1, 9].

For example, on burning plastic and rubber, the colour of smoke is black, and yellow, smoky colour of flame is produced. Kerosene produces green colour of smoke and yellow colour of flame.

These colours are helpful in tentative analysis of the material burnt.

Odour of the ignitable liquid is also an imperative tool for detection. The human nose can smell this ignitable fluid even if they present in trace amounts. No doubt there are lots of subjectivity in sense of smelling, and it usually differs according to the climate. Sense of smell is regarded as a primary investigation tool, and many of the investigators rely on this.

12.13.1 Sniffers

Investigators use portable detector "sniffers" for detecting the presence of ignitable liquids while scanning the fire scene. Sniffers prove best than humans and dogs because they don't suffer from olfactory fatigue. Portable detectors are less expensive and are easily available. The device will not function properly if it is not calibrated or if it doesn't have enough power [22].

12.13.2 Canine Detection Teams

A dog's sense of smell is much powerful as compared to humans. Their sense of smell is a useful asset for selecting and detecting the ignitable liquids. Canine accelerant teams are made all over the world, and they are trained so that they can target and select certain substances specifically. The teams are then taken to the scenes where they target the scents and help in detection. Training and looking after a dog is quite expensive. Being a living organism, dogs might usually suffer from olfactory fatigue, and hence, they are unable to sense the target smell. In such situations, they become less effective. The major disadvantage of canine detection team is that they can't distinguish between ignitable liquids and polymers. NFPA has generated guidelines in which it has been stated that the canine detection teams can

be employed at the scene, but one cannot rely on their findings, and proper laboratory analysis is mandatory for making final decisions [22].

12.13.3 Portable Gas Chromatographs

Portable gas chromatographs assimilating a FID and small packed columns are used successfully for detecting and analysing ignitable liquids and their residues. They have dual property as they serve both as a sniffer and a chromatograph. This device can easily detect the hydrocarbons even if they are present in trace amount. Moreover, this tool can also differentiate whether the source is ignitable liquid or a burnt plastic. The only limitation in this device was that it has poor resolution. New methodology and advancements are made in this tool so that it can aid in future investigation with commendable resolution [16, 22].

12.13.4 Portable Mass Spectrometer

Portable mass spectrometer is a completely new device which is handy, and some of its models are commercially available. These include Spectra Track, EM 640, the MMI and the Kore MS-200. This device provides a high degree of sensitivity and reliability. These results are so rapid that the device provides information on the spot. On the flip side, the device is so bulky and heavy. Moreover, this portable device is quite expensive [22].

12.13.5 Chemical Tests

Certain chemical tests are employed at the scene to detect the quantity of ignitable liquids in air, soil or water even in trace amounts. Draeger tubes are used for performing atmospheric sampling [22]. Species-specific reagents are filled in glass vials, and these reagents are dependent on chemical reactions for deciphering the types of particular chemical constituent in the sample and its nature. A measurable amount of sample is taken into a tube, and if desirable target species are present, there is the change in colour of reagent. The concentration is determined on the basis of length of colour change.

Hydrocarbon kits are also available which are used for analysing soil and water samples only. The basic principle is the same as that of prior test that is colour change. Positive results are indicated by the change in colour. The limitations of this method are as follows: firstly, it is difficult to differentiate between ignitable liquids and burnt plastic, and secondly, it is very expensive [1, 22, 23].

12.14 Fire Scene Examination

Fire scene examination broadly consists of three steps:

- Origin determination.
- Reconstruction of events.
- Cause determination.

12.15 Origin Determination

Origin determination is done mainly by analysing **fire patterns**. Fire patterns are defined as "measurable or detectable physical changes or distinguishable shapes formed by fire effects or group of fire effects". In the absence of any eye witness at the crime site, investigators determine the origin of fire by their expertise and skill. They interpret physical evidences in order to reconstruct fire. So, fire origin determination is completely based on fire pattern recognition and analysis [1, 9, 23].

Fire patterns can be classified on the basis of fire dynamics:

- Plume-generated patterns.
- Ventilation-generated patterns.
- Hot-gas layer-generated patterns.
- Full room involvement-generated pattern.
- Suppression-generated pattern.

Through depth measurement or visual analysis, fire investigators identify fire patterns. The analysis is done on the basis of physical laws of fire dynamics and investigator's expertise and inference. The collected pattern data is analysed by the investigator who identifies the damaged areas that can help in inferring the area of origin.

In 1970, fire patterns are described on the basis of geometrical shapes, for example, V Pattern, U Pattern and horse glass patterns. These fire patterns are useful in tracing fire spread. 3D conical shape given by Kirk is useful in evaluating geometry of fire pattern.

By using fire patterns, many findings can be done including:

- Degree of fire damage along surface of compartment.
- Identification of trends of damage.
- Casual factors analysis for generation of fire pattern.
- Identification of fire pattern process in determining area of origin.

Arson investigators usually investigate "burnt indicators" and stated it as the most common method of establishing arson.

12.16 Floor Patterns

Fire pattern identification on the floor is also a commendable parameter. Some researchers have experimented and evaluated the damage caused to floor surfaces including carpets and wood, with varying volumes of ignitable liquids used in the open. They concluded that it was possible to identify the quantity of fuel used by burn area. Floor patterns caused by ignitable liquids might be minimal because they can be destroyed easily and due to short duration of exposure due to fuel consumption [1, 7, 9].

12.17 Undamaged Areas

The undamaged area is usually not noted. It is recommended for investigators to evaluate the undamaged areas. These areas help in reconstruction of fire. Heart shadowing some areas usually makes them undamaged. Heat shadowing is defined as "the involvement of object which blocks the way of heat or flame from its source to particular surface material which is under examination".

Heat shadowing and protected areas are considered important as they assist investigators in investigating the scene. They determine that fire did not travel from that particular area and, hence, infer that it has not originated from there as well [1].

12.18 Penetrations

Holes in the floor indicate the ignitable liquids being used. Kirk's experiment proved this wrong; he declared that flammable liquids never carry fire downwards. Penetration patterns help in determining the direction of fire spread from top to bottom.

The major limitation in fire investigation is that identification of cause of damage is done by using evidences which are usually burnt location, damage caused, ventilation, dimension of compartment, position and number of fuels all analysed to identify the initial scenario [22].

12.19 Reconstruction of the Fire Scene

Reconstructing the crime scene is important in determining the origin and the events that had occurred. All objects like windows, doors, furniture, etc. are marked on the floor. Other areas of the affected building are also analysed in the same way.

It is important to communicate with fire suppression officials as they can provide detailed information regarding the condition of the affected area. The information includes the presence of unusual things at the scene like accelerants or any unusual odour, what they saw and what they have heard. All witnesses and victims are interviewed and their testimonies are compared.

During the investigations, the scene gets disturbed, so it is mandatory to document everything as it will assist in reconstructing the events [14, 19].

12.20 Cause of Fire

The main question directed to the investigator after the inspection is: What was the cause of fire? The investigator has to monitor the crime scene and inform whether the fire was accidental or deliberately caused by someone.

If the fire is deliberately caused, all evidences of incendiarism like accelerants, devices used or other unusual things are collected properly and documented on the log sheet [9].

Heat exposure is a key factor in creation of damage. It is basically dependent on the temperature difference. This heat exposure to certain materials like wood, plastics, etc. leads to physical and chemical changes [1].

Physical changes:

- Melting.
- Deformation.
- Expansion.
- Loss of tensile strength.

Chemical changes:

- Pyrolysis.
- Dehydration.
- Changes in colour.

If in case the officials are unable to find the cause, then they have to mention or state that the cause of fire is undetermined [1, 7, 22].

12.21 Samples to be Collected for Analysis

While collecting samples from the fire scene, investigators must keep this thing in mind to recover those samples which can yield ample amount of identifiable ignitable liquid residue. The accelerants used for fire spread are generally hydrophobic, and they vanish after the fire is extinguished. They are still detectable up to 3 months, as they seal into porous substances by water. This porous substance saves them from evaporation. On the other hand, water- miscible ignitable liquids are usually not detected because they are not protected by porous substances. Hence, they wash away when fire is extinguished [2, 6, 13, 14].

12.22 Properties of Ideal Samples

- Porous
- Presence of adsorbent material with high surface area
- Thermal distortion
- Encapsulating ignitable liquids

12.23 Samples Commonly Collected for Laboratory Analysis

- Carpet
- Concrete
- Floorings
- Liquids
- Roofing
- Wood
- Soil.

12.23.1 Wood

Wood samples are adsorbent and have high surface area. Wood samples burn very easily and consume most of the ignitable material which is the biggest disadvantage. Charred timber is a preferred sample, as it has a coating of charcoal. Charcoal is the best adsorbent of volatile residues. If in case the originally present ignitable liquid is lost, residue vapours from the scene are later adsorbed.

12.23.2 Carpet

Carpet and carpet padding are the most commonly recovered from the scene. Modern carpets and carpet paddings are made from synthetic polymers or synthetic rubbers. The pyrolysis of these synthetic material during fire leads to generation of diagnostic indicators for the presence of ignitable liquids. Canine detection team and sniffers can easily identify these liquids.

12.23.3 Soot Debris

Smoke produced in the fire scene generally gets deposited on the surfaces and is named as soot particles. Forensic experts are recommended to analyse the soot present or recovered from window panes or doors so that one can determine and identify the ignitable liquids. Smoke residues as an evidence from the fire scene are quite common, and soot analysis can be done by various instrumental techniques including GC-MS, GC-FID and pyrolysis GC.

12.23.4 Concrete

Despite having low surface area and *adsorptivity*, concrete samples are recovered from the crime scene. Diatomaceous earth which is a suitable absorbent is sprinkled on the surface. It has a property to absorb liquids, which are then recovered and analysed.

12.23.5 Roof

Using headspace concentration and GC-FID techniques on the samples recovered from roof shingles, the chromatographic profiles resembled with those of diesel and kerosene. GC-MS techniques also proved to be successful for analysing roof material [1, 22].

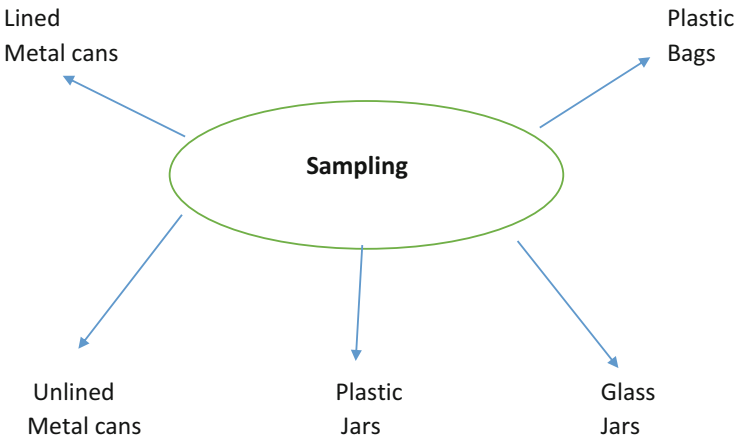
12.24 Sampling Containers

- Glass jars
- Lined metal cans
- Plastic jars
- Plastic bags
- Unlined metal cans

Plastic bags used for sampling are usually made from nylon; nylon is the best material for collection and transportation. Rubber sealed cans are also used as containers for storage. Cans are the most preferred containers for sample collection [17, 22].

12.24.1 Integrity of Containers

It is mandatory to maintain the integrity of the containers used for sampling. Containers of unknown quality should not be employed for collecting samples. Officials should make use of certified containers which are commercially available [9, 16, 22].



12.25 Laboratory Sampling

After obtaining samples from the scene, sample preparation is done. This is done to maintain the volatility of the samples. There is not any specified method for attributing the ignitable liquids because of different physical and chemical properties.

12.25.1 Conventional Method

12.25.1.1 Solvent Extraction

This technique is employed for extracting small container or when sample size is quite less. It is used in cases where there is high affinity of matrix with the sample. There is a high degree of co-extraction of unrelated matrix components. This in turn leads to poor sensitivity and complex chromatograms. Sampling with headspace can partially solve the problem; use of headspace is beneficial for compounds having lower boiling point [19, 24].

12.25.1.2 Steam Distillation

This technique is also not much used nowadays. It is used only when abundant ignitable liquid residue is expected in the suspected sample. Steam distillation technique overweighed adsorption technique as it showed superiority in analysis of low-to-medium-range ignitable liquid, for example, gasoline and gas oil [2, 6, 8].

12.25.1.3 Vacuum Distillation

Vacuum distillation is the variation of steam distillation technique. It is also used rarely. This technique is used for analysing fragile fire debris, for example, burnt documents.

12.26 Disadvantages of Conventional Method

- Time-consuming
- Laborious
- High interference in background
- Key component gets lost

12.27 Modern Methods

Modern methods were made to address the limitations faced in conventional methods [8, 9, 17].

12.28 Direct Headspace Analysis

In this technique, the sample is heated, and the aliquot of vapour headspace is drawn. The sample is further injected into GC. It is generally used as a screening method.

- This technique is useful in dealing with highly polar substances.
- It has poor sensitivity.

12.28.1 Dynamic Headspace Sampling

This technique focuses on the use of inert gas to purge the heated headspace of sample to induce the removal of volatile components completely.

- The subsequent sample gas stream passes through cooled or sorbent packed trap, retaining the volatile components.
- These volatile components are released by heating, and then quantification process is performed.
- In the newer methodology, dynamic headspace is totally automatic. Automatic thermal desorption system is used which is controlled by microprocessor.

12.28.2 Passive Headspace Concentration

Passive headspace concentration is a non-destructive technique and is simple as compared to the dynamic one.

- This technique is quite versatile and can be used according to the type of debris to be analysed.
- The original sample can be re-analysed which is the biggest advantage of this technique.

- Being performed in a closed system, there is no chance of depletion or contamination of the sample.

12.29 Sorbents

Carbon strips: Carbon strip technique is used commonly in fire investigation. The strips do not absorb water or any other gas and are virtually applied to any ignitable liquid and even water-miscible liquids. These are resistant to oxidation.

The limitation is that the strip cannot individualize between target compound and background compound.

Nowadays, porous polymer adsorbents are used. These sorbents are hydrophobic and adsorb volatile analytes without retaining water or Chromosorb, Porapak Q, Carbopack GC, etc. having their own properties and advantage.

12.30 Extraction Solvent

Carbon disulphide (CS_2) is the solvent of choice in fire investigation in forensic scenario. CS_2 has high solubility, and its efficiency in displacing organic molecules is quite high. Despite its merits, it is not much favourable because of its cost, flammability as well as toxicity [2].

GC-MS is the best technique for analysing arson or fire samples; with the trend of this technique, CS_2 is replaced with safer solvents. These solvents have less solubility properties but still are used. These are:

- Methylene chloride.
- Diethyl ether.
- Methanol.
- Pentane.

Out of all, methanol has high solubility.

12.31 Analysis

The analysis of fire evidences starts from the scene itself. Any ignitable liquid or accelerant used for causing or spreading of fire leaves its trace at the location where it is used. The trace evidence might be any mechanical, electric device or chemical. In case of accidental fire also, the cause is found at the origin. The eyewitness gives his/her testimony which helps the investigator solve the case. These evidences include the description of burning characteristics, the part of building which caught fire first. The investigator uses his personal experience, knowledge and skills to find out the area of origin. The official then reconstructs the scene on the basis of clues.

The main motive is to determine whether the fire is accidental or deliberate [6, 21, 25].

The laboratory analysis is based on the samples provided to them. The samples along with legal documents are important for analysis [17].

Most commonly recovered accelerants from fire investigation are kerosene, gasoline and fuel oil. So, firstly, volatile materials are analysed in the laboratory. The fire debris are usually recovered in sealed containers. Unlined metal paint cans are recommended for storing the debris as the cans do not carry any organic material. Rubber ring steal jars are also preferred for the same [14, 17, 21].

Volatile evidences are usually analysed by the head space vapour analysis technique. Petroleum products consist of complex mixtures of lots of aromatic and aliphatic components. They are classified into groups on the basis of properties such as flash point, distillate fraction or boiling point range. Chromatograms formed in headspace vapour residues are different from chromatograms generated by other techniques.

The vapours present in the evidence decide the method which can be used for analysis. Petroleum hydrocarbons are not similar in every laboratory. The brands and grades are classified into different classes [2, 5].

Class I: Brands and grades of automotive gasoline.
Class II: Brands of fuel oil (I) and kerosene.
Class III: Higher fuel oils.

12.32 Analytical Techniques

12.32.1 Gas Chromatography

GC is a commonly used screening technique for determining the suitability of fire samples and for actual identification. Short columns are now introduced which have extremely high performance and can do fast analysis. Multi-column chromatography provides unproved resolution. In this technique, two columns of different sensitivity are connected to the same injector. This technique is more useful than single-column chromatography [5, 9, 22].

12.32.2 Two-Dimensional GC

Co-elution and unknown inert ferments were two barriers in conventional GC. Two-dimensional GC X GC is a recent development which has potential to overcome the demerits faced earlier. The resultant data provide large and detailed information. By use of this technique, separation of complex mixture was increased, and thousands of peaks were resolved. Modulator is the key to this new technology. This modulator connects two columns. The sample gets transferred from the first column to the second one and compresses the analyte into a narrow peak. GC × GC

is usually used for analysis of crude oil spills and contamination. Therefore, it can be extensively used in forensic fire analysis and ignitable liquid detection. This method is able to separate thousand components in diesel samples and up to 300 components in petrol samples [5, 22, 24].

The detectors which are used with GC are:

1. Photo-ionization detector
2. Flame ionization detector
3. Fourier transform infrared
4. Microcells

Substance specific detectors are:

- Oxygen
- Electron capture detector
- Nitrogen phosphorous sulphur

12.32.3 GC-MS

Fire debris analysis is performed by GC-MS technique since many years. Recent developments have introduced a cheap, compact and user-friendly model of GC-MS. 90% of laboratories rely on this method for analytical examinations.

In case highly complex matrices are recovered, this technique enables a high degree of sensitivity. GC-MS is used in analysis of complex ignitable liquids and fire debris. Earlier the chromatograms generated produced a number of peaks, but with these instruments, amount of data is reduced, and rapid identification is possible.

Ideal sample for analysis by GC-MS is petroleum-based ignitable liquid. All the components present in petroleum including alkanes, aromatic hydrocarbons, cycloalkanes, etc. produce characteristic ions for each class of ignitable liquid.

The limitation of this technique arises when the target compound and background material consist of the same mass. During fire debris analysis, when the recovered sample comprises of petroleum based substances, for example a carpet, the carpet pyrolysates contains large amount of alkylbenzene. These alkylbenzenes are found in petroleum-based substances also. This leads to a barrier in identification of target compound.

Rodgers et al. introduced a technique called Fourier transform ion cyclotron (FT-ICR) MS. This technique is used for analysing complex mixtures by its ultra-high mass resolving power, high accuracy and capability of analysing the substances at a rapid rate. Moreover, this method provides a baseline resolution of multiple different elemental composition species. FT-ICR-MS provides molecular formula for all peaks present in the spectrum. This technique provides fingerprints that enable specific identification. Through its discrimination property and identification of fire debris samples and ignitable liquid, it is regarded as forensically viable technique.

Rella et al. combined thermal cold trap desorption with gas chromatography-mass spectroscopy (TCT-GC-MS) to overcome matrix interference effects which were

common in Arson cases in fire debris samples. The thermal treatment employed in technique reduces the matrix interference and provides a high degree of sensitivity [17, 22, 24].

12.32.4 Advantages of TCT-GC-MS

- Little sample destruction
- No need of solvents
- Rapid
- Economic
- Detects trace sample (100–150 mg)
- High efficiency

12.32.5 Gas Chromatography and Tandem Mass Spectrometry

In GC-MS/MS, the sensitivity of the basic process is improved because of the implementation of a new sequential mass spectrometer. This technique enables fragmentation of pathways of different ions to be tracked.

The MS/MS process breaks the phenomenon by targeting the characteristic ion and then subjects it to collision-induced dissociation (CID). It then checks the ion transitions and rejects those ions which do not follow the desired pathway. Hence, the matrix interference affect gets greatly minimized.

MS/MS is more sensitive than MS in analysing alkylbenzenes and naphthalenes. It can also analyse ignitable liquids like gasoline, kerosene, etc. It is capable of identifying residues even from household solvents. Hence, it is best utilized as secondary confirmatory analytical method.

Its ultra-specific characteristics are proven to be the best in reducing interference from GC-MS profiles. Moreover, it is highly efficient in analysing trace evidences. A study resulted that GC-MS/MS can confirm ultra-trace level of highly weathered petrol [5, 9, 22].

12.32.6 Other Technique

Besides GC, there are many analytical techniques which can be used for analysing samples recovered from the fire scene. These methods can be used as complementary methods along with GC.

Refractive index and flash point can be used to isolate the neat volatile liquid samples, if they are available in ample volumes.

Infrared (IR) spectroscopy also provides information regarding chemical structure of liquids after they have undergone through distillation process. A 10-microlitre sample is enough for performing this method [22].

Thin layer chromatography (TLC) and high-performance tin layer chromatography can be used for analysing debris-derived residues of petrol, diesel and kerosene [17, 22].

Volatile compounds are analysed by vapour-based ultraviolet spectroscopy. This technique has a number of merits as it is simple, cheap, rapid, highly sensitive and discriminative and can detect polycyclic aromatic hydrocarbon or alkanes [27–29].

- Cheap
- Rapid
- Highly sensitive
- Discriminative
- Can detect polycyclic aromatic hydrocarbons

12.33 Case Study

In Bharatpur, Rajasthan, a police chowki was found burnt due to unknown reason. A police official was suspected to be misguiding the investigation. The experts went to the scene to know the real cause behind it. This would help in laboratory analysis of debris and wires and to make a final report of the cause.

12.33.1 Observations

- The entire chowki was heat-affected, and firearms were recorded which were completely burnt.
- Those ammunition evidences confused the investigators during initial site visit.
- An aluminium pot used for making tea, and boiling water was found. The pot was melted from the bottom with a circular pattern.
- Two heating plates were also recovered from the scene. Out of these two, one was intact with a copper wire, but the other one was broken, and some extraneous material adhered to the copper.
- The heated plate melted the aluminium pan, and the melted aluminium dripped on the coil on heated plate which led to short circuit and fire.
- The police workers at the chowki connected the heating coil to the main power supply with aluminium. The wires were melted not far from the location of the heating coil. An aluminium wire consists of globules.

12.33.2 Results

The investigation proved that burned police chowki was caused by fire because of negligence. It was not caused by arson or any explosive. The composition of aluminium pan, aluminium pan collected from heating pan and aluminium wire on the heating plate was analysed using chemical testing, specific gravity, trace element analysis and X-ray fluorescence.

The melted material and aluminium pan were made from the same material. Through forensic investigation, the case was solved [26].

12.34 Case Study II

In Himachal Pradesh, there were two buildings of forest sales depot in the complex. Out of the two buildings, one was the office of the forest officer, and other one was a building used for storing coal and timber. One evening after the working hours, when the office was closed, fire was seen in the office and the storage area. The testimony given by the officials was that the office was locked from the outside, and government money worth 1.32 lakhs got burnt because it was placed in wooden almirah. They reported that fire has been caused by short circuiting of the electrical wires.

Forensic experts were called to ascertain the reason behind the cause of fire and whether the money was burnt because of the same reason as stated by the officials.

12.34.1 Observations

- The office and the storage area were completely burnt by fire.
- Water was present all over as the fire brigade tried to extinguish the fire.
- Debris of the burnt area were containing office files, papers and records out of which some were burnt completely and some were partially burnt. Most of the papers were decipherable.
- As stated by the officials, wooden almirah containing money was also investigated. Burnt wooden almirah and some papers were found. The written papers were decipherable.
- Nothing related to currency notes, pins of currency notes were recovered from the site, indicating that money was not placed in the wooden almirah.

The pattern of burning indicated that fire started in the two buildings at the same time but farthest from each other; it was not spread from one building to another. The deposition of soot, burning pattern on the bolts of doors and absence of locks specified that rooms were not locked; they were just blot from the outside. The electrical wires were unburnt and were having rubber covering, and this depicted that fire was not spread from one building to another.

The interconnecting wires were burnt near both of the buildings. When checked and analysed, it was found that there was no sign of electrical short circuit.

12.34.2 Result

On the basis of facts and clues gathered after investigation, it was found that the fire in both the buildings was started simultaneously. There was no sign of short circuiting, and hence, the case was found to be that of arson.

The absence of ash and pins of note bundles indicated that the cash was not present in the cash box or wooden almirah. Also, fire pattern helped in investigating that locks were not present and that doors were blot from the outside. Furthermore, all the printed or written material was easily deciphered as it was not carbonized.

Hence, the case was of arson, and the fire was deliberately caused by the officials [18].

12.35 Conclusion

Arson investigation is a very challenging task as all evidences are burnt because of fire. Arson attacks are planned and arranged in such a way that it can cause so much destruction; it causes so much difficulty for the investigators to find relevant evidence. All the evidences like fire debris are either destroyed or contaminated. The investigators must be familiar with all the principles, protocols and techniques to be employed for analysing evidences. GC proves to be the best instrumental technique for analysing evidences. Portable GC-MS devices are available which are sensitive and have high discriminative power. Many advancements are still going on to make the analysis process more specific, sensitive and easy.

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Psychological Evaluations: An Indoor Game of Criminal Minds

13

Shipra Rohatgi and Aastha Gupta

Abstract

Forensic psychology is the application of psychological practices and principles under the court of law. The chapter explores the principles and techniques that are used in forensic psychology to aid the legal system along with other contributing factors such as mental disorders or other factors involved in commission of crime. Combining the discussion of theory, this chapter also addresses the different roles of the forensic psychologist. Investigative techniques such as polygraph, narcoanalysis/truth serum, and Brain Electrical Oscillation Signature Profiling (BEOS), and forensic hypnosis play a very important role in helping the legal system by providing a corroborative evidence. By exploring the crime scene, the modus operandi used, and place and time of the crime and interviewing witnesses (if present), a lot can be known which can be further used to narrow down the suspect list which is really helpful for the police. This chapter will further address the ways in which forensic psychology is really helpful in both civil and criminal proceedings. The importance of forensic psychology in the court of law is further explained.

13.1 Introduction

This chapter permits a brief understanding on the field of forensic psychology which includes understanding and analyzing investigative techniques used, the kind of psychological evidences, and the behavior of the suspect or criminal which can be

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really helpful in understanding the crime scene. Forensic psychology contributes a very important role in helping the legal framework. Forensic psychology can be basically defined as practicing psychology within the court of law.

Even though "forensic psychology" is a relatively young field, the practice of intertwining law and psychology dates back to the 1800s. There is absolutely no doubt of contribution of forensic psychology to the execution of justice.

The father of forensic psychology is "Wilhelm Wundt." In Germany (1879), he established his first lab, and since then, forensic psychology has grown with immensurable contribution by other professionals. Another expert, "James Mckeen Cattell," has performed a number of analyses on psychological evidences.

"Alfred Binet" inspired by the Cattell's work studied the conclusions of other experiments of psychology applied to legal and system of criminal justice. His testing of intelligence was conjointly vital in the discipline of forensic psychology as several future tools of assessment were formed based on the work done by him. "Hugo Munsterberg" (1908) published *On the Witness Stand: Essays on Psychology and Crime* where he discussed the flaws of eyewitness memory, the role of hypnosis within a legal context, and detection of false memories.

Another important contribution in this field in 1917 was made by "William Marston" who is known as the "father of the polygraph" despite the contributions made by other experts before him. Marston remained the primary advocate for the use of polygraph lobbying in the courts. He published a book in the year 1938 called *The Lie Detector Test* where he explained the theoretical part and use of the polygraph. There was an advertisement produced by Gillette company where Marston was also casted who claimed that the usage of the polygraph has shown that razors produced by the company are better than other razors in the market.[1]

13.1.1 Forensic Psychology and Criminal Investigations

When there are few or no physical evidences left on the crime scene, then investigators generally give their attention in discipline of forensic psychology to understand and find what and how it occurred. The very known techniques of forensics that are used for investigation include fingerprint analysis, shoe print casting, or analyzing evidences such as hair and fiber. Recently, analysis of DNA as a forensic technique has greatly improved in solving crimes, but investigators turn to forensic psychological techniques when these evidences are very few or not present. Forensic psychology techniques such as polygraph, Brain Electrical Oscillation Signature Profiling (BEOS), and narcoanalysis/truth serum have been playing very important roles in criminal investigations. Some famous cases where one or more forensic psychological techniques have been used are the Veerappan case (2001), Nithari killings (2006), Abdul Karim Telgi case (2007), Aarushi Talwar case (2011), and the infamous narcoanalysis of Kasab and his confession about Mumbai terror attack on the Taj Hotel.

13.1.2 Criminal Profiling

Profiling of criminal or criminal profiling also goes by another name as offender profiling. It is the procedure of making deduction about characteristics of perpetrator from their actions during a crime. It helps in identifying the offender or perpetrator based on the analysis and mode in which the crime was committed. It is done on the basis of crime scene characteristics. Criminal profilers rely on psychological and behavioral evidences to find the offender's likely characteristics. Criminal profiling is largely prepared by the police with the help of forensic psychological experts. Because of psychological/criminal profiling, police or investigators are able to link the crimes and even narrow down the suspect list which thereby helps in solving the case more efficiently.

Criminal profiling has gotten people's attention from various famous movies like "Silence of the Lambs," "Hannibal," or "Red Dragon" or web series like "Criminal Minds" or "CSI" where offender profiling has been associated with the prediction of expert in understanding the offender's mind. However, the important thing to be kept in mind is that criminal profiling aids forensic investigation and is not a forensic technique because:

- It helps in giving a bigger picture on the likely characteristics of the offender and not the indication of the offender.
- Presently no particular scientific evidences are present that can support and provide the validity of the criminal/offender profiling in solving criminal cases (Fig. 13.1).

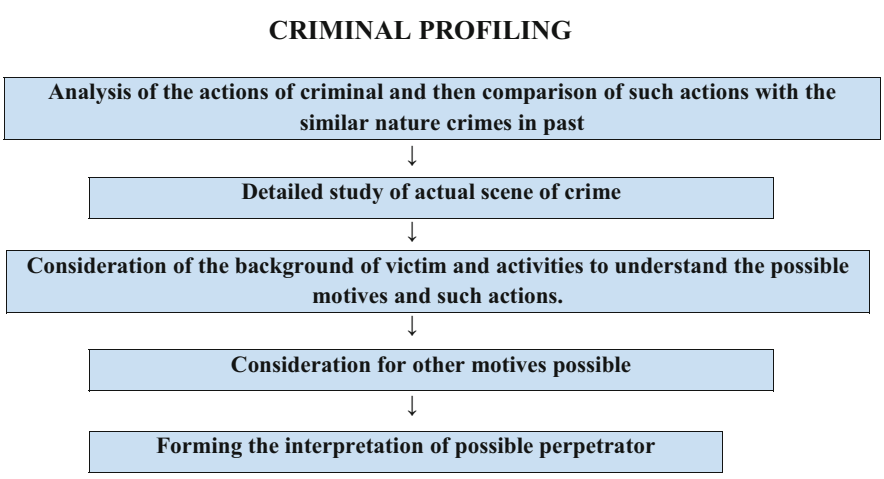

Fig. 13.1 Five steps of criminal profiling

13.2 Behavior Analyzed in the Crime Scene

Many things contribute to the success or failure in generating the profile or investigation of criminal, and there is need of an approach that is more empirical and systematic in nature which plays an important role for the decision-making process. Opposite to the approaches which are deductive in nature where generalizations guide the conclusions, the inductive approach is an empirical approach where scientific analysis derives conclusions.

For instance, general principles are derived by the methods that are inductive for understanding the behavior of serial killers by examining the facts empirically from an outsized number of cases that are solved in order to understand the behavior that has shifted with the time.

Opposite to the processes that are deductive in nature, which commence with assumption of the behavior, inductive profiling depends on the data collected from the scene of crime, reports of the police records, psychological assessments, reports given by the examiner, and victim reports which are empirically analyzed to check an assumption.[2]

13.3 Theories of Criminal Behavior

Many scholars have attempted to create various theories in order to explain crime systematically. Some of these scholars have even tried to modify the existing theories so that various parts of theory could fit together and exempt out the inconsistencies and contradictions of the positions.

This is done in order to formularize a theory which could provide explanation for crime of different types and categorize different types of offenders.

For example, occupational crime such as white-collar crime is different when compared to common thief's petty crime. Similarly, many theories have been formulated to explain the difference between female crime and male crime. Crimes committed by juvenile delinquents are also explained differently from the crimes committed by adults.

13.3.1 Classical Theory

The classicists defined crime as a behavior which breaches social contract, which is destructive not to the state as such but to the safety and property of those people in the community whose commitment to "contract in" established the state authority. Classicists not only focus on the conditions and influences but on the unlawful act itself. A renowned Italian thinker named "Beccaria" who was influenced by intellectuals like John Howard propounded this theory in 1764.

The main assumptions of Beccaria's classical explanation are as follows:

1. The behavior of a man is rational and purposive. This behavior is based on hedonism or on the principle of pleasure-pain, which means he constantly avoids pain and choses pleasure over it.
2. Each crime should be assigned a punishment, and the measure of this punishment should be damage done by a crime to the public welfare. Any pleasure derived from the commission of crime should always be outweighed by the pain.
3. Extreme and deterrent punishment should not be given, and this punishment should be crime proportioned, pre-determined, prompt, and communal. Instead of corporal punishment, more use of imprisonment should be there while insuring the use of fair trial. Along with this, there should be total abolishment of any kind of torture.
4. Legislation must be equally applied to all citizens.
5. Legislatures should clearly authorize the law and determine particular punishment for its violation. Judges should only determine whether or not an individual has committed a crime or has done any breach of law and never interpret the laws according to the ideas they have. In other words, whether an individual is innocent or guilty, deciding the punishment of a guilty person should be decided by the courts.[3]

Criticisms against this theory are as follows: Assumption of classicists is based on the point that all men are free, rational, and equal and not one of them has any kind of rank priority and no earlier disabilities or any kind of superiority, but this ideal model does not resemble in any way in the real world, and even this fact has been accepted by classicists. If men are equally rational as given in this classical theory, then why do individuals violate the law at all? Criminals are to be treated equally, and no differentiation should be made on the basis of age, sex, or intelligence. The nature of crime has been given no importance in this classical theory, that is, whether the crime is felony or misdemeanor. Similarly, no importance is given to the type of criminal, for example, whether the criminal is a first-time offender or habitual or a professional offender. Explanation of the person's behavior is simply done according to the doctrine of "free will," and the principle of "utilitarianism" helps in suggesting the punishment which fundamentally studies crime in the abstract and lacks the scientific perspective in empirical and objective approach. Lastly, there is not a single provision for criminal acts that are justifiable.

13.3.2 Theory of Evolutionary Atavism

Cesare Lombroso, an Italian physician and professor of clinical psychiatry and criminal anthropology, propounded the theory of evolutionary atavism in 1876. He is also known as the "father of criminology." This theory is also called as the "theory of physical criminal type" or "theory of born criminals."

According to him:

1. Criminals represent a definite "born" type.
2. Identification of this type of criminal can be made by specific physical abnormalities or peculiarities such as presence of asymmetrical face, ears which are mainly larger than average, excessively lengthy arms, compressed nose, a pulled back forehead, feathery and crispy hair, apathy toward pain, defects of the eye, and other physical anomalies.
3. The stigmata are the symptom of atavism and not the cause of crime.
4. An individual who is the criminal type cannot abstain from commission of criminal acts unless he lives under exceptionally favored conditions.
5. Criminals differ from non-criminals not only in physical anomalies, but they can also be differentiated according to the type of crime they commit.

At first, Lombroso came out only with one type of criminals which were "the born criminals" given by Ferri, but later he classified the other two types of criminals which are:

- *Criminaloid or occasional criminals*: These types of criminals differ from born criminals solely in degree and who coddled in crime due to precipitating aspects in the environment, that is, if they have or got an opportunity for the commission of crime.
- *Criminals by passion*: These are criminals who are in complete dissimilarity when compared with born criminals in points of anxious and emotional perceptiveness and also in the criminal motives such as love or passion.

The main criticisms against this theory are:

1. Collection of facts by Lombroso was narrowed to organic factors, and he ignored the factors such as psychic and social.
2. The above method for describing the criminals was mainly descriptive.
3. The research samples taken by Lombroso were generally very small and were not representative as it was only taken from the population of prisons.
4. There is no use of sophisticated statistical analysis.
5. Operational definition of their terms was not always clear and concise.
6. Presence of logic science errors because of the assumption that institutionalized populations represented criminals.
7. The approach that was used while formulating this theory has no anticipating value because many who have the anomalies attribute to criminals, but that doesn't mean that they become criminals, and many of them who do not have these anomalies do become criminals.[4]

13.3.3 Differential Association Theory

Differential association theory was proposed by Edwin Sutherland in 1939 and elaborated in 1947. He believed that delinquency is a learned behavior just like

any other behavior. The theory indicates that individuals become predisposed toward criminality because of an excess of contacts that advocate criminal behavior. Due to these contacts, a person will tend to learn and accept values and attitudes that look more favorably on criminality.

His main thesis is that individuals encounter many inharmonious and inconsistent social influences in their lifetime, and many individuals become involved in contacts with the carriers of criminalistic norms and a consequence become criminals. He called this process "differential association."

Sutherland proposed nine propositions for the differential association theory:

1. Criminal behavior is learned.
2. Learning of the criminal behavior occurs from interaction with other people through communication.
3. Within the intimate personal groups, learning of the criminal behavior occurs.
4. The learning of the criminal behavior includes:
   - Approaches of committing the crime, which are very easy
   - The particular cause of motives, drives, rationalizations, and perspective
5. The particular direction of motives is learned from definition of the legitimate code as favorable or unfavorable.
6. An individual turns into delinquent because of the presence of excess number of definitions favorable to breach of law over definitions unfavorable to breach of law.
7. Differential association may differ in frequency, period, priority, and severity.
8. The learning of criminal behavior by indulging with criminal and anti-criminal patterns includes all of the mechanisms that are present in any other learning.
9. Even though criminal behavior is a description of general needs and values, it is however not explained by those general needs and values since non-criminal behavior is an expression of the same needs and values.[5]

The criticisms against differential association theory are:

1. The theory was criticized, questioned, and claimed to be not testable by Sheldon Glueck.
2. There seemed to be a lack of reasons in theory proposed by Sutherland to explain acts of deviance that are not learned.
3. The ideas proposed by Sutherland seemed to be difficult to be put into action and evaluate quantitatively. Therefore, Akers and Burgess revised Sutherland's theory in their theory of social learning theory.

13.3.4 Social Learning Theory

Ronald Akers is an American criminologist known for social learning theory. According to him, in order to commit crime and gain the skills for commission,

individuals should develop motivation and associate with such people. Aker's social learning theory states that individuals, by observing and learning from the social factors present in their day-to-day lives, are able to learn the deviant behavior.

Robert Burgess and Ronald Akers in 1966 collaborated to revise Edwin H. Sutherland's differential association theory of criminal behavior. To show that discriminative cues activate the criminal behavior, Burgess and Akers cut down the nine propositions of Sutherland's theory to seven propositions which are as follows:

1. Learning of the criminal behavior occurs according to the principles of operant conditioning.
2. Learning of the criminal behavior occurs both in nonsocial and social situations.
3. The principal parts of learning happen in groups.
4. Criminal behavior is learned by including peculiar techniques and attitudes which is a purpose of the active and existing reinforcers and the existing reinforcement contingencies.
5. Behavior is reliant on the application of reinforcers which is based on norms.
6. Criminal behavior is a function of rules which are discriminatory for criminal behavior.

The forte of criminal behavior is dependent on the rate of recurrence and likelihood of its reinforcement.[6]

Social learning theory is also based on operant conditioning. Operant is a behavior that results in some consequences which have effect on future behavior. The theory is built on the postulation that the "primary learning mechanism is social behavior is operant (instrumental) conditioning in which behavior is shaped by the stimuli which follow, or are consequences of the behavior."

Direct conditioning and imitations of other are important in determining this behavior. Rewards or positive reinforcement as well as avoidance of punishment, or negative reinforcement, strengthens it. The determination of whether the behavior is deviant conforming depends on differential reinforcement, defined as "past and present rewards or punishments for the behavior and the rewards and punishment attached to alternative behavior."

These explanations help in reinforcing the behavior and serve as prompts for behavior. The more positive the explanations society have of a given action, the more expected they are to participate in it. These definitions are learned from peer groups and from family but also may come from schools and other groups.

13.3.5 Tarde's Imitation Theory

This theory is formulated by Gabriel Tarde (1843–1904). He presented significant interest in social problems and countered Cesare Lombroso and the positivist school.

According to him, individuals are not "born criminal"; instead, they become criminal. He saw criminal conduct as the outcome mainly of social influences, a belief that establishes one of his greatest contributions in the field of criminology.

Social-process theory of criminal behavior given by "Tarde" is reflected in his belief that altogether, the "important aspects of social life are carried out under domination of example." Upon this belief, he framed the "Theory of Imitation." He described the method of attaining criminal and non-criminal behavior.

13.3.5.1 Developing Theory

Tarde distinguished between fashion and custom. Fashion is representative of imitation that occurs in crowds or cities where the interaction is close and regular. Custom can be described as the phenomenon that happens in small towns and rural areas where interaction is less close or frequent and variations are fewer. Fashion may uproot and create new custom.

Tarde also gave three laws of imitation which are:

- **First law of imitation:** *Men imitate one another in proportion as they are in close contact*.
- **Second law of imitation**: *Inferior imitates the superior*.
  Peasants replicate royalty; small-town and rural population imitate the acts of residents living in city. Tarde penned: "Infectious epidemics spread with air or the wind; epidemics of misconduct trail the line of telegraph."
- **Third law of imitation:** *The law of insertion*.
  When two equally exclusive fashions come together, then one of the fashions can be replaced by the other. When this take place, then there is deterioration in the older method, and the newer method increases.[7]

13.3.6 Labelling Theory

This theory was propounded by Howard Becker in the year 1963 and is also known as "social reaction theory." This theory does not explain or deal as to why a person became a criminal, but instead it throws a light on why a society labels some people as deviants or criminals. It also tries to explain criminal professions in relations of the damaging social communication and social interaction produced by stigma.

"Crime" and "Deviance" are always defined by social audiences, and Becker described those who make these social rules as moral entrepreneurs. Labelling a particular group as "outsiders" by social groups creates deviance. Individuals are given various kinds of social labels that define the individual as a whole. A person's self-image is reduced by stigmatizing him or her by negative labels, and it is the social groups that create definition of negative and positive labels. Criminal behavior may actually be maintained and amplified by these labels.

So, this theory emphasized that labelling actually causes deviant behavior, and when an individual is labelled as "deviant," he or she might conclude that this is the behavior that others might expect from them, and they end up becoming the same.[8]

13.4 Stages of Labelling (Fig. 13.2)

There can be many consequences of labelling such as:

- Stigma is produced by labelling.
- Condemnation or degradation starts getting carried out in ceremonies like trials and media.
- The individual starts self-labelling which involves captivating those defiance and characters which basically reflect upon how an individual is viewing himself or herself which is based upon how others see them.
- Few of the labelled individuals may also connect with cliques such as deviant cliques or other outcast peers.
- Labelling may also cause retrospective reading which means reassessing the past of a person in order to find the suitable or the current generalized label, and these labels often become a person's personal identity.

Research suggests that evidence supports the target of labelling specially for the poor and powerless people, and these people are victimized by law and system of

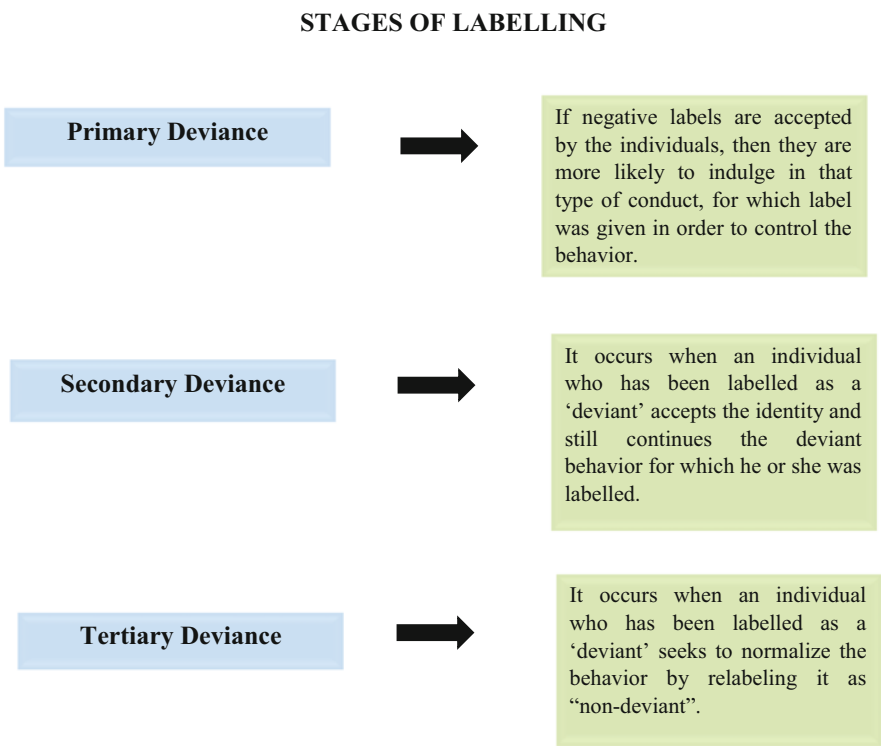

Fig. 13.2 Three stages of labelling

justice. It has been seen that there is presence of contextual discriminations in the justice system. Empirical evidence supports those labels which are negative and which affect the self-perception of the person and often provide cumulative disadvantage thus provoking repeated behaviors.

Criticisms that are made against this theory are that it disregards the beginning of deviant behavior and is not even able to stipulate the condition which must occur previously to an act or if a person is labelled and that too negatively labelled. Along with this, it has been seen that this theory fails to explain the differences in rates of crime.[9]

13.4.1 Rational Choice Theory

The theory of rational choice states that a person makes a rational choice by using rational calculations and thus are able to achieve outcomes that are affiliated with their own particular objectives. An individual's best, self-interests are also related to such results. With the help of using the theory of rational choice, one can also predict what will be the end outcomes which can help in providing individuals with the finest benefits and satisfaction given the available alternative needs. Many economic assumptions that are mainstream and theories have supported the theory of rational choice. Rational choice theory is generally discussed and is mainly related to the notions of rational actors, the rationality postulation, and self-interest.

The theory of rational choice is based on the idea of participation from rational actors who are the people in an economy making rational choices or supportive of rational calculations and available rational information. Rational actors formulate the idea of rational choice theory and are what makes an effective rational choice theory. The theory of rational choice assumes that people are rational actors' information which is rational in order to actively take full advantage in any situation and therefore constantly try to minimalize their losses.

Much of these ideas can be traced back to the economist Adam Smith. Smith, who wrote in the 1700s, tried to make sense out of how people come to make choices, especially economic ones. Rational choice theory has found its modern home in an article written by the Nobel Prize-winning economist Gary Becker (1968). As a part of broader studies to understand certain behaviors existing in society, economists may use the rationality assumption. The rationality assumption assumes that every person is likely to be rational actors making choices that are rational based on the theory of rational choice in order to understand what best results are there for themselves and their self-interests.

For instance, a good illustration of rational choice theory is white-collar crime. An investment banker chooses to skim money from the account of his clients and then skins the loss, and then the banker uses that cash to fund his/her lavish lifestyle. The criminal of white-collar crime premeditated and then weighs that choice of himself or herself. He then decides the personal advantage of stealing the money which overshadows any chance of theft being discovered by him or her.[10]

Criticisms against the rational choice theory: Several economists do not believe in the theory of rational choice. Dissidents have pointed out that people do not make rational utility-maximizing decision every time. For instance, the discipline of behavioral economics is built on the idea that people often make decisions which are irrational and explores why they do so. Nobel laureate Herbert Simon proposed the bounded rationality theory, which says that individuals are not always able to gain all the information they would need in order to make the best decision. Christopher Simms of Dalhousie University in Halifax, Canada, showed that when individuals are anxious, they mostly fail to make rational decisions. According to him, the theory has no scope of explanation for decision made by mentally challenged individuals.

For instance, political parties in favor of the Brexit vote was held on June 24, 2016. Promotional campaigns which were constructed on emotion rather than rational analysis were used.[11] These campaigns were semi-shocking, and the result of the vote was unexpected, when the European Union was officially left by the United Kingdom. The financial markets then responded with shock, which increased short-term volatility, as calculated by the CBOE Volatility Index (VIX).

13.4.2 Social Control Theory

This theory was propounded by Hirschi (1969) and is also known as the social bond theory. According to this theory, the delinquents fail to form or maintain a societal bond consisting of attachments, commitments, involvement, and belief. Everyone has the potential to violate the law. Sociologists apply the term "social control" to those mechanisms by which a normative social system is maintained by society. So social control basically means a control of society over individuals.

"Self-control" refers to a strong moral sense that prevents a person from hurting others and violating social norms. "Walter Reckless" argued that a strong self-image insulates an individual from criminogenic influences of the environment. Similarly, even "Howard Kaplan" suggested that young individuals or youths who have poor self-concept are likely to engage in criminal behavior.

There are many factors which suggest why a social control is necessary or needed to maintain an order which is a must for every society. A social control is also necessary in order to regulate an individual's behavior and therefore establish social unity. This control also helps provide a social sanction and check cultural maladjustment. "Hirschi" also linked the onset of criminality to the weakening of the ties than to bind people to society thus establishing a social bond. His concept of social bond comprises four elements which are:

1. **Attachment:** It refers to the sensitivity and interest in others. Factors such as development and attachment with family, friends, co-workers, and the community make an individual sensitive, therefore increasing and strengthening the companionship.

2. **Commitment:** It basically refers to investing time, energy, and effort into the conventional activities. It is only rational for a person to be concerned with his or her self-respect. A person commits toward family, career, success, and future goals.
3. **Involvement:** When an individual is involved in activities like sports, school, and other recreational pursuits, he is less likely to be indulged in criminal actions. This happens because when a person devotes energy to a productive task, he or she reduces chance to offend.
4. **Belief:** It is moral respect for the law and social values. It is seen that people with strong and high moral values or belief in law are less expected to indulge in any sort of criminal activity. These beliefs are based on the constant social reinforcement. A fundamental understanding of right and wrong is very important in reducing such criminal activities. These beliefs include honesty, morality, fairness, patriotism, responsibility, etc.[3, 12]

Some of the arguments that have been made against this theory are that this theory was not able to explain all the modes of criminality and is therefore restricted in scope. Bonds do change over time, and not all elements of bonds are equal as well, that is, some of the individuals are much involved and still not attached. The major criticism that Hirschi's theory faced was the notion that delinquents are detached loners, but it is also seen that people with lots of connections also commit crimes (e.g., corporate crimes). Some individuals are very much attached to deviant groups, and therefore wrong direction of association might be calculated (Fig. 13.3).

13.5 Personality Disorders and Criminal Behavior

Research suggests that rates of personality disorder are higher especially antisocial personality disorder which is found among individuals in correctional and forensic mental health settings when compared to the general community.

A deep-rooted relationship between personality disorder and violent reoffending that an occurrence of personality disorder has been included as a risk factor in tools calculating risk assessment.

The Hare Psychopathy Checklist-Revised (PCL-R) which is often used to check psychopathy which is a specific subtype of antisocial personality has become a well-established tool to predict danger of violent reoffending. It is very important to comprehend the relationship between personality disorder and offending in order to provide the correct treatment and to prevent future reoffending.

Symptoms of Anti-social Personality Disorder

1. Indifference of right and wrongdoing
2. Persistent lying or trickery to exploit other people
3. Being callous, skeptical, and disrespectful to others
4. Using charisma or wit to deploy others for personal advantage or pleasure
5. Egotism, a sense of dominance and being awfully opinionated

Theory	Propounder	Year	Main thesis the crime is result of
Classicist	Beccaria	1764	Man's rational motivation Pleasure-pain principle
Atavism theory	Lombroso	1876	Physical stigmate
Differential association theory	Sutherland	1939	Association with & social influences of criminalistic norms.
Social Learning Theory	Ronald Akers	1966	Learning and observing through the presence of social factors.
Tarde's imitation theory	Gabriel Tarde	1843-1904	Criminal behavior is learned, people are not born criminals. They become criminals
Labelling theory	Howard Becker	1963	Labelling certain groups as 'deviants' or 'outsiders' by society may cause deviance.
Rational choice theory	Gary Becker	1968	People use rational objectives that are aligned with their personal benefit.
Social control theory	Hirschi	1969	Delinquents fail to maintain any form of societal bond.

Fig. 13.3 Different theories and the cause of crime

6. Repeated problems with the law, as well as criminal behavior
7. Frequently violating the rights of others through pressure and deceitfulness
8. Impulsiveness or failure to plan ahead
9. Resentment, significant irritability, distress, aggression, or violence
10. Absence of empathy and lack of guilt about harming others
11. Taking unnecessary risk or risky behavior with no respect for the safety of self or others
12. Relationships that are abusive or poor
13. Failure to ponder on the negative consequences of behavior or to learn from them
14. Being constantly careless and frequently failing to accomplish work or financial commitments

Adults with antisocial personality disorder primarily show symptoms of conduct disorder before the age of 15.

Signs and symptoms of conduct disorder include serious behavioral problems such as:

1. Being aggressive toward people and animals
2. Causing damage or destruction to property
3. Deceitfulness
4. Theft
5. Serious violation of rules

People with antisocial personality disorder repeatedly violates the law and become criminals. Such individuals lie, conduct themselves abruptly or aggressively, and have problems with drug usage and alcohol. Due to the presence of these traits, such individuals are unable to fulfil responsibilities associated with family, work, or school.

Risk factors that are likely to increase the risk of antisocial personality disorder are:

1. Diagnosis of conduct disorder in childhood
2. Presence of antisocial personality disorder or other personality disorders or mental health disorders in family history
3. Being exposed to abuse or neglect during childhood
4. Not being stable or violent or chaotic family life in childhood [13]

Ways to avoid antisocial personality disorder from developing in individuals who are at risk are still not present, and it is assumed that this personality disorder has its roots in childhood. Early warning signs may be spot by parents, teachers, caregivers, and pediatricians. Early diagnosis may help children who show symptoms of conduct disorder be given early intervention to prevent them from becoming future offenders.

Many researches have proved that offending is related with personality disorder. It has been found out that rates of personality disorder are altogether high in serious criminals, but the part played by personality disorder could also be greater in some crimes than others, for instance, rapists compared with child molesters, men who murder their fathers instead of their mothers, men who murder their children with mothers who murder their children, and in less severe stalking behavior with those who get convictions.

The important point to be remembered here is that such researches are able to show a link between personality disorder and offending but show us nothing of the casual link. Any act of criminal behavior usually arises from interaction of various factors such as predisposing characteristics of individual, circumstantial factors, for instance, a physical and social context.[14]

Section 84 of the Indian Penal code deals with the act of a person of unsound mind: "Nothing is an offence which is done by a person who, at the time of doing it, by reason of unsoundness of mind, is incapable of knowing the nature of the act, or that he is doing what is either wrong or contrary to law." [15]

This is a modification of the Mc Naghten's rule. The burden of proof lies upon the suspect. He must prove in a court of law that he was of unsound mind at the time of doing the act. The degree of unsoundness must be such that he did not know the nature of his act, or he did not know that his actions were either wrong or contrary to law.

13.6 Intelligence and Offending

Intelligence has been found to predict wider range of antisocial and criminal behaviors including violent and chronic offending. Lesser people with profound intellectual disabilities commit many criminal acts since arts of crime assume mens rea; if they go into the justice system, they are probably diverted to mental health, intellectual disability, or forensic psychological state service system through the courts.

Many previous researches investigating the role of cognitive and affective empathy to behavior of offending found that the connection between low empathy and offending vanished after controlling intelligence and social economic rank (Jolliffe and Farrington 2004). It is done not to neglect the notion of empathy but relatively to understand the emotions of other people which could also be chief function of intelligence, which eventually links with criminal activities (Jolliffe and Farrington 2004). Roughly the research that has been done with the intention to look or point at the roots of offending behavior, predominantly juvenile delinquency which continues to spot at the origins of offending behavior, and more particularly juvenile delinquency that remains to spot the low IQ as a interpreter of the offending behavior (Diaz et al. 1994).[16]

Such people are protected under the Right of Persons with Disabilities Act, 2016. The Act substitutes the Persons with Disabilities (Equal Opportunities, Protection of Rights and Full Participation) Act, 1995. It fulfils the requirements to the United Convention on the Rights of Persons with Disabilities (UNCRPD), to which India is a signatory. This Act came into power during December 2016. Disability has been well defined based on developing and dynamic concept. The types of disabilities have been increased from existing 7 to 21, and the Central Government will have the power to add more types of disabilities. The 21 disabilities are given below:

1. Blindness
2. Low vision
3. Leprosy-cured persons
4. Hearing impairment (deaf and hard of hearing)
5. Locomotor disability
6. Dwarfism
7. Intellectual disability
8. Mental illness
9. Autism spectrum disorder
10. Cerebral palsy
11. Muscular dystrophy
12. Chronic neurological conditions
13. Specific learning disabilities
14. Multiple sclerosis
15. Speech and language disability
16. Thalassemia
17. Hemophilia

18. Sickle cell disease
19. Multiple disabilities including deaf-blindness
20. Acid attack victim
21. Parkinson's disease

People with "benchmark disabilities" are defined as those certified to have at least 40 percent of the disabilities specified above.[17]

13.7 Socioeconomic Factors and Crime

Crime accompanies social life from the very beginning and occurs in every society, in every stage of its development irrespective of its structure, system, or even period. Undoubtedly, crime may be a consequence or a result of many social and economic problems which constantly change.

Crime and changes within the structure of crime both suffer from such elements as the degree of economic development, socio-form of government that functions in a given country, the progress of industrialization and urbanization, transformations in social organization which are age related to members of the society, and eventually migrations. Transformations could also be administered during a revolutionary way or throughout an extended period of time. These changes can also happen rapidly because of some raging changeovers and sudden changes which can take place in a community.

Changes within the number related to the factors such as, sex, age, structure, migration (demographic changes), and even their shared impact associated with the economy, system of power, education, health protection, religion, and crime. Economic situations such as poverty may transform into criminal activities with the rise in unemployment. A very important point to be remembered is that unemployment is naturally associated with economy which may have a different dimension.

Unemployment can be further classified into structural, cyclical, long-term, and frictional unemployment. When individuals suffer from unemployment, due to the social and demographic factors like gender, age, or educational level, this could have various links and impact on offending activity.

Many analyses have been done on police statistics which depicts that the very best intensity of the criminal activity occurs among individuals of less than 30 years of age and unemployed. If a person is suffering from long-term unemployment, he then starts to suffer from results of such circumstances, namely, a way of segregation, prejudice, and eventually the lack of hope to find a source of income that is legitimate.[18]

Such study has shown that unemployment brings on crime against property instead of violence. Nevertheless, an important point to be noted here is that the rise in job loss in different ways may affect specific social groups by the increase or decrease in their criminal activity.

So, four specific relationships between unemployment and crime can be outlined which are:

1. Some perpetrators associate their legitimate work with their criminal activity. Legal occupation is then used as a cover-up for illegal activity or occupation. During this event, the case of unemployment may lessen the "gray zone" business, because the legitimate work during this situation gives a way of cover-up or security for carrying out the illegal or criminal activity.
2. There are variety of misconducts, probable to be conducted only during activities being employed legally, for instance, "Bribes are handed over to officials or higher authority, "employee theft." In those cases, the number of crimes of abovementioned type will be inhibited by unemployment, instead of increasing them.
3. Children, especially differentiate between two choices which are either being employed legally or being involved in an unlawful activity. If there is shortage of job or work, then the will to require a revenue from the sources that are illegal could also be conclusive. Being unemployed, during this viewpoint, may cause a rise in illegal activity or a crime.
4. There are many individuals for whom unemployment is firmly associated with their style of living. This group of individuals treat with their style of living. This group of individuals treat legitimate occupation as a situation that is abnormal to those people who are not a part of the market. For such people circumstances such as shortage of employment is a component of their cultural individuality, and the illegtimate activity in their case is accepted socially as their source of income. When such situation happens, a rise in joblessness or unemployment will not have any effect on the development of criminal behavior.

13.8 Investigative Techniques Used in Forensic Psychology

The discipline of forensic psychology remains an early branch of industrial psychology within the early twenty-first century. It has been documented by the APA in 2001 and recertified in 2008. In 1991, Specialty Guidelines for Forensic Psychologists were accepted by the American Psychology of Law Society, APA Division 41. It makes use of different investigative techniques to detect deception. An important point to be kept in mind is that these techniques provide an aid to investigation but are not a substitute for the investigation. The forensic psychological evidences are used as corroborative evidences in the court of law.

The different forensic psychological techniques that will be discussed below are:

1. Polygraph
2. Narcoanalysis or narcosynthesis
3. Brain Electrical Oscillation Signature Profiling
4. Forensic hypnosis

13.8.1 Polygraph

Polygraph can be defined as a device that is used for recording of changes in blood pressure, pulse, respiration, and skin resistance as indication of emotional disturbances specially of lying when an individual is subjected under questioning. The word "polygraph" was derived from the word *poly* meaning "many" and *graphs* meaning "writing chart." Therefore, polygraphy can be described as the scientific method of detecting deception, using a polygraph instrument. The polygraph is employed to check or question a person with the aim of detecting deception or verifying truth of statements through a visible, perpetual, and instantaneous recording of an individual's cardiovascular and respiratory pattern as a minimum instrumentation obligation.

A polygraph (mentioned commonly as a lie detector) is a device which measures and records various physiological responses such as:

- Blood pressure
- Pulse
- Respiration
- Skin conductivity

These physiological measures are checked while the subject is asked and responds to a series of queries, on the idea that untruthful answers will produce distinguishing measurements.

The polygraph records physiological changes which are produced by the sympathetic nervous system while an individual is under questioning. Within the US federal government, a polygraph examination is also known as a psychophysiological detection of deception (PDD) examination. Other different technologies also are utilized in the sector of lie detection, but the polygraph machine is the one that is the most famous.

13.8.1.1 History of the Polygraph

The notion that deceitfulness produces physical side effects has long been appealed. In West Africa, people who were accused of a criminal offense were compelled to pass an egg of a bird to at least one another. If an individual broke the egg, then the person was considered to be guilty, and this was supported by their thought that a person's nervousness was responsible. In ancient China, the suspect was asked to hold a couple of rice in his or her mouth during a speech of prosecutor. Since it was believed that salivation ceases sometimes of emotional anxiety, the individual was considered guilty if the rice was dry by the end of the speech.

Cesare Lombroso (1895) employed the very first scientific instrument for detecting deception known as "hydrosphymograph," which recorded the changes in pulse and blood pressure when accused were questioned about their participation of a specific offense.

"Pneumograph," an instrument that graphically records the subject's inhalation and exhalation to detect deception, was successfully developed by Vittorio Benussi.

He therefore demonstrated changes in breathing patterns that accompanied deception.

There was a research conducted by William Marston in 1917 which addressed the "sphygmomanometer," which was accustomed to find periodic intermittent vital sign interpretations during the period of an examination. An instrument recording both blood pressure and galvanic skin response, it was invented by Dr. John A. Larson (1920) of the University of California, and it was first used by the Berkeley Police Department for the law enforcement work under its nationally well-known police chief August Vollmer. Additional work on this device was done by Leonarde Keeler.

The term "polygraph" for the first time was used by James Mackenzie in 1906 in his invention of the "ink polygraph," which was used for medicinal reasons. A second paper was written by Mackenzie in 1915 on this topic in 1915, while he was finishing his undergraduate studies. He got admitted into Harvard School of Law and graduated in 1918, re-publishing his earlier done work in 1917.

According to their son, Marston's wife, Elizabeth Holloway Marston, was also involved within the development of the systolic blood pressure test: "According to Marston's son, it had been his mother Elizabeth, Marston's wife, who suggested to him that 'When she got mad or excited, her blood pressure seemed to climb' (Lamb 2001). Although Elizabeth isn't listed as Marston's collaborator in his early work, Lamb, Matte (1996), et al. refer directly and indirectly to Elizabeth's work on her husband's deception research. She also appears during a picture taken in his polygraph laboratory during 1920s (reproduced in Marston, 1938)."

A famous character of magazines which is "Wonder Woman" inspired by Elizabeth Marston was created by William Marston. Wonder Woman carries a magical rope which was modelled upon the test of systolic blood pressure. Although Elizabeth isn't listed as Marston's collaborator in his early work, Lamb, Matte (1996), et al. refer directly and indirectly to Elizabeth's work on the research done on the deception by Marston. She also appeared during a photo taken in his polygraph laboratory during the 1920s (reproduced in Marston, 1938).

Despite the contributions made by predecessors, Marston was the self-proclaimed "father of the polygraph." Marston remained a primary advocate of the instrument, petitioning for its use within the system of courts. He also published a book in 1938 entitled *The Lie Detector Test*, wherein he documented the knowledge and use of the polygraph. In the year 1938, he appeared in an advertisement produced by the Gillette company claiming that Gillette razors were better than the competition as shown by polygraph.[19]

There are two types of polygraphs – analog and computerized polygraph. Analog polygraphs are polygraphs shown in movies, which contained a single strip of scrolling paper scribbled by little needles, while computerized polygraphs have sophisticated algorithms and computer monitor replacing the scrolling paper.

13.8.1.2 Components of the Polygraph

Cardio-sphygmograph: It measures the blood pressure and heart rate of the subject. This is measured with the blood pressure cuff that is wrapped around the arm of subject. This cuff remains inflated when the subject is under questioning.

Pneumograph: It records the respiratory rate of the subject. This component of polygraph has two tubes, one of which is wrapped around the chest, and other tube is wrapped around the abdomen of the subject.

Galvanograph: The amount of perspiration produced is measured by this component of the polygraph. Galvanometers are electrical sensors which are attached to the fingertips of the subject. Galvanometers are attached to the fingertips because these are the areas which contain high density of sweat glands, which makes them a best location to measure the perspiration.

13.8.1.3 Principle Behind the Use of the Polygraph

The nervous system of the human body contains:

1. *Central nervous system (CNS)*
2. *Peripheral nervous system (PNS)*

The central nervous system contains the brain and spinal cord. It is referred to as central because it is involved in combining the information received from the entire body of an organism and then coordinating the activity. The peripheral nervous system contains all those nerves that lie outside the CNS. Its role is to basically connect the CNS to organs, limbs, and the skin.

The peripheral nervous system is further divided into:

1. **Somatic nervous system:** The primary role of this nervous system is to carry out the sensory and motor information to and from the central nervous system.
2. **Autonomic nervous system:** The autonomic nervous system is in control of regulating a variety of bodily processes that take place without conscious effort. It maintains blood pressure, controls breathing rate, influences digestion and urination, and modulates sexual arousal. The autonomic nervous system can be further divided into **sympathetic nervous system** and **parasympathetic nervous system**.

Sympathetic Nervous System: The neurons of this nervous system emerge from the thoracic and lumbar regions of the spine. The nervous system is typically activated when there's need for response to emergencies. The sympathetic division of the autonomic nervous system regulates the fight or flight responses by releasing neurotransmitters like epinephrine or norepinephrine. It basically controls the body's response to the perceived threat, and the body accelerates, tenses up, and becomes more alert. Contraction increases in the circulatory system, and thus heart rate increases. Bronchial tubes dilate in the pulmonary system. Muscles contract and pupils dilate. There is a decrease in stomach movement and secretions in the gastrointestinal system. Saliva production is also decreased in the salivary glands,

and the adrenal gland releases adrenaline. Glycogen to glucose conversion increases, and there's decrease in urinary output.

Parasympathetic Nervous System: The parasympathetic neurons are related to cranial and sacral regions. It helps in maintaining normal body functions and conserves physical resources. This branch of nervous system monitors the body's response while at rest and activates the response of rest and digest and neuron pathways are longer and slower. The parasympathetic nervous system basically counterbalances the general body response. Bronchial tubes constrict, and there is decrease in heart rate. The musculoskeletal system relaxes, and pupils constrict. Stomach movements increase, and there is increase in secretions of GIS; there is also increase in urinary output. The neurotransmitters released here are acetylcholine. The parasympathetic response is associated with enhancing growth and reproduction.[20]

13.8.1.4 What Happens when We Are in Stress?

Stress is a response which can be either biological or psychological which is experienced on encountering a danger that we feel we do not have the resources to deal with. A stressor is a stimulus (or threat) that causes stress. Our body judges a circumstance and then decides whether or not the circumstance is stressful. This decision is formed supporting sensory input and processing of the information (i.e., the items we see and hear within the case) and also in stored memories (i.e., what happened the last time we were during a similar case). This stressor also can be physical within the sort of exertion and may even be emotional which may include fear, lie, anger, excitement, detection, etc.

If the case is referred to as being stressful, then the activation of hypothalamus is seen. The hypothalamus within the brain is responsible for the strain response. When a stress response is activated, it sends signals to two other structures: the pituitary and also the medulla.

The medulla is also activated by the hypothalamus. The medulla is a component of the ANS. The ANS is the region of the peripheral nervous system that acts as an effect system, maintaining homeostasis within the body. These activities are generally performed without conscious control. The hormone adrenaline is secreted by the medulla, and this hormone makes the body ready for a fight or flight response reflex including the increase in pulse rate.

Adrenaline causes stimulation of the sympathetic nervous system and decreased activity within the parasympathetic nervous system. It also causes bodily changes like decrease in digestion and increase in sweating, pulse rate, and blood pressure. Once the "danger" or "threat" is over, the parasympathetic branch takes control and brings the body back to a balanced state.[21]

13.9 Psychological and Physiological Symptoms of Guilt

Sweating and change of color: Anger is indicated by sweating along with the flushed face, embarrassment, or extreme nervousness. Shock of fear is indicated by sweating along with the facial pallor. Sweating of hands indicate tension. A pale face may be a more common guilt sign. Nervous tension causes reflex inhibition of secretion of saliva which causes dryness of the mouth, and because of this there is continuous swallowing and licking of the lips. On the account of the dryness of the throat apart from the mouth, the person will continuously swallow saliva from the mouth which causes frequent upward and downward movement of the Adam's apple**.**

Fidgeting: It is when the person is consistently moving within the chair, pulling his ears, rubbing his face, picking and tweaking of the nose, crossing or uncrossing the legs, touching the hair or eyebrows, rubbing his or her eyes, biting, or snapping of fingernails. These indicate that there is nervousness.

It has also be seen that a subject who is guilty frequently utters such expression—"I will die if I am lying," etc. Such statements are made to form powerful and substantial proclamation of innocence. Sometimes, the subject may very strongly proclaim that it is not possible for him to do anything like that since he is a religious man, and this is something that cannot be done by him.

The guilty subject doesn't wish to look right at the investigator or have a face-to-face interaction for distress that his guilt may see in his eyes. He will rather consider staring the ground or ceiling. The guilty subject sometimes will use the expression of "I don't remember anything like that" expression when responding to be elusive or to evade committing something prejudicial to him.

Examination room: Important points that should be kept in mind while conducting the test is that the examination room should be private and free from any sort of external noises or distractions. It should not contain any sort of decors like paintings or showpieces. The room should have good ventilation and should be properly lit. Only the polygraph desk and the subject's arm chair should be present, and there is no need of any sort of furniture. It is preferred to have a presence of one-way mirror along with the sound system so that the test can be monitored properly and precisely to the authorized audience.

Test subject: Before conducting the polygraph test on the subject, the examiner needs to examine whether the subject is fit for the test. If there is presence of any temporary illness or any sort of condition, for instance, sickness, pain, infection, any sort of physical discomfort, or mental exhaustion, or if the subject is under the effect of alcohol or any sedatives, then the test should not be conducted till the subject recovers his normal physical or mental condition.

A subject is considered unfit for the polygraph if there is presence of any permanent physical illnesses like certain conditions of the heart, respiratory problems, or addiction to any narcotic drugs. It should also be checked whether the subject has undergone any treatment before he or she was asked to undergo a polygraph test, as this may affect the results of the polygraph test. It is also seen that the subject may react falsely to the polygraph test or may even have a flat

emotionless chart if he or she has been under prolonged interrogation, and this is the reason why a person's daily habit should not be upset more than necessary.

13.9.1 Three Phases of the Examination or Polygraph Test

Before conducting the examination of the subject, the examiner also has an initial interview with the investigator to obtain the detailed and accurate information regarding the case and the subject, for instance, the details that only the investigator and the subject know, information regarding the crime scene, any past police records, result of any lab test, etc.

(a) **Pre-test interview:** This is the initial and informal interview between the subject and the examiner which lasts for 20–30 min. The main purpose of this interview is to condition the subject psychologically for the test and to explain the purpose of it. It is necessary to tell the subject of his or her constitutional rights, and a free, voluntary consent is given by the subject for the conduction of test.

(b) **Actual interview and recording of the test:** There are four approaches that are used while conducting the test. For all the approaches, some common things should be kept in mind: there should only be 12–15 number of questions that should be asked to the subject; three charts are at least taken; and each test should last not more than 4 min.

- **Control question test:** Control Question test is also known as comparison question test. It was initially developed by Reid in 1947, and Backster further conceptualized it. This is the most common approach that is used in polygraph. CQT consists of five phases:

  Pre-interview phase: It is premeditated to obtain info about the accused and the crime scene. The examiner asks the subject some basic biographical information including information about physical and mental problems and discusses the accusations against the examinee.

  Stimulation test: Here it is proposed to persuade the examinee that the polygraph is able to detect the lie. The notion that polygraphs are 100% precise may supplement concern in a guilty suspect when answering questions related to the crime relevance. The card game is often used here. The subject is asked to pick up a playing card from a deck of cards to make note of it and then return it to deck. The polygrapher then shows the subject several cards and asks every time whether this is the card that the examinee has seen or not while the examiner examines the polygraph charts.

  Formulating question phase: Here, the examiner frames the questions that will be asked during the polygraph test and discusses with the subject. Here, three types of questions are asked. *Irrelevant or neutral questions* are general questions: Is your name Patrick? Do you live in the UK? Sequence typically contains three relevant and three probable

lie or control questions. *Control questions* deal with acts that are related to the crime under investigation but don't refer to the crime in question. For example: Have you ever stole anything in 10 years? *Relevant questions* are specific questions about the crime. For example: Did you take that watch?

Test proper: It is vital that subjects are not preoccupied during the polygraph test. Every and any distraction causes a change of physiological response that will be perceived by the polygraph and may influence results.

Scoring phase: It is the reading of charts of the polygraph, which can be done through approach that is global or numerical. The numerical method is an effort to score the charts systematically while reducing the effects of sources other than the polygraph charts in making of the decision. Comparisons are made between the reactions to the control and relevant questions. There are four possibilities. If there is no difference in physiological response, then a score of "O" will be given; if there is noticeable difference, a score of "1" will be aligned, and strong and dramatic differences are allocated with "2" and "3," respectively. If the reaction detected is stronger to the relevant question than the control question, a negative score (−1, −2, −3, −4) is given. If the observed reaction is weaker in comparison to the relevant question than the control question, a positive score (+1, +2, +3, +4) is assigned.

- **Directed lie test (DLT):** In this approach, directed lie questions substitute control questions that are standardized and can be questioned in any case. For example: In the early 15 years of life, have you ever lied? And before age 24, have you ever broken even one regulation? The examiner presents these directed lie questions by informing the subject that they are designed to see how the subject physiologically responds when he or she lies.

  Subjects will be told to answer "NO" to the directed lie questions and to reason about such examples in which they did they tell a lie. The principle behind this is that Guilty Subjects are thought to be mostly concerned with relevant questions and are anticipated to show the strongest responses to these questions. Innocent subjects are believed to be most concerned about the directed lie test.

  DLT is more standardized than CQT test and is believed to be 80% correct. This test is scored by the conventional 7-position scoring system used in the tests, with the total score being the sum across the three-examination parameter for each question on all charts which will finally tell us whether that person is hiding something or not.

- **Guilty knowledge test or concealed information test:** This test compares responses which are physiological on the multiple-choice-type questionnaire given about the scene of crime, one option of which contains knowledge only the investigator and the guilty subject would know about. The fundamental postulation of the GKT is that the right choice has a special meaning for the

knowledgeable subject only; thus, this subject will show more physiological response to the relevant answer. So, if a suspect's physiological responses to the relevant options are consistently greater than to the control options across a number of questions, information about the event is inferred. Here two types of items are taken:

Critical items: These are present one for each test. These items are options or choices that only guilty subject will notice, and the innocent subject will not have any idea regarding it.

Irrelevant items: These are present in five for each test and should be similar to the critical item but in plausibility.

The important and relevant pieces of information is known only to the investigators and persons involved in the crime, for instance, modus operandi used, specific actions that took place, or entry and exit point. Minimum number of keys that should be present are three, but four or more keys are preferred. There is decrease in false positive and increase in accuracy by adding a greater number of keys. Keys are randomly placed in the list of buffers except on the first slot. The first slot is like a neutral or sacrifice relevant. Questions are reviewed with the subject in random fashion and not the order they will be given on the test. First item is not scored as it is considered neutral. If the highest reaction is seen on the critical item, it gets a 2. If there is second highest reaction on the critical item, it gets score of 1. All others scores are 0. The number of tests is the recommended cutting score.

- **Relevant-irrelevant test:** The relevant-irrelevant test equivalences the examinee's responses to the questions that are relevant and irrelevant. A relevant question is one that deals with the issue under the investigation. These questions include asking whether the subject committed the target act or has any knowledge about the culprit and perhaps questions about actual pieces of evidence that would convict the guilty subject.

  An irrelevant question is one that is fashioned to aggravate no emotion (e.g., "Is today Thursday?). Questions that are irrelevant are placed in the first position of list of questions because the responses that are physiological that trail the demonstration of the first question are assumed to have no diagnostic value. Guilty subjects are anticipated to show stronger reactions to relevant than to irrelevant questions; innocent subjects are expected to react similarly to both types of questions. So, if arousal (relevant) $>$ arousal (irrelevant) $=$ Lying [22, 23]

(c) **Post-test interview:** This interview is taken after the polygraph test has been performed on the subject and is done to clarify the findings of the test and to know whether there is presence of any other explanations for the examinee to respond to relevant questions other than the information about the scene of the crime. This interview is also taken to obtain other information for the law administration purposes if the outcome of test suggests deception.

Limitations of the polygraph test: Even though it is a helpful investigation aid, it is never a substitute for any investigation. It is a lie detector, and not a

scientific diagnostic device. It does not determine facts, but only records answers to that which the subject knows to be true and is only as precise as the examiner is competent [24].

13.9.2 Narcoanalysis/Narcosynthesis

Narcoanalysis is derivative of a Greek word meaning "anesthesia." "Horsley" coined this term. It is an analytical and cathartic method that uses drugs which are psychotropic in nature. This technique is used in forensic psychology for investigation purposes. Narcoanalysis is also sometimes known as truth serum or drug hypnosis or narco interview technique. The hypnosis is induced with barbiturates or other psychotropic drugs as a means of liberating repressed feelings. This method is effective in two ways, either as therapeutic or rehabilitative. Narcoanalysis cannot be performed on the subject below.

The history of narcoanalysis can be traced back in the 1990s where it first reached mainstream in 1922, when Robert House, a Texas obstetrician, used the drug scopolamine on two prisoners. Later in time, American Armed Forces and Intelligence Agencies sustained to research with Truth Drug mostly Barbiturate during and afterwar. It was then widely used in the 1940s. Marijuana was used as a truth serum by the Office of Strategic Service (OSS), a US government intelligence agency shaped during World War II.

Objective behind the use of narcoanalysis: The objective of the test is to excerpt information from the suspect when she/he is in hypnotic state, and the entire responsibility lies on the doctor along with the forensic psychologist. This test therefore helps extract masked information relevant to crime and helps in eradicating the innocent person.

The principle used in this test is that the imagination of subject is neutralized by making him semi-conscious. In the semi-conscious state, it becomes hard for the person to lie, and answers would be limited to facts she or he is already aware of. The answers are supposed to be unprompted as a semi-conscious individual is incapable to deploy the answers, and further efforts are made to obtain probative truth about the scene of crime. The test is conducted in the presence of a forensic psychologist, psychiatrist, physician, anesthetist, audio-videographer, and writer.[25]

Procedure of the Narcoanalysis Test

1. **Pre-test interview:** This interview is the same as the pre-test interview of the polygraph test where the subject is informed about the purpose and procedure of test and is appraised about his or her constitutional rights. The informed consent from the subject is taken.
2. **Pre-narcotic state:** In this stage of the interview, the anesthetist induces narco and maintains the pre-narcotic state through the interview. A drug with 5% or 10% solution of sodium pentothal is injected gradually in the antecubital vein NMT 1gm/grain per minute till the person appears relaxed and in a state of good contact. When the individual's speech starts overrunning, the interview begins.

3. **Semi-narcotic state:** In this phase, the subject appears to be flushed and decelerating and with slurred speech. The interview is then facilitated by the forensic psychologist and psychiatrist. The subject is allowed to sleep and then awakened. Once the subject wakes up, the anesthetist checks the subject, and he or she is then allowed to drink tea or coffee. The entire narco-interview is audio- and video-recorded.
4. **Post-test interview:** It is done to clarify any additional findings, and the subject is told about what he or she has spoken during the interview [26].

On the 5th of May 2010, the Supreme Court of India decided the following point with respect to narcoanalysis which was Article 20(3) of the Indian Constitution**:** *No person accused of any offence shall be compelled to be a witness against himself.* The result of the test cannot be acknowledged as an evidence if they have been attained through the use of compulsion.[27]

13.9.3 Brain Electrical Oscillation Signature Profiling

It is a new technique used in the area of forensic psychology which provides information on the action committed by the subject rather than detecting deception in the statements of the subject. It is basically an EEG technique by which a subject's participation on the scene of the crime is detected by eliciting electrophysiological responses.

BEOS is a forensic investigatory tool for signature extraction of electrical oscillation from the background of electrical activity of the brain of an individual by presenting probes. This electrical signature contains reference to "Experiential knowledge" in individual to an act executed by the individual which is stated in form of "probe." It is a noninvasive, memory-based test with a high degree of sensitivity which makes it a reliable technique.

The methodology was developed by C. R. Mukund, a neuroscientist, former professor, and head of the Department of Clinical Psychology at the National Institute of Mental Health and Neurosciences (NHIMAS) in Bangalore (India). He worked on the project "Normative Data for Brain Electrical Activation Profiling" granted to DFS (Gandhinagar) by the Technology Information Forecasting and Assessment Council (TIFAC), Ministry of Science and Technology, Government of India. His work is built on studies that were previously done by other researchers including J. Peter Rosenfeld, Lawrence Farwell, and Emanuel Donchin.[28]

Principle: This forensic investigatory tool is based on collective principles of encoding, storage, and memory retrieval from the brain. The process of encoding occurs only when the person is involved in the participation of the particular event or action. This action can be later recalled in any point of time but is dependent on the efforts which are voluntary and intentional, which would eventually trigger the remembrance of the event. BEOS is therefore based on the principle that an individual who has participated in the vent or in the execution of the events will

show responses when presented cues known as "probes" representing personally acquired knowledge of the event in form of "experiential knowledge."

13.9.3.1 Important Points in BEOS Profiling

Experiential knowledge: It is made up of:

- **Sensory-motor information:** It consists of a combination of sensory information and motor acts and responses. Sensory information is acquired through recognition of external and internal situations, for example, eyes detecting light and colors or ears detecting sound waves.
- **Motor acts and responses:** On the other hand, motor acts and responses consist of motor activities which are influenced by the mixing of conscious and unconscious experience. A characteristic feature of emotions is change of psychomotor activities.
- **Proprioceptive information:** "Proprioception" or "kinesthesia is mainly a sense of self-movement and position of the body. It is sometimes also referred to as the "sixth sense." Proprioception focuses on the awareness of the body and its movement and behaviors. This information is cognitive, while kinesthetic information is more behavioral. The signals of proprioception have an influence directly on an experiential fabric which is then constituted by an individual's emotional awareness.
- **Emotional experience:** Events or experiences that have an emotional arousal are derived from greater vividness and clarity than past events without emotional loading. Presence of emotions enhances consolidation as well as extraction of the routine information. Since every experience has a relation with emotion, these psychological states of emotions can be described with specific names such as flight and fear, anger, sadness, happiness, etc. Every emotion is linked with specific mental state and behavioral experiences.[29, 30]

13.9.3.2 Difference between Knowing and Remembrance

Knowing	Remembrance
• It refers to recognition of familiarity, e.g., "I know Patrick"	• It is the recall of episodic and autobiographic details of an individual's life, e.g., "I saw Patrick on MG road"
• This can only occur when an individual intentionally recalls or is in the presence of external cues	• Remembrance only occurs when it is cued by stimulus and is automatic
• Brain areas involved: dorso-frontal cortex	• Brain areas involved: ACC, ventral brain, medial temporal cortex, and orbitofrontal cortex
• It is mainly the process of sharing information with others	• It occurs because of autobiographic episodes and experiences that produce EK
• Memory used here is only for the knowledge that is conceptual	• It is related to recalling of events in life which are autobiographic episodes and information with reference to time and space

Category 1	**Neutral probes**	These probes are used for detecting the semantic processing of the subject. Eg: Monkey jumps, 2+2 = 4
Category 2	**Control Probes**	These are the events confirmed by both, that is the subject as well as the investigator. Eg: occupation, marriage etc.
Category 3	**Target-A probes**	Events that are thought to be committed by the suspect according to the investigator.
Category 4	**Target- B probes**	Events that occurred according to the version of the suspect, which the investigator doesn't agree with.

Fig. 13.4 Categories of the probe

Probes: The probes induce the psychological state alike to the state an individual had at the time of occurrence of events. There are four types of probes that are used to trigger remembrance (Fig. 13.4):

Principle of Probes

1. Deciding the scenarios.
2. Designing probes for each scenario approx. for 60–70 min.
3. Probes are sequentially arranged.
4. Length of the sentence (5–6 s).
5. Probe should contain up to seven words.
6. What each probe can indicate.
7. Deciding the codes for every probe based on the effect of its cuing.
8. Response of the brain is in 10 s; the maximum length of the probe is 3.5 s[31]
9. Total epoch length = 10 s (duration of pre-probe epoch is 3 s and probe epoch, 7 s).

Neuropsychological Principles for Designing the Probe

- Sequence of events
- Sequence of components of events
- Sequence of actions and fixing contextual specificity of actions and experiences
- Description of components of events
- Sequence and description of perceptual components
- Description of emotional arousal
- Verbalization of actions, perceptions, emotions, and intentions

Stages of BEOS Profiling

1. Interview the investigator and the subject.
2. Designing probes and scenarios.
3. Probe recording and event markers for different scenarios and presentation schedule of probes.
4. Familiarize the subject or suspect with scenarios and probes.
5. Probe recording is done using the VASP (visual and auditory stimulus package) in the computer.

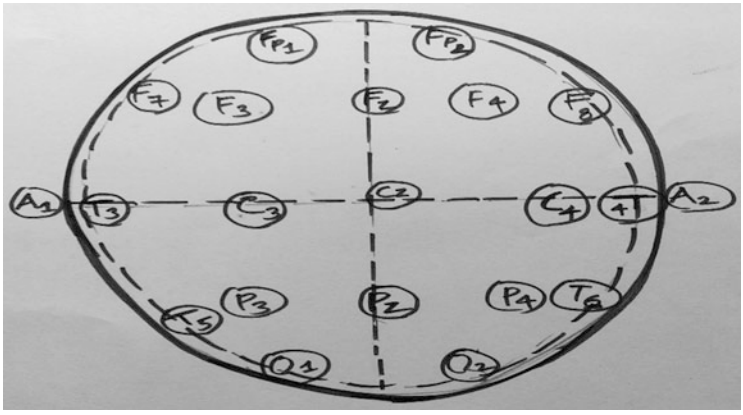

Fig. 13.5 10–20 system of EEG electrode placement

6. Familiarize the subject with instructions details of the test which includes asking the subject to stay calm with closed eyes and listening to probe alertly as he or she will be later on asked to recall the probes. The subject is asked not to respond to any of the probes. The subject is also instructed to inform the examiner if there is a need to pause the test. Instructions are also given for the posture and position of the head.
7. Obtain the written informed consent of the subject (Fig. 13.5).

Data analysis: An automated analysis is carried out by the system which produces an automated report in form of PDF. The report contains the list of probes, ID of probe used, and result derived by the analysis of program. The number of probes which produced experiential knowledge and other results are calculated from each of this report for additional statistical analysis [32].

13.9.4 Forensic Hypnosis

Hypnosis can be well defined as change of consciousness and concentration in which the individual displays a sharp state of suggestibility with the awareness being maintained. Forensic hypnosis is the use of hypnosis in the process of investigation and can be used in both civil and criminal cases.

13.9.4.1 Session of Forensic Hypnosis

When the subject enters the room, right then and there, audio and video taping of the session begins. The hypnotist initially greets the subject which is an informal meeting. After this, pre-session interview which is the initial interview commences where the subject's personal information is taken. The hypnotist also explains about the hypnosis and steps of its conduction. Memory of event before conducting hypnosis is recorded. The next step is induction which means that the subject is

made to relax. The next step is to clarify all the details and findings while the subject is under hypnosis or to ask any additional questions. Once the hypnosis session comes to an end, the subject is brought out from hypnosis. The audio and video recording of subject are stopped once the subject leaves.

Reasons as to why hypnosis is not admissible in court:

- Hypnosis lacks the general scientific acceptance and reliability in differentiating truth from false statements.
- There is always a possibility that the subject who is hypnotized can deliberately fabricate the statements or can pretend to be hypnotized.
- The panorama that the hypnotized subject in his or her heightened state of suggestibility can produce the distortion of the fact.
- The whole process of hypnosis is dependent on the skill and state of mind of the examiner, and that is too subjective to permit the admissibility of hypnosis [33].

13.10 Different Roles of the Forensic Psychologist

There are many roles that a forensic psychologist can play apart from just working with the forensic investigating tools. The forensic psychologist also plays a significant role in defining whether the defendant is competent to stand trial, that is, whether the defendant is able to understand and comprehend the process of legal proceedings that are charged against him and if he is able to cooperate efficiently with his defense counsel. These professionals are able to narrow down the list of possible suspects and therefore help in solving criminal cases by deducing the role of the suspect, witness, and accused in crime with the help of various psychological aids.

The forensic psychologist plays the role of expert witness which means reformulating the findings of psychological report into the legal language of court, thereby providing information to the legal personnel in such a manner that is easily understood by him or her.

Clinical assessments of suspects, witness, or accused are very important since these assessments help in evaluating the individual's past, present, and future psychological status which in turn can also be used as legal fact finder to aid a variety of law setting.

Sometimes, forensic psychologists are appointed by the court to assess the state of mind of the defendant's and to assess the criminal responsibility at the time of offence. These professionals may also be called to provide sentencing recommendations or any other knowledge requested by the judge, for instance, information regarding mitigating factors, assessing future risks, and evaluating credibility of the witness.

Forensic professionals can also work in criminal or family law and also civil cases like cases of child custody, deciding guardianship, or even setting land disputes. The field of forensic psychology can be very beneficial in preventing crimes as well;

professionals in this field may be called upon to provide rehabilitation of convicts and help them become law-abiding citizens.

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Competence of Evidences: A Pragmatic Approach in Court of Law

14

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14.1 Legal Classification of Evidences

The Indian Evidence Act of 1872 defines "Evidence" under Section 3. According to Section 3 of IEA, evidence is identified as oral evidence and documentary evidence. The documentary evidence here includes electronic evidences and records submitted to the court, while oral evidences here refer to statements submitted of which the court allows witnesses to present, and such evidence should also be associated with the case or the matter of fact under enquiry. This evidence is non-inclusionary of other material evidences like weapons of offence, the proceedings of identification, court made statements, and so on.

Evidence under the Indian Evidence Act of 1872 includes:

- All testimonials of witnesses and written statements.
- Recording of videos or audios.
- Photographs submitted.
- Physical objects, such as hitting weapons or clothes or used in the offense committed.
- All data and media digital evidence.

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- Laboratory result of semen or blood and even evidence used for demonstrating to the jury about some complicated topic using models or some form of display.
- Statements which are permitted and considered admissible by the Court provided from the witness in front of or even before magistrate, discussing about the concerned matter of dispute under doubt.
- All documents produced for inspection as per the order of the Court including electronic records.

Competent evidence are those evidences that are legally accepted and approved in a lawsuit which help prove the matter in concern or dispute. For instance, if there is an ongoing trial of murder, the fingerprints present onto the weapon can be considered as competent because it is related to the trial.

There are different types of evidences:

1. **Direct Evidence**
   Direct evidences are evidences that directly disprove or prove the fact in question. Without any detail or reason to connect to the fact, such evidence establishes the validity of the fact.
2. **Circumstantial Evidence**
   Circumstantial evidences unlike direct evidences use other relevant facts and details to prove or disprove the fact under question. These circumstantial evidences are referred to as "relevant facts" in the IEA. This includes documentary and oral evidences.
3. **Analogical Evidence**
   The less utilized evidences that come into play when no other statistics are available are analogical evidences. When no other material is present to represent a fact, these evidences are utilized.
4. **Anecdotal Evidence**
   Anecdotal evidence as the name suggests are anecdotes or personal perspective of events of an individual. These can be used for topic buildup. This is also one of the least utilized evidences as the claims can be contradicting at times.
5. **Character Evidence**
   As the name suggests, this evidence indicates the probable character of the individual in question. This type of evidence may be utilized to ascertain the guilt of an individual. This type of evidence can be subjective and can be based on the individual's past actions and behaviors.
6. **Demonstrative Evidence**
   As the name suggests, demonstrative evidences are evidences that are used to demonstrate facts to the judge or before the court. This can include evidences like demonstrative charts. These evidences assist in proving or disproving facts.
7. **Electronic Evidence/Digital Evidence**
   The definition amendment of evidences due to the Information Technology Act, 2000 resulted in the inclusion of electronic or digital evidences as evidences. Any evidence obtained in digital or electronic form can come under this category such as stored images, data generated, and so on. Such evidences must be collected using appropriate means and within a reasonable time limit.

8. **Documentary Evidence**
   Documentary evidences are defined under Section 3 of IEA. Documentary evidences are evidences that are expressed using letters, signs, or alphabets on any surfaces or substances. Such documents are used to prove or disprove any facts in court.
9. **Exculpatory Evidence**
   Exculpatory evidences as the name suggests exculpate the accused of their guilt and aid them in establishing their innocence. Any evidence that can aid the defendant in proving his/her innocence is considered as exculpatory evidence.
10. **Forensic Evidence**
    Forensic evidence can be classified as physical evidences and biological evidences. All the inorganic and nonliving evidences come under physical evidences like impression evidences and so on. Biological evidences include evidences like body fluids, entomological evidences, botanical evidences, and so on.
11. **Hearsay Evidence**
    The hearsay evidences as the name suggests are evidences which the witness has neither heard nor seen personally. This type of evidence is considered as weak evidence. These kinds of evidences are considered irrelevant, and its authenticity can be questioned.
12. **Physical Evidence**
    Evidences that are found in the crime scenes are referred to as physical evidences. These evidences can range from impression evidences, other evidences that cannot be seen with the naked eye, to that which requires a microscope to view. These examinations of evidences are crucial for forensic analysis.
13. **Prima Facie Evidence**
    Prima facie evidences are presumptive evidences that establish basic facts, and the existence of which are quite questionable. These evidences are quite sufficient to establish a fact and raise suspicions unless rebutted or disproved.
14. **Statistical Evidence**
    Statistical evidences as the name suggests refer to data that are used when something is proved or disproving a fact using numbers or when statistical data is used to test the strength of the presented evidence.
15. **Testimonial Evidence**
    Testimonies in the forms of written or spoken statements presented to the court of law are known as testimonial evidences. Testimonial evidences are subjected to credibility test by the court before they are deemed admissible or inadmissible.
16. **Judicial Evidence**
    Any evidences obtained by the court of law that aid in proving or disproving any facts before the law are known as judicial evidences.

17. **Non-judicial Evidence**
    Evidences mainly out of court confessions made by the accused in the presence of a person that is yet to be accepted by the court are known as non- judicial evidences. Once the court of law accepts these evidences, then they are classified under judicial evidences.
18. **Primary Evidence**
    Any top-class evidences that need no prior notice for admissibility before the court of law are known as primary evidences. This can be any authentic document that is required to be inspected by the court of law after submission. These evidences are mandatorily submitted to the court before any other secondary evidences, and only in the absence of these evidences can secondary evidences be presented before the court of law. Section 62 of Indian Evidence Act defines primary evidences.
19. **Secondary Evidence**
    Secondary evidences are defined under Section 63 of the Indian Evidence Act. Secondary evidences are those evidences that are presented before the court of law in the absence of primary evidences after justifying the absence of primary evidences. These evidences are considered to be inferior evidences that need prior notice to the court before submission.
20. **Real Evidence**
    Any evidences that are not derived from any witness or document that can be presented as tangible evidence before the court of law that the court can inspect by itself and that can be backed by an expert witness is known as material evidences or real evidences.
21. **Oral Evidence**
    The fourth chapter of the Indian Evidence Act contains the provisions related to oral evidences. Any creditworthy words spoken by mouth are considered as oral evidences. Such evidences are considered doubtful if the provided statements are contradictory. These evidences do not require the need of any document evidence to be proved.
22. **Scientific Evidence**
    Scientific evidences are empirical evidences that are obtained from careful scientific research. Such recognized evidences need to be researched in accordance with the set standards. These scientific evidences are admitted by discretion by the court of law.
23. **Substantive and Corroborative Evidence**
    Any considerable evidences that need no corroboration to be proved are known as substantive evidences, while concrete substantive evidences are known as corroborative evidences. These evidences can be either circumstantial or direct or both. Corroborative evidence is used to ascertain the significance of substantive evidences, and its entire existence is dependent on the substantive evidences.

14.2 Legal Admissibility of Evidence

An evidence has no practical value in the court of law if it does not meet the legal admissibility requirements. If an evidence is legally inadmissible, any fact that it ascertains would be inadmissible too and hence might lead to unjust judgements.

Admissibility is generally stated as the quality of being acceptable in the court room. According to law and order, anything which is admissible in the court to confirm or deny a certain fact is termed as evidence. Not all evidences are being considered in court, and only the reliable ones are accepted. Therefore, they are demonstrated in front of court to prove their point either in favor or denial.

14.2.1 Conditions for the Admissibility of Evidence

Section 20 of the Indian Evidence Act, 1872:

> *Admissions by persons expressly referred to by party to suit. — Statements made by persons to whom a party to the suit has expressly referred for information in reference to a matter in dispute are admissions.*

Evidence under this section covers all the documents including electronic documents issued during inspection according to court's order. Every statement made by the witnesses with respect to the dispute, in front of magistrate or before, is either approved or admitted by the court.

The Exclusionary Rule

> The two opponents – prosecution and defendant – are trying to prove their perspective in the ongoing case, so they provide evidence to prove guilt without any concern. Before the trial begins, they have equal opportunity to take a look at each other's evidence and can raise doubt regarding them even bore or during the court proceedings. If in some criminal cases the evidences are violating constitutional rights, the defendant has the right to raise an objection.

The criteria for legal admissibility vary marginally between countries. The American and Indian laws that pertain to the legal competence of an evidence are discussed below, where the laws are set forth based on the reliability of the expert who is ascertaining the significance of an evidence, through scientific testing and such, rather than the actual evidence itself.

14.2.2 American Law

14.2.2.1 Frye Standard

Also termed the "General Acceptance test," it was devised by the Federal District Court of Columbia, USA. It stated that:

> *Just when a scientific principle or discovery crosses the line between the experimental and demonstrable stages is difficult to define. Somewhere in this twilight zone the evidential forces of the principle must be recognized, and while courts will go a long way in admitting expert testimony deduced from a well-recognized scientific principle or discovery, the thing from which the deduction is made must be sufficiently established to have gained general acceptance in the particular field to which it belongs.*

It dictated that if a principle or scientific procedure was established enough in the relevant scientific community, it could then be acceptable for the courts as well [1].

This was first enacted in a case (Frye vs United States, 1923) where the admissibility of a lie detector test was debated. The judges ruled against admission of the lie detector test results as it had not achieved justifiable scientific recognition in the respective scientific field. Post this, Frye's standard became the predominant test for deciding evidence admissibility across the courts in the USA by the early 1970s.

14.2.2.2 Daubert Standard

While the Frye standard covered the admissibility of most forensically relevant evidences, admissibility of testimonial evidence of an expert was not very well defined. This led to the formulation of the Daubert guidelines [2].

In the case of Daubert vs. Merrelll-Dow Pharmaceuticals Inc., 1993, the US Supreme Court concluded that the Federal Rules of Evidence (as enacted in 1975) superseded the Frye standard, whose text or history did not incorporate the Frye guidelines.

Federal Rule of Evidence 702 (1975) originally stated:

> *If scientific, technical or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training or education may testify thereto in the form of an opinion or otherwise.*

While the Federal Rule of Evidence 702 was referred to during the Daubert case, the confusion caused in the successive cases of General Electric vs. Joiner (1997) and Kumho Tire Co. vs. Carmichael (1999), due to the ambiguous wording of Rule 702, led to its amendment to generalize the standard to all expert testimony rather than just those pertaining to scientific testimony [3].

The Rule 702, as amended in 2000, stated that:

> *If scientific, technical or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training or education may testify thereto in the form of an opinion or otherwise if*
>
> - *the testimony is based upon sufficient facts or data,*
> - *the test is the product of reliable principles and methods, and,*
> - *the witness has applied the principles and methods reliably to the facts of the case.*

The wording of Rule 702 was further amended in 2011 to the following:

A witness who is qualified as an expert by knowledge, skill, experience, training, or education may testify in the form of an opinion or otherwise if:

- *the expert's scientific, technical, or other specialized knowledge will help the trier of fact to understand the evidence or to determine a fact in issue;*
- *the testimony is based on sufficient facts or data;*
- *the testimony is the product of reliable principles and methods; and,*
- *the expert has reliably applied the principles and methods to the facts of the case.*

The Daubert guidelines for admissibility of evidence dictated that while the general acceptance may be a criterion, there are additional aspects to be considered. It also considered:

- The reliability of the scientific assessment of the technique/s.
- Whether it has been peer reviewed/published in relevant publication.
- Known/potential error rates.
- Maintenance of standards and controls for the technique's operation.

This focused on the reliance and reliability of an evidence and required the judges to employ a gatekeeping duty to determine the reliability and importance of the evidence submitted to the jury. The judge needed to determine the reliability of the expert's methodology as well as the expert's application of the said method to the respective case [4].

Daubert's guidelines for evidence admissibility were adopted by all Federal courts and most States except a few which still follow Frye standards [5].

14.2.3 Indian Law

14.2.3.1 Indian Evidence Act (IEA), 1872

Enacted in 1872 under the British Rule, this act codified a standard for rules of admissibility of evidences in the court of law. Till this date, it remains largely unchanged except for a few minor amendments.

The Act has 3 parts and 11 chapters of which Part I, Chapter II (Section 5 to Section 55) deals with the Relevancy of Facts. Part II deals mostly with the different kinds of evidences and their importance in the judicial proceedings.

In Sec. 3 (Part I, Chapter I), the definitions are given for various important terms, including:

- Evidence: *"Evidence" means and includes —(1) all statements which the Court permits or requires to be made before it by witnesses, in relation to matters of fact under inquiry; such statements are called oral evidence; (2) all documents*

including electronic records produced for the inspection of the court; such documents are called documentary evidence.

- Relevancy: *One fact is said to be relevant to another when the one is connected with the other in any of the ways referred to in the provisions of the Act relating to relevancy of facts.*

Sec. 45 of the Indian Evidence Act is an important and pertinent section that relates to what could be admissible as Expert Evidence. Sec.45, IEA states that: *When the court has to form an opinion upon a point of foreign law, or science or art, or as to identity of handwriting (or finger impressions), the opinions upon that point of persons especially skilled in such foreign law, science or art, (or in questions as to the identity of handwriting or finger impressions) are relevant facts.*

It means that an opinion of a third party (such as an expert) can be relevant when the opinion pertains to five situations, namely, foreign law, science, arts, handwriting, and finger impressions. But the opinion of an expert does not trump the direct evidence/eyewitness accounts, as the expert's statement is just an opinion that the court will regard but is not bound to. In cases of opposing statements of the eyewitness and the expert opinion, if the eyewitness and their account is believed to be credible, then the conviction is done on the basis of the evidence, i.e., the eyewitness account [6].

The expert can hence not be tried for perjury as she/he is not a witness and her/his statement is only an opinion not evidence.

Under this law, opinion of a medical expert, such as the doctor who conducted the postmortem examination, is not a substantiative evidence but is reliable as long as there is nothing that is "inherently defective" in the report. The Courts cannot substitute its own opinion for that of the doctor.

The role of an expert is that of an advisor who explains the technical aspects to the Court so that the Court can then form its own judgement on the matter, after which the expert's opinion becomes the opinion of the Court.

14.2.3.2 Sec.293, Code of Criminal Procedure

It is another important section that governs the admissibility of reports of Government scientific experts. In India, the forensic labs function under the Government of India, and hence the scientists in the labs are government employees. Sec 293, CrPC deals with the admissibility of the reports presented by these scientists [7].

The section states:

Reports of certain Government scientific experts.

1. *Any document purporting to be a report under the hand of a Government scientific expert to whom this section applies, upon any matter or thing duly submitted to him for examination or analysis and report in the course of any proceeding under this Code, may be used as evidence in any inquiry, trial or other proceeding under this Code.*
2. *The Court may, if it thinks fit, summon and examine any such expert as to the subject- matter of his report.*

3. *Where any such expert is summoned by a Court and he is unable to attend personally, he may, unless the Court has expressly directed him to appear personally, depute any responsible officer working with him to attend the Court, if such officer is conversant with the facts of the case and can satisfactorily depose in Court on his behalf.*
4. *This section applies to the following Government scientific experts, namely:*
   (a) *any Chemical Examiner or Assistant Chemical Examiner to Government;*
   (b) *the Chief Inspector of Explosives;*
   (c) *the Director of the Finger Print Bureau;*
   (d) *the Director, Haffkeine Institute, Bombay;*
   (e) *the Director, Deputy Director or Assistant Director] of a Central Forensic Science Laboratory or a State Forensic Science Laboratory;*
   (f) *the Serologist to the Government.*
   (g) *Any other Government scientific Expert specified by notification by the Central Government for this purpose.*

As per this law, the official reports submitted by any of the officers mentioned in the section are accepted by the court as valid evidence and do not require the officer themselves to be examined, unless specifically requested.

Despite these laws, there is still confusion regarding admissibility of certain evidences like DNA test reports, and ultimately it is up to the discretion of the judge to allow or reject the admission. As a general rule, all relevant evidence is admissible unless there are some contradictory circumstances.

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Penology: A Friend or Foe for Criminal Justice System

15

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Abstract

If we talk about penology module in the past, same two questions were asked, namely: "What is penology?" "Is it just about punishment and prisons?" Attempt is made to donate an educated reaction to the primary address that continuously gives an unequivocal "NO!" to the moment. In this manner, penology is and ought to continuously be respected as being so much more than a fair discipline and detainment facility. Looked at in its least complex shape, it is a department of criminology managing jail administration and treatment of wrongdoers. Penology is concerned with the practicality of those social shapes defined and attempts to dodge wrongdoing through the concealment or obstacle of criminal point utilizing the fear of teaching. Consequently, the thought of penology deals with the treatment of prisoners and the ensuing rebuilding of sentenced guilty parties. It also includes perspectives of probation (recovery of wrongdoers within the community) as well as prison science relating to the secure detainment and retraining of wrongdoers committed to secure education. Penology is concerned with numerous subjects and hypotheses, including those concerning detainment facilities (jail change, detainee mishandle, prisoners' rights, and recidivism), as well as hypotheses of the purposes of discipline (discouragement, revenge, debilitation, and recovery). Modern penology concerns itself basically with criminal recovery and jail administration. The word rarely applies to hypotheses

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and qualities of teaching in less formal circumstances such as child-rearing, school, and working environment remedial measures.

15.1 Introduction

The exciting improvement of human rights contributes to the assignment of changes in penology as a science. Presently, the fight against culpability livelihoods disciplines and security measures and flawless social procedures, the bunch of penology ought to be behind the times. Penology is made up of two separate words: the Latin "poena" (meaning punishment) and the Greek suffix "logia" (meaning the study of), making it the study of punishment. Typically, definition to the word refers to which it widens a small nature for word by expressing that "they think about the discipline of wrongdoing and jail management"? Key in both is the word discipline, which is characterized as the "infliction or burden of a punishment as reprisal for an offense". In conclusion, we understand penology as cruel these days as a substitute which we call "science of fight against culpability" which implies the idealize procedures of avoidance and treatment regards culpability. So we might clarify, to start with, the shirking and after that the treatment in terms of whether its technique is definitive, legitimate, or official [1, 2].

As the detainment with by manhandling within the treatment of the prisoner, reformers endeavored to dispose of these misuses and their endeavors driven that comes after almost:

1. The possibilities of the prisoner to claim justice in front of the court.
2. The disciplinary sanction could not be applied unless after a disciplinary regular trial in which the prisoner's defense is heard.
3. The suppression of any discrimination due to race, religion, or social position.
4. The freedom of religious belief and performing its rites.
5. The interdiction of any cruelty or savage treatment.
6. The abolition of flagellation.
7. The suppression of the iron chain which was attached to the prisoner's legs.
8. The teaching of a job that the prisoner exerts after his release or the improvement of the job exercise when the prisoner has yet a job.
9. The prohibition of medical experiments on prisoners without their consent.
10. The attenuation of freedom restrictions before release.
11. The right of vote in the general elections.
12. The right of studying and passing instruction examinations.
13. The right of maintaining contact with the exterior social ambient of relatives and friends by receiving their visits exchanging correspondence with them, and going out of the prison for a short stay.

Penology may be a portion of the criminal equity framework in which an indicted individual is punished for his criminal offense. Discipline could be a need to

diminish the event of offense. It may include a fine or detainment. We all are great and awful in a few ways. But in case an individual does something awful in terms of legitimate equity at that point he is breaking a few rules and directions and as a result punishment is given to him, for example, if an individual commits a murder. We are going to consider two scenarios: one, where an individual is not rebuffed, and the other, where the individual is rebuffed. When an individual is not rebuffed for the wrongdoing he has committed, he might wander around freely and might commit the same offense once more. In the event that he is rebuffed on time, then he will not commit the offense once more; indeed individuals living within the community will get to know that on the off chance that they commit something illicit, at that point they will be rebuffed [2–4].

15.2 History

As a matter of reality, penology owes its roots to Cesare Beccaria, the classical school of criminology. This Italian criminologist fought that the defense of teaching must anticipate potential guilty parties and it is not fair to rebuke the blameworthy party. A while later, in the midst of the conclusion of the nineteenth century, particular speculations of teaching were propounded centering on the focuses and targets of the teaching [5].

15.2.1 Cesar Beccaria's Views About Penology

Cesare Beccaria was an Italian legal scholar, edification scholar, and rationalist. In 1794, he composed On Wrongdoings and Discipline. In this book, he talked against torment and the passing punishment, but he was most celebrated for laying an establishment of penology, which bargains with the suppression of criminal exercises and discipline of violations committed. Beccaria was most celebrated for announcing that "a discipline ought to fit the crime." He implied a few things by this but most imperatively he had two fundamental focuses. The primary way he said was that the discipline ought to fit the wrongdoing which means that the seriousness of the discipline ought to parallel the seriousness of hurt coming about the wrongdoing, This does not implies that somebody was a killer, which they thought to put to passing. Beccaria freely condemned the passing punishment since he said that the state does not possess the proper lives which is not an awfully valuable frame of discipline which is not required. He stood for a more obstructive work of the corrective framework. When he said that it must coordinate the harms of the offense and parallel the hurt of the wrongdoing, he was more alluding to the lines of discipline and imprisonment. His momentary point was that the discipline ought to be seriously sufficient to exceed the delight of committing the wrongdoing. For example, somebody might see a sexual attack as pleasurable, and hence the discipline has to be seriously sufficient for that individual to think, "Wow, the discipline is cruel, it's not worth committing this crime." On the off chance that this was not the

case, a sound, individual would weigh the pickup with the result, and decide that the wrongdoing is worth committing in any case. Individuals speed since speeding tickets are basically fines; in the event that they were to be booked and imprisoned, I am beyond any doubt that individuals would speed less. Nothing is usual how it ought to be, it is fair that foremost outright case; the issue with this point is that even though it applies to levelheaded individuals, it does not truly work on others [5].

Inside the treatise, "On Violations and Disciplines," Beccaria composed a brief chapter on foreseeing wrongdoing since he thought that dodging wrongdoing was better than rebuking them. He gave nine standards that should be considered to effectively prevent wrongdoing. To avoid wrongdoing the following are required:

1. Society must make sure that laws are clear and simple.
2. Society must make sure that the entire nation is united in defense.
3. Laws should be not against classes of men, but men.
4. Men must fear laws and nothing else.
5. There should be a certainty of the outcome of crime.
6. Members of society must have knowledge because enlightenment accompanies liberty.
7. Virtue should be rewarded.
8. Perfect education should be ensured.
9. Direct the interest of the magistracy as a whole to observance rather than corruption of the laws [5].

15.2.2 Jeremy Bentham's View About Penology

Jeremy Bentham was the mental pioneer and the genuine author of English utilitarianism, who is profound intrigued in undertakings secured the period from the American Transformation to the Change Charge of 1832. He was born in a wealthy lawyer's family in 1748 in London. From an exceptional childhood, Bentham was insightful and hypercritical. He learned Latin when he was just 3 years old. He moreover learned Greek and French and afterward he gave himself to consider statute and legitimate logic. He got his degree of graduation at the age of 15 from Queen's College Oxford. He was intuitively intrigued by science and had an unmistakable ability for reflective brain research. From his youth he appeared to have an energetic commitment to social welfare, distinguishing himself in creative ability and deciding to apply to the social sciences the strategies that were being worked out in normal science.

Bentham held that punishment ought to be preventive and remedial instead of coercive and retaliatory. It ought to be calculated to anticipate the spread of fiendish and to secure the expansion of great. Discipline must not be delivered where it is incapable, unfounded, unnecessary, or unrewarding. It ought to be clearly legitimate and proportionate to the offense committed but it must be adequate to secure its closes. It needs to be able to avoid the wrongdoer from rehashing the offense. It

ought to be individualized, subjectively and quantitatively, to suit the person's wrongdoer. The basic principles of punishment are:

(a) Equable
(b) Exemplary
(c) Frugal of pain
(d) Remissible
(e) Compensatory
(f) Reformatory
(g) Popular
(h) Certain and not severe

Agreeing to Bentham, a significant test of the ampleness of teaching was its capacity to secure open welfare. He acknowledged that the English criminal law was brutal. He was in favor of the alteration of the criminal and detainment offices and suggested the building of his ethical Panoptical, a wheel-shaped building for the lodging and fitting recognition of the guilty parties [5].

15.3 Punishment in Medieval and Modern/New India

15.3.1 Punishment in Medieval India

Within the Mughal period, the judges thought it fit and best to take after Quranic statutes; discipline was discretionary with the officer who endeavored the case and might anticipate any outline. Inside the run, the show of Akbar, data was issued to the common agent asking him "to look for after what the witnesses removed by complex ask, by the thinking around of physiognomy and the work out of foresight." Predominant official officers were the specialists to undertake criminal cases. Shasta Khan, amid his organization of Bengal, held open durbar every day for regulating equity and changing wrongs. The Kazi went to the hearing of cases by the attempting officers and helped them to reach choices concurring to the statutes of the Quran. Where the Quran was quiet on a point in issue in a case, the attempting officer connected his possessed caution in arriving at a choice. There was a right to request to the Head. The criminal law of the Mughal period was started within the statutes of the Heavenly Quran, and the declared thought in rebuffing the transgressor was that of educating his righteousness.

The most noteworthy court in Mughal India was that of the Sovereign himself. The Sovereign was the wellspring of equity. Akbar was famous for committing a few hours within the day to hearing cases of offers, Shah Jahan had his court every Wednesday in the Diwan-e-Khas, and Aurangzeb administered justice in his private chamber [5].

The Maratha framework of the organization of equity was straightforward and suited to desires of the time. The law was not codified but was based on ancient traditions and treatises like Mitakshara and the Code of Manu. Trials and vows were

permitted in deciding the blame or blamelessness of the charged. The Legal and the Official were not isolated. For murder, the punishments were fines, seizure of property, and detainment. Discipline was implied for being utilized as a remedial as it were. Fines were forced concurring to the budgetary capacity of the wrongdoer; installment by installments was too permitted. The guilty party was not unnecessarily sent to imprisonment for nonpayment of the fine. Witchcraft was rebuffed with fines, detainment, and excommunication.

For adultery, the discipline for female guilty parties was slavery and corrective subjugation, but such people were discharged in the event that a few connections stood surety for their future great behavior. In some cases, mutilation has requested the input of servitude. For the male guilty party, the discipline, by and large, was fine, and in some cases detainment. The condition of the slaves was to, disdain toward hard labor they were put to, moderately well. Punishments for forcible marriage were reallocation of property and excommunication. Now and then the guilty party was let off with a fine, but burglars and hoodlums were ruined [5].

15.3.2 Punishment in Modern or New Penology

With cutting-edge criminological progressions, particularly inside the field of penology, it has been expansively recognized that teaching must be in degree to the gravity of the offense It has been recommendation that reconstruction of the criminal rather than his launch from society is more purposefulness for his rebuilding with this point to see that penologists have centered their thought on individualization of the Cretan through treatment procedures. These days, antiquated primitive techniques of teaching such as mutilation, branding, hanging, burning, stoning, whipping, evacuation, starving the criminal to passing, or subjecting him to pillory or superb teaching are completed left out [5].

The pillory was a technique of corporal teaching underneath which the blameworthy party was subjected to open mock by revealing him to teaching in open places. Differing beautiful disciplines were given for differing infringement. For example, cutting off hands for burglary, taking off the tongue for the offense of equivocation, debilitating for an ambush, shaving off the head of a woman in case she committed sex wrongdoing or whipping her within the open street, and comparative other modes were common shapes of superb teaching in the midst of the center ages. Modern penologists have substituted unused shapes of restorative sanctions for the antiquated techniques of sentencing. They come up with modes of teaching which is commonly consolidate the burden of budgetary fines, separation of the blameworthy party briefly or until the end of time through detainment or externment, or stipend by way of hurts from the wrongdoer in case of benevolent hurt.

The credit for presenting these penological changes goes to famous criminologists, like Beccaria, Garofalo, Ferri, Trade, Bentham, and others who defined sound standards of discipline and made all-out endeavors to guarantee recovery of wrongdoers so as to make them valuable individuals of society once more. Garofalo emphatically prescribed “transportation” or “banishment” of certain

sorts of guilty parties who had to be isolated from society. Advanced correctional frameworks may constrain the discipline of transportation inside the nation itself. Of late, open correctional facilities, parole, or probation are being goal utilized for longtimers so that they can earn their vocation within the institution [5].

Briefly communicated, it is by and large well recognized that expectation of wrongdoing and confirmation of society are the foremost objects of teaching. It, along these lines, takes after that no single speculation of teaching will serve the genuine reason. Punishment may be craftsmanship which includes the altering of retaliation, debilitation, and reestablishment, in terms, not because it was off the court but the values in which it takes to put and inside the altering of these reasons of disciplines, to begin with, one and after that, the others get to compliment with ongoing conditions change.

Advanced penological considering favors rationalization of instruction by taking into thought the differing approaches in their appropriate centers of seeing and utilizing them to suit the given circumstance and prerequisite of the reprobate in understanding the appeal of individualization [2, 5].

15.4 Understanding Factors Influencing Penal Policy

1. Teaching can be distinguished from other forms of torments or sufferings which are not a response to our wrongdoings, e.g., horrifying helpful treatment. Teaching rests on moral reasons and is the expression of moral condemnation in response to infringements. Feinberg J (1994) in a bit called "The expressive work of teaching insinuates to censure or condemnation as to the characterizing include of teach contains ordinary significance". It may be a scheduled contraption for the expression of demeanors of contempt and outrage. A key incorporate of teaching is that it rests on a moral foundation communicating a moral judgment. It is cleverly based on reasons. It stems from a definitive source more often than not the state. A key issue to be addressed has been why a few acts are criminalized and not others and why society bargains brutally with a few wrongdoings and gently with others. The most common questions posed are the following:
   - What particular response is made to an action or behavior and why, i.e., what to punish?
   - If the response is penal which particular penal option is selected, i.e., how to punish?
   - What is the particular level of penal response, i.e., how much to punish?
2. Penology is additionally concerned with questions of uniformity, reasonableness, and equity which must also be considered within the approach, e.g., whether a few bunches are chosen for harsher discipline or in case clearly unbiased approaches have a differential effect, e.g., separation against race(s) or financial implementation or the rationally disarranged, ladies and children, etc. the notion of equity is not clear but encapsulates decency to all individuals of the community counting casualties and wrongdoers, and sticking to adjust between their

competing interface. Which is a Typical foundation of the current criminal justice framework.

3. Human rights have suggestions for both the hypothesis and hone of discipline in defending particular disciplines, in accepting the equity of discipline, and in moving forward guidelines in correctional education, e.g., regard for supports, treatment of remand detainees, safeguard, right for a reasonable trial, and assumption of guiltlessness. This rule may act as a control on legal watchfulness and hindering aberrations in sentencing.
4. There are moreover impacts on the corrective approach which may reflect the political and ideological standards supporting the correctional arrangement. Political measurements raise questions around control, like how much control a government must execute through the improvement of the law. In financial terms, wrongdoing discipline is costly in monetary terms and includes a significant impact on the corrective approach. The fetch of preparing guilty parties is colossal, in this manner there is progressively a move to cut costs by presenting, e.g., community punishments and when choosing what to rebuff, a few offenses may be uneconomic to rebuff, such as minor encroachment and now and then it is way better to utilize lighter sentences.
5. Impact of open conclusion on correctional arrangement: This may be a key variable in forming the reaction to wrongdoing and clutter. It can be communicated through appointive choice, e.g., hanging, deadly infusion, open conclusion surveys, and letters of compliance, and judges who see themselves as apportioning well-known equity as agents of the open. For the criminal equity framework to be viable, it must have authenticity within the eyes of the public. In some cases, this causes an issue where a government responds to the ethical freeze by giving harsher sentences, which do not succeed in controlling wrongdoing. It is additionally troublesome to recognize precisely the open supposition on issues of wrongdoing and discipline.
6. Prison populace: These are expanding in number and it is felt that there is a requirement for options to care such as community punishments and fines. Be that as it may, this requires open mindfulness and data on wrongdoing levels, sentencing choices, and policies. The public has to be persuaded that elective guardianship will be compelling and to be mindful that the more noteworthy utilize of detainment will as it were possibly influence wrongdoing rates.

15.5 Approaches in Penology

Like in criminology, penology may also be approached from various points of views. These may be denoted as follows:

(a) **Administrative Penology**—The definitive staff utilized for custodial capacities in detainment offices should be competent individuals trustworthy of their obligation to society. They must be well educated and conferred entry-level arranging at a few points as of late taking the work. Organizations of

old-fashioned investigators, social aces, and media people need to be benefitted for making a distinction of the detain aces in carrying out their helpful computer program engineers. Detain watches and confines chairmen owe a phenomenal commitment to keep the prisoners underneath control and phenomenal vigil on detainees who have no devotion to the detainment.

(b) **Scientific Penology—**Individualization of detainees ought to be the challenge of privatization and the ampleness of rehabilitative methodologies is basically subordinate to releasing up the custodial and disciplinary conditions keeping in light the character needs of each detainee. The organizations of restorative stars may be utilized for the relentless helpful treatment of prisoners in detainment. The detain environment has had to be helpful rather than restorative.

(c) **Academic Penology—**Scholastic penology is in a general sense realistic in character, and its crucial reason is the spread of penological data. It limits itself with the speculative data of penology.

(d) **Analytical Penology—**It centers on finding out as fair-mindedly as conceivable the ampleness of existing restorative courses of action and techniques and prescribes measures for making strides interior to the system. Hence, it makes a fundamental examination of restorative measures and offers courses of action for the beneficial organization of accommodating regard.

The basic appearance of central cutting-edge penology is that the sentences allowed need to be proportionate to the gravity of the offense. In working the sentencing system, the law should get a handle on the therapeutic contraption or debilitation based on the veritable system of the case. The nature of the wrongdoing, the way in which it was coordinated and committed, the method of reasoning of the commission of wrongdoing, the conduct of the censure, the nature of the weapons utilized, and all other factors going under circumstances are basic substances that have to be taken into account at a few points as of late sentencing the blamed. The court must not keep in light the rights of the casualty to the wrongdoing but in expansion the society at clearing despite considering this burden of a sensible sentence. Permitting sentences lacking affectability for the censured would do more harm to the esteem framework and weaken the open certainty interior to the ampleness of the medicinal framework [2].

15.6 Principle of Penology

The vital points of corrective science are:

- To examine the moral bases of discipline, and the thought processes and purposes of society in dispensing it
- To make a comparative consideration of correctional laws and strategies through history
- To assess the social results of the approaches in drive at a given time [2]

15.7 Theories of Punishment

15.7.1 Retribution Theory

Revenge is the primary hypothesis of discipline. Its essential point is that the blameworthy party got to get as much torment and persevering as brought about by him on his casualty to reduce the angry presumptions of the casualty and the community. The casualty plays a basic part in rebuking the blameworthy party. These kind of sharpen livelihoods to be require to put in the midst of the old-fashioned and medieval periods of time. Amid those days there were no corrective arrangements and the ruler was the head of the state; it is much simpler to get it when it is put this way: "an eye for an eye" and "a tooth for a tooth." Everything was on the premise of exact retribution and retaliation. Most of the casualties were utilized to rebuff the guilty parties by themselves.

15.7.2 Deterrent Theory

Impediment or debilitation refers to something that debilitates a few individuals or something, restrain anyone from taking action, weakens a few individuals from taking action or dodge something happening especially by making people feel puzzled or on edge.

Making aware the blameworthy parties that in case he commits wrongdoing he would be kept for corporal disciplines in open makes fear in rapscallions. In this speculation, strict teaching is given so that it is an outline for miscreants and they may not commit wrongdoings.

15.7.3 Preventive Theory

As Krishna says, preventive logic of discipline is based on the suggestion "not to vindicate wrongdoing but to avoid it." In the event that a criminal is restricted or executed, or something else is crippled, such discipline will deny the criminal capacity or opportunity to commit wrongdoings and anticipate society from that future hurt (Krishna). This hypothesis advances the discipline to keep the wrongdoer absent from society, which is able to eventually declare the criminal exercises within the community. Gruffly it says to keep a perilous individual in jail or excommunicate.

15.7.4 Reformative Theory

This speculation claims that a criminal can be changed into an extraordinary city as the law stands by giving him competent treatment in the midst of his detainment period. He is in need of a doctor-cum-guide and not of the adjustments officer.

Transgressors are treated more like determined who are persevering with mental and social issues. He must evaluate his behavior with the help of the masters to be a commonplace person once more.

15.7.5 Rehabilitation Theory

The ultimate and the preeminent cutting-edge speculation of teaching is rebuilding. After serving the sentence the blameworthy party got to get back to society. For living his life with regard to the jail office and with the association of NGOs provide them a put in society. These molds are especially crucial to set an outline that the blameworthy party can remember their past life without any disfavor and weight [6].

15.8 Criminology

Criminology may be a teacher that investigates and assembles the information of wrongdoing and criminal behavior. The word "logy" in criminology stands for pondering. The fundamental point of criminology is to evaluate the exceptional nature of the wrongdoing, measurements of wrongdoing, criminal behavior that motivates the person to commit a wrongdoing, and anticipation of wrongdoings. Criminology may be an exceptionally intriguing subject that incorporates the logical principles to survey criminal behavior and the brain research behind it. It moreover bargains with the sociological perspectives of wrongdoing that conjointly clarifies the reason behind the event of violations in our society [6].

Criminology in a general sense is classified into three energized branches. These are human science which deals with the social point of view of the infringement, criminal etiology which deals with the causation of wrongdoings, and penology which deals with the instrument of expectation of wrongdoings. The objective of criminology is to supply a codified, organized, and assistant subject that will endeavor to find the cause of infringement and will inevitably grant ways and cures on how the wrongdoings can be reduced or murdered.

Criminology is basically regarded as craftsmanship or social science since unlike science it does not work on codes or for the most part characterized benchmarks. There is not any genuine speculation that can be given with regard to criminology [6].

Criminal law and criminology are the aces of that possess individual spaces. They have their particular specialty. Criminal law is related with the legal and criminal equity framework, adolescent equity framework, law authorization organizations, and remedial education such as jails and restoration centers. The main objective of criminal law is to arrest the hoodlums and to supply equity to the equity searchers. It works on a settled guideline.

Criminology could be an entire other teaching that looks to investigate wrongdoing and its propensities from the grassroot level. It does not ponder criminal inclinations but moreover considers the coordinated impacts of wrongdoings on

society. It moreover looks to ponder the adequacy of discipline and recovery offices [6, 7].

15.9 The Birth of Criminology

Criminology had a quick and crucial effect on the criminal equity framework from the minute it was born. In the event we take encounters of Henry Handling (approximately) the locale of London or on the other hand, off chance that we cite Beccaria's popular book distributed in 1764, it can be opined that these logical discoveries shaped the premise of the creation of the proficient police and indictment benefit, the collection of information on wrongdoing, restrictions on the deal of liquor, of the utilize of social approach implies in wrongdoing anticipation. It was Beccaria and after that Bentham who depicted the reasoning of discouragement that criminal law is built on up to the display day. For translating the relationship between science and criminal equity a special wonder first described by Lawrence Sherman is additionally vital to discuss. Concurring to him, criminology was born twice within the eighteenth century: to begin with as an observational science and after that as hypothetical science.

Criminology as an observational science reformulated the organization framework of criminal locale. Be that as it may, its hypothetical line—be that as it may critical logic comes about as it has been driven within the works of twentieth-century masterminds—had small effect on the working of criminal equity framework of the time. As Sherman says, social sciences and criminology maybe played the part of the faultfinder and not the innovator, and this choice had long-term results with respect to the gathering of logical conclusions. Criminology as hypothetical science, precisely with its level of reflection, was not able to affirm its explanations specifically, and its scientific adages may well be addressed alluding to passionate reasons as well. Criminology as a teaching was blended with belief system and logic. The advanced improvement of criminology as a hypothetical science may clarify the truth that the working of the criminal ward is still an unexplored region and we still know little about the effect the working of the criminal ward has on the security of the community.

Social sciences legitimize their esteem on the off chance that, as associated science, they are able to demonstrate the convenience (or ampleness to preserve a vital avoidance of hurt) of the present-day courses of action they work out. The rise in wrongdoing levels at the conclusion of the ultimate century has once more affirmed the centrality of criminological inquiry. As Sherman says, associated criminology is great not because it was able to supply more comprehensive data about the ampleness of wrongdoing organization but in addition it serves a crucial task and provides a definition of more palatable superior approaches [6, 8].

15.10 Indian Criminal Courts Hierarchy

India's law framework is the biggest and complex framework in the world. It has different sorts of courts, tribunals, gatherings, etc. due to its assorted nature; the proficiency of the equity conveyance framework is high. Each legal body determines its control from the Constitution of India. The Indian legal framework is the gatekeeper of the Constitution of India, in conjunction with being an overseer of the basic rights of the citizens [9].

The criminal equity system may be a system underneath which the body of government is facilitated at keeping up social control, avoiding and directing wrongdoing, or underwriting those who mishandle laws with criminal disciplines and recuperation endeavors. The Indian criminal value system acknowledges changing the miscreants by rebuking their completely different conduct. The faulted wrongdoing ceaselessly features a few securities against misusing of arraignment powers. Criminal value systems are outstandingly unmistakable around the world depending on the country. The Indian criminal justice system works in two tiers (Fig. 15.1):

1. **Trial court**—At each area level, there is a sessions court where the trial is conducted and judgments are passed.
2. **Appellate courts**—The offers from lower courts are being tuned to the reappraising courts; the tall courts and preeminent courts are the reappraising courts where the judgments of lower courts are being challenged.

There are more powers and functions for criminal courts in India and that will be discussed later.

Hierarchies of criminal courts in India are as follows:

- Within the hierarchy, the **Supreme Court of India** is the most noteworthy and last court of request beneath the Constitution of India. It is the most noteworthy sacred court. Being the Pinnacle Court of India, it was set up under Article 124 of Portion V and Chapter IV of the Constitution of India.

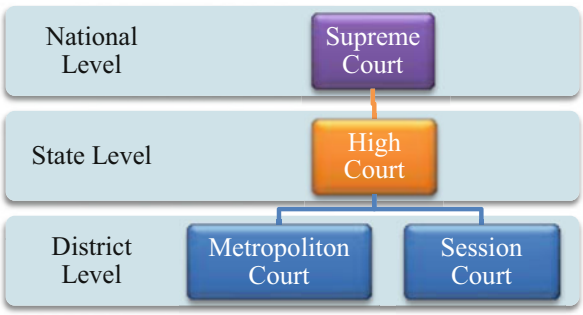

Fig. 15.1 The powers of criminal courts in India

- **High Courts of India** are inside the minute number in a pecking arrangement. There are distinctive tall courts at the state and union region level, which nearby the unique court of India at the national level contain the country's lawful system. Each tall court has domain over a state, a union locale, or a bunch of states and union spaces. In our protected plot, the tall court is careful of the total organization of value inside the state. They are managed by the Constitution of India and are bound by the judgment of the apex court. It is the court of record [9].
- Lower courts in India are being further classified as follows:
  1. **At district area**
     (a) **District courts**
        - Sessions court
        - Judicial magistrate first class
        - Judicial magistrate second class
        - Executive magistrate
  2. **At metropolitan area**
     (a) Metropolitan courts
        - Sessions court
        - Chief metropolitan magistrate
        - Metropolitan magistrate first class

15.10.1 Supreme Court

Supreme Court, in addition to being known as the Summit Court in India, is the foremost vital master over all the courts in India. The Summit Court has diverse powers which are deduced from the Constitution of India and they are as follows:

1. **Highest Court of Appeal**—The Summit Court is the foremost lifted court for an offer in India. It has the control to tune into offers from all the cases lying inside the distinctive tall courts and subordinate courts of our country. It is the most elevated court of offer inside the full country under Articles 132, 133, 134, and 136.
2. **Federal Court**—As per Article 131 the Supreme Court has the power to resolve any disputes which arise between two or more states of India.
3. **Custodian of the Constitution**—Because it is the Supreme, it has all legal powers to tune in and select the issues related to the Constitution, and because it is the Preeminent Court, it can interpret the Constitution on the off chance that any issue rises of elucidation [9].
4. **Power of Judicial Review**—Article 137 of the Constitution gives the control to the Incomparable Court that it can review any sanctioning passed by the Parliament and any judgments passed by lower courts in India [9].

15.10.2 High Court

1. **Original Jurisdiction**—It encompasses a special jurisdiction given under Article 226 of the Constitution to issue any writs to any person. The introductory district of the tall court additionally extends to the things of admiralty, probate, conjugal, and hate of court cases. The High Courts have as well full powers to make rules to control their commerce concerning the organization of value. It can rebuke for its disdain under Article 215 of the Constitution.
2. **Appellate Jurisdiction**—The ward of the High Court amplifies to all cases beneath the state laws.
3. **Revisional Jurisdiction**—It has provisional jurisdiction conferred under the Civil Procedure Code, 1908, and Criminal Procedure Code, 1973.
4. **Supervisory Jurisdiction**—This recommends the control of common superintendence of the High Court over the things of all the subordinate courts and tribunals but those overseeing with the orchestrated powers working inside the state. In exercise of this power it may:
   1. Call for return from courts
   2. May issue general rules and prescribe forms for regulating the practice and proceedings of courts

Reshape in which books and accounts are being kept by the officers of any court. This control has made the High Court tried and true for the full organization of value inside the state.

15.10.3 Lower Courts

1. **Court of session**—In India, there are locale courts underneath specific state governments for each locale or one or more districts together considering the number of cases and individuals diffusing interior the run. These zone courts are arranged at a neighborhood level. At the zone level, the area judge or extra district judge works outward on both the fundamental side and reappraising side in charitable and criminal things rising inside the range. The regional and money-related ward in charitable things appears set in concerned state establishments around mindful courts. On the criminal side, the ward is exclusively deduced from the criminal methodology code, 1973. As per this code, the most noteworthy sentence a sessions judge may allow to a convict is capital discipline.
2. **Chief Judicial Magistrate, Judicial Magistrate First Class, and Metropolitan Magistrate**—Legal judges are named and controlled by the tall court and discharge legitimate capacities. Under Section 11 (3) of the Code of Criminal Methodology, 1973, the High Court may give the powers of the lawful judgment of the essential lesson or the minute lesson on any portion of the lawful good thing about the state, working as a judge in a thoughtful court.
3. **Judicial Magistrate of Second Class**—Lawful judges are assigned and controlled by the High Court and discharge legitimate capacities.

4. **Executive Magistrate**—In India, the executive magistrates are appointed and controlled by the state government and discharge executive functions, i.e., maintenance of law and order. Unless otherwise defined by the district magistrate, the jurisdiction and powers of every executive magistrate extend throughout the district or the metropolitan area, as given u/s 22 of Cr.P.C [8, 9].

15.11 Criminal Justice Systems Around the World

Criminal justice frameworks can be straightforwardly classified as common, mindful, Islamic, or communist law in nature. Be that as it may, in these days different areas ought to get half-breed models that combine components of particular legitimate frameworks. A few of these frameworks share a common set of center values. In advancement, most criminal esteem frameworks have gotten a handle on a legitimateness. Almost in every criminal esteem, the framework is made of comparable performing skilled workers (Fig. 15.2).

Britain takes after a totally different leveled structure in its lawful system. Much obliged to this legitimate system, the country is known for the way it manages wrongdoing and conscious things. The British courts are categorized into three wide regions or levels which work in a dynamically organized way. Each of these

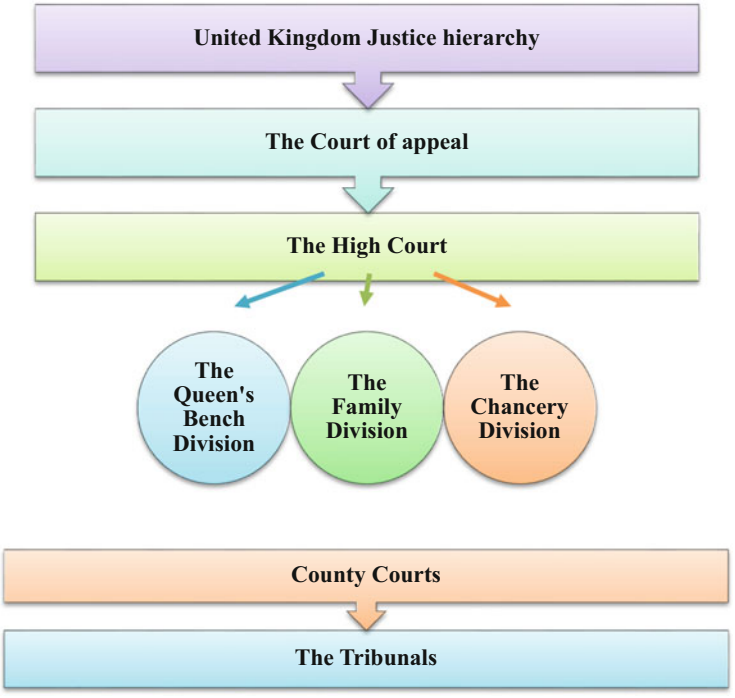

Fig. 15.2 British court hierarchy

divisions is capable in itself and is free of the others. You will get a better knowledge about the chain of command of British courts after the given information [8, 9].

15.12 The Supreme Court of Britain

In Britain, the foremost hoisted court is the Unique Court. This will be the extreme court of offer and value in Britain's court system and any decision given by it cannot be challenged or fought by anyone inside the country.

15.13 The Court of Appeal

The second highest court inside the country is the court of offer. There are two essential divisions interior to this level and they are the criminal division and the conscious division. The UK aware court division of offer takes all cases which are aware in nature and coming from the tall court, the area courts, and the tribunals. The choices taken by the court of offers are official on itself and other courts but not the exceptional court. The criminal division on the other hand tunes into all criminal cases coming from territory courts, tribunals, and tall courts.

15.14 The High Court

Third in line in the British court hierarchy is the High Court. This is the highest court of appeal within different states and this is further divided into three main divisions which are given as follows:

- **The Queen's Bench Division**—This is often the division of the High Court of Britain which handles cases that are related to tort and contracts within the zone. There are numerous other little courts that come beneath this division and a few of them incorporate the trade court, regulatory court, commercial court, admiralty court, and innovation and development court.
- **The Family Division**—Another division inside the High Courts is the family division which houses the divisional court, companies court, and licenses court.
- **The Chancery Division**—Usually, the third division inside the High Court of Britain is the one that houses the court dealing with all cases related to family law and marriage, etc.

15.15 County Courts

At the fourth level of the chain of command in Britain are the locale courts. The region taken care of by these courts is benevolent in nature and these courts are spread over 92 towns/cities generally in the UK. They handle all the litigations and

cases related to benevolent law. District and circuit judges handle all those cases and demands which come to territory courts from lower courts and tribunals [8, 9].

15.16 The Tribunals

The lowest level of courts in Britain is the tribunals. These are in a general sense those uncommon courts that handle cases and offers that are related to commerce, child welfare, relocation, etc. These are those cases that are managed by the tribunals inside the UK and there are apportioned tribunals for particular districts [8, 9].

15.17 Senior Courts of the England and Wales

These courts were formed by the Act of Judicature as Supreme Court of the Judicature. This comprises as follows:

- **Court of Appeal**—Court of Request incorporates two divisions such as the UK Respectful Court Division, which listens to issues from the High Court and the Province Court alongside other prevalent tribunals, and the Criminal Division which is offered from the Crown Court related to a trial of genuine crime.
- **High Court**—High Court is regarded as the respectful court to begin with asking and a respectful and criminal reappraising court for the cases from subordinate courts.
- **Crown Court**—Crown Court hears criminal cases for both appellate and original jurisdictions.
- **Subordinate Courts**

  In the UK legal system hierarchy, the Subordinate Court comprises as follows:

- **Magistrates' Courts**—In magistrates' courts issues are directed by a lay magistrate's seat or an area judge sitting in each region of nearby equity. No juries are there in these courts.
- **Family Proceedings Courts**—Family procedures courts listen to family claims which incorporate care cases. They moreover have the specialist to provide selection orders. It is not open to the public.
- **Youth Courts**—The youth courts bargain with the guilty parties who are between 10 and 17 years old. These courts are administered by a bunch of extraordinarily prepared grown-up judges.
- **County Courts**—These courts are nearby courts which are there in 92 cities and towns of Britain and Ridges. These are administered by a circuit or locale judge [8, 9].

15.18 Special Courts

There are some other special courts which are as follows:

- **Coroner's Court**—Coroner's court deals with the cases of death in suspicious conditions.
- **Ecclesiastical Courts**—This is too often an extraordinary court which bargains with the things of the properties of the Church of Britain.

15.19 Other Courts

The other courts comprise as follows:

- **Military Courts**—Directed by military workforce in things related to court-martial.
- **Election Courts**—Listens to petitions regarding an upcoming election.
- **Patents County Court**—Bargains with certain basic mental properties [8, 9].

15.20 The Relation Between Criminology and Criminal Justice System

As I have delineated, criminal law and criminology both play a pivotal role within the discovery and examination of violations. Both are associated and intensely depend on each other to complete each other's implications. Criminal law is a thought -about present set up, characterized as legal sets of legal guidelines. These characterized measures are formed upon the introduction of considerations and estimations that have been collected by criminologists over a period of time. The criminal value system has been heightening influenced by criminology. It has played a major role in advancing various guidelines of criminal laws.

The objective of criminology is the advancement of all-inclusive characterized sets of guidelines and definitions to wrongdoings, examination of wrongdoings, treatment of punks, and shirking of wrongdoings. Criminology to root as old as the criminal value system and it is more frequently than not the think-about of the law. Criminology does not focus on the criminal value system and other benchmarks related to criminal law, or possibly it takes an essential approach to law and how it is utilized. It places less complement on benchmarks related to criminal law and value. The association between criminology and criminal law is confusing. They for the most part cover and rule each other's capacities. If we perceive criminal justice system in this broad sense, it has four main tasks:

(a) Defending the community and society
(b) Operating the system of jurisdiction or prosecution
(c) Executing punishments
(d) Helping the reintegration of perpetrators

Based on possible consonance or lack of consonance between them we can talk about three models:

15.20.1 Cooperation (Service) Model

We are able to do conversation approximately this show of chance that the political circle requires and employments the discoveries of criminal sciences when working out for wrongdoing arrangements. It bolsters observational investigation, recognizes the accomplishments of the calling, and regards the flexibility of investigation. Agents of a proficient organization make proposals concurring to their best information with respect to wrongdoing approach to be utilized as well as its particular components, from which lawmakers can select from, taking proficient viewpoints into thought, and they bear the political duty for the proficiency of executed wrongdoing control measures.

15.20.2 Submissive Model

This demonstrates political partners that tamely apply proficient recommendations without recognizing their political obligation. They take off the definition of wrongdoing control approach objectives to the specialists, obscuring proficient and political obligation. Political duty is settled in proficient duty and it is inconceivable to see where one closes and the other starts. A case for such a show within the history of criminology was "treatment policy"—the arrangement of the welfare state, which considered wrongdoing control as the only proficient master issue.

15.20.3 Disillusioned (Dismissive) Model

This is a model in which political control acts freely, both with respect to the definition of the rules of wrongdoing arrangement and the set of measures to be utilized. This sort of wrongdoing approach considers wrongdoing control and the definition of measures as an exclusively political issue and does not let criminology skill impact wrongdoing arrangement. Rather than letting autonomous criminology specialists obtain information with respect to the current patterns of wrongdoing and the efficiency of wrongdoing control, there is a solid centralization of this work as a portion of administrative exercises.

Criminology has finished up as an associated social science by taking up the portion of analyzing the criminal value system. In the past decades, we might see that with regard to regulatory wants in wrongdoing administration in most countries instead of "why" and "what" the question of "how" was the prerequisite.

By giving a reply to these sorts of questions, from connected science, criminology is turning to an adapting/conforming science. Impartial criminology free of values and legislative issues is certainly a figment for two reasons. since criminological

inquires about have to be grounded on some scientific hypothesis; and moment since the confirmation of speculations is additionally done by inquire about. Concurring with Roger Hood, I myself moreover think that it is not the errand of criminology to characterize the objectives of wrongdoing policy.

When characterizing the suggestions of wrongdoing control, capable and political obligation is isolated between criminology as a science and authoritative issues as the performing craftsman applying wrongdoing control. Behind the relationship of science and ward, it is the cooperation/service appear that best serves the perfect division of capable authority and commitment and the usage of consistent revelations inside the sharpen of locale [6].

15.21 Difference Between Criminology and Criminal Justice System

Criminology considers wrongdoings and other perspectives related to them whereas criminal equity is the application of criminology. The criminal equity framework is not one of specialist. It comprises different multi-lexicon units that work from ground level to the upper first class level. Criminal law looks to discover the causes of wrongdoing, implement laws, explore the wrongdoing, rebuff the guilty parties, and give equity to those who are casualties. It moreover looks to restore guilty parties. A legal science master by and large applies measurable science to follow the offender; he is said to be a master in both criminal laws and criminology since he thinks about and observes criminal behavior and additionally explores crime-related viewpoints [6].

The criminal justice framework and criminology both are related to each other. They both are interlinked. Both criminal law and criminology are required for lawyers and law necessity organizations who deal with the brain activity about guilty parties and distinctive points related to infringement. Criminologists in a general sense focus on the plan, behavior, and sociological perspectives of wrongdoings. They tend to observe the plans in criminal behavior [10].

Criminal equity is a set up legitimate framework that examines violations, captures offenders, keeps them, and prosecutes the blameworthy ones. Criminal law is clearly interlinked to law authorization organizations since their fundamental work is to recognize the infringement and capture people suspected of committing any offense. On the other hand, criminology is the nitty-gritty that considers approximately the root cause of the wrongdoings, their starting, the mental slants of a criminal, and infringement on society and diverse parameters.

Criminal law and criminology are the pros that have specific spaces. They have their specific claim to fame. Criminal law is related to the lawful and criminal value system, youthful value system, law prerequisite workplaces, and therapeutic instruct such as correctional facilities and rebuilding centers. The foremost objective of criminal law is to capture the hooligans and to supply value to the value searchers. It works on a settled principle.

Criminology could be an entire other teaching that looks to investigate wrongdoing and its propensities from the grassroot level. It does not consider criminal inclinations but the coordinated impacts of violations on society. It moreover looks to consider the adequacy of discipline and restoration offices [6].

15.22 Quest: Is Criminology Good for Criminal Law?

Criminology has numerous branches such as legal specialists, mental specialists, penmanship and fingerprint investigation specialists, criminal brain research, and numerous more. Amid the examination stage of any wrongdoing, there are specialists from different areas who are required to unravel the riddle of wrongdoing and to infer the particular clue. Frequently a criminal or a charged takes off different clues or signal cards behind him amid the commission of wrongdoing. These signals can be obtained by the particular individual who is a master in that specific field. Penmanship specialists and fingerprint specialists can effortlessly derive whether the specific penmanship has a place to the charged or not. These specialists who have a place in the different strata from criminology regularly cut the time and bring us closer toward the tackling of a wrongdoing mystery.

Criminal law or criminal equity framework employs customary or standard procedures that turn around the same center and by and large incorporate the same theories and systems which have been there since the bygone. Though criminology has been progressing and liberating itself from time and is up to date and works as a standard with the latest advancements, it has been steady in understanding various well-known cases in the world. Criminology comprises a number of disciplines such as drawing on techniques and procedures that have been made in both common and social sciences [6].

Separated from these techniques, criminology as well manages various sociological and mental speculations to induce the individual behavior of guilty parties. It is especially profitable from the vision of criminal equity law. Through these speculations, natural and mental contrasts of the criminals underneath diverse social circumstances can be found and this might help maintain a strategic distance from the cause and rate of infringement in the future.

Criminology is a fundamental and indistinguishable portion of criminal law. Consequently, this ought to not emerge within the first place, whether criminology is sweet for criminal law or not? Without criminology, it would be inconceivable for law requirement officers to solve the profound and covered-up riddles of violations. There is not any inclusion of a single figure of criminology in criminal law or maybe it spins around criminal law. All of these components portray the characteristic complexity of criminology and hence it leads to the conclusion that no single figure can choose the person's behavior or the root cause of any wrongdoing. It is the amalgamation of numerous variables that work in a synchronized way [6].

15.23 Sentencing Standards Arrangements and Procedure

A. What is sentencing?
- Hearing that forces a criminal endorsement or punishment
- Comes at the conclusion of the trial

B. **Types of sentences**

1. **By Sentencing Terms Concurrent vs. Consecutive Sentences**
   - Concurrent sentence when arraigned of more than one offense, sentences begin the same day and the miscreant is released when the longest sentence is served
   - Consecutive sentence when arraigned of more than one offense, transgressor begins serving time for the minute sentence after the essential sentence had been served [5]
2. **By Statutory Basis**
   (a) **Indeterminate Sentencing**
   - Treatment has to fit the blameworthy party; hold the miscreant until he is restored.
   - The administering body sets the most noteworthy lawful sentence.
   - Judge sentences to a slightest and most extraordinary term.
   - The parole board chooses when the blameworthy party is "cured" and can be discharged.
   - Met states inside the USA utilize unclear sentencing.

   (b) **Determinant Sentencing**
   - Sentence to a fixed term of time; amount of time served is determined at sentencing.
   - No release on parole or use of time off for good behavior.

   (c) **Different Types of Determinate Sentencing**

   I. **Sentencing Guidelines**
   - Offense reality and criminal record are utilized to decide the sentence for an offender.
   - More serious offences receive longer sentences.
   - Offenders with long records receive longer sentences.

   Two types of sentencing guidelines:
   - Presumptive—guidelines must be followed.
   - Voluntary—guidelines only suggest sentence.

   II. **Mandatory Sentencing**
   - To guarantee certain discipline for the offender
   - Require imprisonment for particular crimes
   - Statutes that indicate the least sentence in jail for all wrongdoers sentenced of a certain offense

III. **Habitual Offender Statutes**
- Mandated enhanced punishment for chronic, multiple, or career offenders
- Three strikes laws, for example

15.24 Sentencing Procedures

1. **Seriousness of offence best predictor of sentence**
   - The more serious a crime, the more likely the person will go to prison.
   - The more serious a crime, the longer a person will spend in prison.
2. **Prior Record**
   - The longer the record, the more prominent the probability of a long sentence or jail sentence.
3. **Extra Legal Factors**
   - Age: Offenders under 20 or over 50 are less likely to receive prison or long sentences.
   - Gender: Women are less likely to be incarcerated when compared with men.
   - Social class: Holding offence constant.

Poor folks are more likely to be incarcerated than are middle class or rich folks mainly because they cannot afford private attorneys and are more likely to be detained before trial [5].

15.25 The Five Rules of Punishment

Penologists inquire about vital philosophical factors regarding almost all forms of discipline, direction, and control. We consider the following three:

- What is state punishment?
- Are state punishments necessary and justifiable?
- Do we need to punish at all?

The penal sanction must:

1. Create human suffering
2. Arise as a coordinate result of the execution of an offense
3. Only be coordinated at the individual who embraced the offense, i.e., the offender
4. Be the purposefulness creation of other people in reaction to that offense
5. Be delivered by an authorized body speaking to the encapsulation of the rules or laws of the society in which the offense was committed

15.26 Restitution and Victim-Offender Mediation and Victims' Rights

15.26.1 Relationship Between Penology and Victimology

It must be noted that criminology is one of the branches of criminal science which are concerned with the social consideration of encroachment and criminal conduct. It centers on finding the causes of encroachment and compelling measures to combat them.

Penology bargains with care, treatment, anticipation, and control of wrongdoings as too the different modes of sentencing and restoration of criminals.

The basic concern of casualty is to seek value for casualties of wrongdoing who are gone up against with distinctive issues. It deals with the rights and claims of casualties of wrongdoing and their dependents. The focus is on diminishing the sufferings of wrongdoing casualties and giving them compensatory and other reliefs.

15.26.2 Right of Victim

(a) **Reparation:** Reparation is clearly the preeminent comprehensive suggestion of compensating individuals and bunches whose rights have been harmed. Reparation recognizes that veritable wrongs have been done and, hence, that the hurt person is entitled to remedy and alter.
Reparation is commonly related to paying monetary payment. Though this outline of reward is the basic means to offset hurts persevered, India should not disregard other, nonmonetary, forms of reparations. Concurring to the fundamental measures and rules, reparation joins "restitution, stipend, rebuilding, fulfillment, and guarantees of non-repetition."

(b) **Victim assistance:** Isolated from compensation, casualty offer assistance that shows up to be a frantic requirement for casualty rebuilding. This would consolidate but is not obliged to the taking after organizations like crisis intervention, counseling, emergency security, criminal value advancement, and emergency transportation.

(c) **Right to engage an advocate of his choice:** The court is enabled to allow the casualty to lock in an advocate of his choice to help the indictment under Segment 24(8).

(d) **Right to prefer an appeal:** The casualty ought to have a right to incline toward a ask (proviso to region 372 was inserted by the Change Act of 2008) against any orchestrate passed by the court clearing the faulted or sentencing for a lesser offense or driving missing reward, and such offer might delude the court to which an ask generally lies against the organization of conviction of such court. In development supporting cash-related reparations for casualties, unused sanctioning got to besides address other needs of casualties, tallying helpful and mental care, budgetary care, incite security and security, and long-term restoration [11].

15.26.3 Mental Health in Prison

For the past 15–20 years, there has been more focus on the mental health of prisoners across the world [12]. This area is investigated from time to time including self-inflicted injuries, depression among prisoners, attention-deficit hyperactivity disorder (ADHD), etc. [13]. The solutions to these problems are very necessary and many researches are going on for the same. According to the study, every third prisoner in England suffers from mental health disease. A special care team is created to give proper treatment to these people who would not otherwise receive it [14, 15].

15.26.4 Psychological Impact on Juvenile

Focus on the mental health of juveniles or adolescents is less as compared to adults. Researches in Australia suggest that about 32% adolescents commit suicide in prison. About 48,963 adolescents were considered for the study. Out of these, 1452 deaths occurred in which 458 were suicides in prison. There can be many reasons for suicide like separation from family and friends, drug abuse, and mental stress. One reason is architectural hazards. Due to the presence of points to put ligature, people find it easy to commit offence leaving the prison unsafe. Prison suicide is due to both individual factor and society or criminal justice factor. One should not focus only on the individual factor as criminal justice system factor also plays an important role [4]. After coming out of the prison, juvenile finds it more difficult to adjust in the society, which leads to poor mental health, drug abuse, early mortality, and isolation from the society and social media. It results in poor health outcomes during adult phase [9]. Also the students studying with them make distance from them which directly affects their mental health [16].

15.26.5 Psychological Impact on Adults

According to a study conducted by James and Glaze, about half of the adults in the prison are suffering from some mental health disease including stress, schizophrenia, depression, and dementia [17]. According to many studies, parts of the subpopulation present in the prison have a history of mental health disorder [18]. About 44–46% of people were found to be suffering from dementia as the major mental health disorder [19]. Suicide rate in prison is higher but mortality rate after release from prison is also high due to both drug-related disease and self-inflicted injuries. The number of women is higher as compared to men in this scenario [20].

15.26.5.1 *Quest*: Does the Punishment Fit the Crime?

The approaches which are hypothesized by these three branches, i.e., criminology, penology, and casualty logy, are executed through the office of criminal law. Broadly, all these taken together constitute the subject matter of criminal science. For example, in a few social orders robbery is rebuffed by chopping off the thief's

prevailing hand. A penologist would be fascinated by why this specific discipline is utilized, for this specific wrongdoing, inside this culture and society. He might look at in-depth what it implies for the rebuffed cheat to return to society, presently lost a hand. He seems moreover to be curious about the part of the individual dependable for regulating such a discipline, or how that discipline came to existence for wrongdoing, inside that society, within.

Without a question, the more unmistakable issue penologists think around is the passing teaching and the preface behind its utilization, as well as the way in which it is carried out. Not every nation has the passing teaching and the utilize changes over around the world borders and over time. Numerous nations, and without a doubt numerous states interior they Joined Together States, have as of late negated the passing teach as a technique of educating. The open doubt has moved over a long time with respect to its utility and it is determinedly being changed.

15.26.5.2 *Quest*: Do Prisons Rehabilitate or Dehabilitate Offenders?

After nearly 200 years of prison experiment in the UK, it seems that a sentence of imprisonment is more likely to increase, rather than to decrease, future offending.

1. The imprison environment is dehumanizing and reestablishing. The innate torments of detainment are likely to be counterproductive. It may Help, the act of detainment may lead to the embedding of brain investigate that propels the expulsion of rejecters; in this way, building obstacles to positive learning.
2. The jail may be a "school for scoundrels." Jails are colleges of "crime" and detainees can learn unused abilities from their peers.
3. Can we learn how to live when we are free though we are in imprisonment? People act in a startling way in imprisonment from how they do on the outside. Detainment may undoubtedly lead to people losing aptitudes that are fundamental for adjusting on the outside. The harms of detainment are additionally likely to compound any social or mental issues that the prisoner may have had a few times as of late coming to jail.

In current applications of the term, an individual who considers penology may select to focus on the differing strategies of instructing utilized by social orders, such as detainment, and the method of reasoning behind them. Penologists focus on how to educate for the same wrongdoing shifts from nation to nation and why.

15.27 Crime Prevention

15.27.1 Concepts of Crime Prevention

Terms such as "prevention," "control," and "discouragement" are habitually experienced within the writing on wrongdoing. Be that as it may, their meaning regularly changes from one content to another. In this regard, penology is like most social sciences in that area; there are few thorough and generally acknowledged definitions.

Rather, certain terms and ideas take on a common meaning, with more assertion on utilization than definition.

Wrongdoing as characterized by the Oxford word reference is an activity or disallowance which constitutes an offense and is at blame by law or in other words an act that is illegal or the forbiddance of a commitment that is inquired by an open law which makes the transgressor at danger for educating by that law and which as well causes despicable harm to the peace and attestation of society in common. Penology routinely focuses on the utilization of criminal disciplines as a suggestion for preventing people from committing infringement and briefly for all time devastating those who have presently committed wrongdoings from reoffending [21].

"Wrongdoing anticipation" may be a term that is frequently utilized uncommonly broadly. It has served to legitimize various contrasting programs. In this way, suggestion is to censor children's TV raise the slightest wage or require school supplication all have advocates who fight that these measures will contribute to a lessening in wrongdoing and wrongdoing. Though most of the elemental goals on criminal behavior are set up by such instruction as to the family, schools, media, and other powers that instruct common social values, there are other instructions included, more clearly related with the shirking of wrongdoing. The preeminent clarity of these is the criminal value system, with its concepts of debilitation and its components for the fear of transgressors, the assertion of their fault or guiltlessness, and the teacher and in a perfect world the recuperation of those found reprehensible of manhandling the law [21].

Currently, the approach of penologists to crime prevention centers around five major considerations, namely:

(a) The miscreant is essentially a human being. In this way, a more critical thrust needs to be on the freedom of the Cretan for his remaking.
(b) The address of detainment is to bring around prisoner's resocialization through the strategy of restoration.
(c) There is a more essential prerequisite for administrative participation inside the shaping of medicinal course of action and subjection of medicinal hypothesis and sharpen to run the emergence of law inside the organization of criminal justice.
(d) Control of misconduct infers an environmental translation of sociological issues. Subsequently, to hold in check the frequency of wrongdoing, the conditions conducive to culpability must moreover be kept under control.
(e) There is a requirement for "socializing" the organization of criminal value by more conspicuous open participation and interventions by operators of the community, both in criminal court procedures and inside the execution of sentences. Consequently, criminal value and the community must be brought closer together, since those who judge and those who are judged are both parts of the same society. Social bolster inside the organization of criminal value is conceivable through the introduction of a jury system, advantaged officers, people's assessors, specialized guides, and definitive sheets [22].

15.27.2 Crime Control

Wrongdoing is not seen since it was one issue in a given society. Or perhaps it could be a significant parcel of understanding a nation's sociocultural, political, and money-related circumstances. In some cases, particular social issues stem from "uncoordinated and unsystematic" social changes, particularly among those who are incapable to change to ever-changing progressed circumstances. Speedy social modification ceaselessly tends to make social disorganization, which consolidates wrongdoing. In despise toward of the truth that each society experiences distinctive degrees of social change and coming about increases in wrongdoing and wrongdoing rates, rates of change differ from one society to another. Another chance that ordinarily is given without a question to the case, at that point it is conceivable to induce it society by looking at changes in wrongdoing and wrongdoing rates where they are utilized as a record for measuring societal changes and their characteristics [21, 22].

One of the incoherencies of cutting-edge, open-course activity is that overwhelming contribution to the criminal esteem framework may, over the long term, cause an increase in wrongdoing. This could be done routinely since contributing to outside social control takes nonattendance from supporting works that create self-control by socializing individuals and giving them a stake in society. Essentially, contributing as well much to outside control and as well small to nurturance may trigger a horrifying cycle in which down and out nurturance increments the number of individuals who are likely to commit encroachment; more wrongdoing invigorates contributing to outside controls and energizes diminished financing for supporting programs, and decreased financing for supporting programs produces more potential criminals [22].

Evading this pickle requires a balanced approach to wrongdoing control that consolidates both exterior strategies that ruin criminal acts and diminish criminal openings over the near term and supporting methods that act over the long term to decrease the concentration of blame in society. In any case, keeping up this alteration is troublesome since media emotionalism, political comfort, and open anxiety make an ask for quick fixes to issues that are complex and significantly embedded. In case of any adjusted approach it was gotten to be doable wrongdoing control courses of action are guided by an add up to an efficient understanding of wrongdoing. The same formative organic guidelines that organize the life sciences can donate such an understanding [21, 22].

15.28 Prison Reforms in India

These days' detainment is not just cruel to pound grinder or breaking rocks but the sense has broadly been changed. Certainly, the condition of the advanced jail framework is way better than that within the more seasoned times but at that point once more still much remains to be wiped out the heading of jail changes for compassionate treatment of detainees. In India, jail changes did not materialize out

of the social development but were essentially a result of the most noticeably awful conditions of treatment confronted by the political sufferers in detainment facilities amid the period of their detainment. They more than once propelled challenges with the jail specialists and made all conceivable endeavors to see that the rigors of jail life are lightened and detainees are humanely treated. The first ever comprehensive consideration was propelled on this subject with the arrangement of the All India Imprison Committee (1919–1920). It is in fact a major point of interest within the history of jail changes in India and is apropos called the turning point of modern jail changes within the nation [3].

15.29 Protection Provided to International Diplomats

All-inclusive Arbitrators consolidates against crime,; A Head of State, tallying any portion of a commonly mindful body performing the capacities of a Head of State underneath the structure of the State concerned, a Head of Government or a Serve for Farther Issues, at anything point any such person is in a farther State, as well as people of his family who go with him; It as well consolidates; any specialist or official of a State or any official or another administrator of a all inclusive organization of a between time character who, at the time when and inside the put where wrongdoing against him, his official premises, his private settlement or his infers of transport is committed, is entitled congruous to widespread law to exceptional security from an ambush on his person, adaptability or regard, as well as people of his family forming parcel of his family.

Wrongdoing committed against such high-profile individuals is remarkably much unsafe to broad peace and coordination. Day in and day out, butcher, seizing, or snare upon the individual or opportunity of such individual takes place. Each state party has made this encroachment culpable by fitting disciplines that take into thought the validity of such an act.

But are these punishments and penalties effective and how such situations should be tackled to maintain international decorum is a serious question which needs to be immediately addressed.

State parties can set up a region over the infringement committed. Steady coordination among portion states can in addition be utilized as a preventive degree to stop such unlawful works. States parties ought to take all practicable measures to dodge courses of action in their personal districts for the commission of those wrongdoings interior or outside their spaces, exchanging information and arranging the taking of administrative and other measures as appropriate to expect the commission of those infringements.

The state party which has reason to recognize that a charged culpable party has fled from its region, communicate to all other states concerned, coordinate or through the Secretary-General of the Joined Together Countries, all the critical truths with respect to the wrongdoing committed and all open data with respect to the character of the declared rapscallion.

15.30 Recent Development

Concurring to the cutting edge hoodlums can be debilitated by unforgiving disciplines as a taken a toll of breaking the law. It has been preeminently observed that developing countries like India focus more on disciplines rather than their practical execution. The slight utilization of laws and unforgiving disciplines lead to a culture of open and private brutality, noncompliance, and exemption, as can be observed in India these days.

The laws relating to social courses of action such as Article 377 on homosexuality or cheeseburger bans and dissent laws, which are picking up ubiquity all over the country, go with overstrict disciplines. Without a doubt in non-prohibition states like Delhi, the possession of a few cases of ale, or a collection of more than nine bottles of single-malt bourbon, appears to reach one to a detainment term of 3 years. Included in list for keeping up criminal censuring under portion 400/500 IPC. Input is in general sense of charitable off-base that was criminalized within the middle of the British period when duels pointed at guarding honor and reputation posed a risk to open organizes. The prerequisite of the hour is that India has to make strides inside the transport of speedy esteem in mindful criticism cases, rather than holding criminal insulting [2].

15.31 Relationship Between Penology and Criminal Justice System

We all know that crime is not something new which has arrived now but it has been preexisting since very long time. Our elder generations have certain rules to regulate these crimes. According to law, crime is doing something wrong which is illegal or violating in terms of criminal justice system. Earlier, in the ancient time, the punishment of any crime committed by a criminal was limited to the person against whom that crime or offence had been committed. Nowadays, crime committed against any person is taken against the whole society or community and even state. Earlier, people believed more in myths and religious facts and even forgot the crime and criminal. Mental state of the victim was not given attention but now everything has changed. Victim's mental health is very important to the criminal justice system. It has now become important to give punishment to a criminal for committing any crime, thus introducing the branch penology. The punishment is decided by a group of people who have knowledge of law and legal system. This bunch of people make the criminal justice system. There is another term which is defined with these terms, victimology, i.e., study of behavior of victim after occurrence of a crime which includes their behavior with society and with their own [23, 24].

Penology and the criminal equity frameworks continuously come hand in hand. These two are fragmented without each other. Penology is the ponder of the treatment of individuals in guardianship and the avoidance of wrongdoing. Penology essentially bargains with the discipline given to guilty parties who commit

violations. On the other hand, criminal equity framework is the bunch of authorized individuals who are included in avoiding and lessening the rates of wrongdoing. The criminal equity framework includes police, judges, and legal counselors primarily. Criminology which incorporates rules and directions to anticipate wrongdoing too plays a critical part in both penology and criminal equity framework.

Penology includes the discipline of wrongdoing against a person who possesses will which is more altogether, detainment. Almost nine million individuals are within the guardianship of law around the world. The jail is utilized for individuals included in law and its utilization is expanding day by day [25]. People are kept within the jail against their will away from their family, companions, and society. There is a certain reason for keeping hoodlums in jail: to improve their behavior, to require them absent from unlawful dos, to form them mindful approximately of the off-base deeds, etc. [26].

During early days, criminals were treated very badly. They were beaten by the authorities for their crime and were made to do labor work. People involved in criminal justice system would have thought that by doing these things, behavior of a criminal can be improved. He might change his mind of doing crimes and get back to the right path in life. Many researchers came and they applied new rules for these criminals. Some of them thought that treating them in bad way is best whereas others focused on the mental and physical health of prisoners. They used to motivate them to do things that they like and give them an idea to earn money. Many prisoners started doing handicrafts to pass their time which makes them happy and as a result there was an improvement in their mental health [27].

There has been an improvement in the working style of prison as well as in the architecture. Earlier many prisoners used to live in a single prison. But as time passed, each prisoner had their single cell where they can do their work efficiently and with full concentration. There were many negatives of the single cell. As prisoners were alone at their own, many cases of suicide were noted and this situation was going up regularly [10].

Earlier individuals had to spend a longer time in jail but with progression within the criminal equity framework, the period of detainment was settled concurring to the wrongdoing committed. The time period can be expanded or diminished depending on the circumstance of the detainee. With the progression in time, a few hoodlums were sent to government or central jails, basically for a longer term; a few were sent to neighborhood detainment facilities for shorter lengths; and a few were sent to the house of redress who did not commit the major wrongdoing or who committed the wrongdoing out of carelessness [28].

As we know, some criminals were sent to house of correction for some days. There they take care of themselves, improve behavior, learn new things, and come back as a new improved person [29].

Not all detainment facilities are for grown-ups; there are a few specialized jails for females and adolescents or youthful wrongdoers. Each and every detainee is given uncommon treatment and care these days. Government specialists focus on the mental and physical well-being of detainees. They organize numerous exercises to

create them upbeat and sound. They are too prepared and given the chances for advancement [30, 31].

It is exceptionally critical to know whether the utilization of jail has any impact on the rate of wrongdoing. The connection between detainment and wrongdoing is exceptionally complex. It completely depends on the person and environment of the jail. On the off chance that both of them back each other, at that point there are chances of diminishing the wrongdoing rate. But in case any of the variables contradict, at that point it creates an issue [31].

The criminal equity framework is composed of offices of government which have all rights to apply the law and take lawful activities against any wrongdoing and criminal. The criminal equity framework is exceptionally imperative for each and every person living in a nation. It makes a difference to give equity to casualties and ensures common individuals from savage packs and wrongdoers. It is able to say that criminal justice framework is social control. As there are numerous awful and perilous things going on in our society, to control them criminal equity framework comes into play. They have the complete right to convict and rebuff individuals for their wrongdoings. They exceptionally try to begin with step which is the enlistment of complaint to last choice is exceptionally critical and in this criminal equity framework plays a noteworthy part.

The criminal equity framework is gigantic because it forms, treats, and rebuffs wrongdoers. Each step needs a group or gathering of individuals. The major components of this framework are police, courts, and restorative houses or organizations. Police, courts, and these adjustment offices are distinctive government organizations having distinctive parts to play, and hence have diverse strategies. Each one of them is imperative for giving equity to a casualty and fathoming criminal cases [32].

We commonly see police patrolling around on roads and streets. Their main role is to assist people when needed, investigating crime and identifying criminals and suspects. They provide medical support in case of emergency [33].

Courts are considered a major part of the criminal equity framework as the choice, trials, and sentencing forms of a wrongdoing take place there. They moreover make any doubt that offenders must not endure some time recently any discipline is given to them by the equity framework. The criminal equity framework can be particular which suggests that it is not continuously that individuals committing the same wrongdoing will have the same discipline. Discipline depends on the criminal, his/her history and behavior, and conjointly on the degree of wrongdoing committed by him/her [34].

It is not conceivable that any wrongdoing can be attempted in any court. There is a framework for court in India that incorporates preeminent court, high court, local court, and extra area court, subordinate judge lesson I, subordinate judge lesson II, metropolitan courts in little cities are for little cases. Incomparable court is at the most elevated level of the chain of command. The lower court is permitted to do the trials of cases and beginning handle. In the event that is conceivable, they can wrap up the case by making a choice that can be in favor/against the wrongdoers. The discipline given by the lower court is generally fine.

Criminal justice system believes that a criminal must go to the prison for moral reasons. They deserve to be punished after what they have done. Prisoners are separated from their normal way of living the life [35].

Indictment plays an imperative part within the criminal equity framework. These are advocates or attorneys from the state to bring equity to the casualty. They stand against the respondents who speak to the contradicting party or criminal side. The prosecutor is mindful of charging the guilty party against the wrongdoing and giving a final decision. The choice can be charge/fine, detainment for a specific time period or for a lifetime, and passing sentence. The passing sentence is primarily given in more incredible violations.

Each victim is given an attorney for their trials. They may take the attorney and even refuse if they can arrange one on their own. Thus, poor people who are not able to arrange any lawyer will get full help from the justice system.

Remedial institutions or offices are the places where wrongdoers are kept for a specific time period. They are taken care of by the government specialists there. These are moreover named correctional facilities. These restorative houses are moreover utilized for the ones who are holding up for their trials or choices. There is a partitioned government office, known as a jail for the offenders who have committed lawful offense violations. Individuals who are sentenced to life detainment or detainment up to 7 years or more are kept in detainment facilities. Detainment facilities change from imprison in terms of both engineering and procedures. These are more secured; one can see long dividers, wire, or glass work on the external divider boundary. The populace in jail is expanding day by day as the rate of wrongdoing as well as the time of detainment is expanding [36].

Numerous detainees are moreover cleared out early depending on their change and behavior. They are taken under the supervision of a few qualified individuals after their discharge to society. They must follow certain rules and directions after discharge. In the event that they are found damaging those rules, lawful activities are taken against them.

In this subtopic, we have considered both penology and criminal equity framework. These are interrelated and total when together. The criminal justice framework gives discipline to the hoodlums or guilty parties for their wrongdoing. On the other hand, penology is the discipline for those offenders ought to go through and it too makes a difference for them to make strides. Both penology and criminal equity framework anticipate the event of violations by giving a lesson to other individuals living in a society [37].

15.32 Summary

15.32.1 Can Penology Be Seen as a Friend?

Within the current climate, there are numerous questions encompassing our criminal equity framework. A few individuals have addressed the numerous roles that are played by our officers in blue. No matter your political views, one thing is evident:

The US criminal equity framework plays an enormous role in our society. Keeping communities secure should be the number one need. In the event that you want to make your community a more secure, you will be considering a career in law requirement or criminal equity.

At its center, there are three primary components of the criminal equity framework: law requirement, courts, and redresses. Each of these branches must run appropriately to preserve law and arrange inside a society [31, 38–44].

The criminal value system is orchestrated to communicate "justice for all." This gathers ensuring guiltlessness, sentencing miscreants, and giving a sensible esteem handle to assist coordination over the nation. In other words, it keeps our citizens secure. Without the criminal esteem framework and all those who work within it, there may be demolition, violence, burglary, and threat all around. But criminal equity specialists—those in law prerequisite, rectifications, and courts—are arranged to acknowledge that we are prepared to travel locally, to work, and/or out in open, securely and safely.

Through the criminal equity framework, when an individual has broken the law and committed wrongdoing and is found culpable of that wrongdoing, they are held dependable for their criminal behavior and works. More frequently than not, through the utilization of a legitimate choice, the blameworthy party is sentenced to encounter a certain discipline to ensure the evasion of any and all future criminal development. The sheer reason of sentencing is not to rebuke the criminal and ensure the disheartening of future criminal development but to keep the public secure. The criminal equity framework covers upon society from top to foot since after you immure some person, you are not fair influencing them. This criminal equity framework is so much greater than the normal citizen realizes and so much more interconnected [31, 38–44].

15.32.2 Can Penology Be a Foe?

Wrongdoing is to be controlled not by changing the working of our teaching, but maybe through expanded watchfulness on the allocation of tenants, either autonomously, collectively, or both, in securing themselves and the social organizing. We may make changes in the workings of numerous principal instructions in society in the event that we are to genuinely address the wrongdoing issue [31, 38–44].

Traditionalist penologists will see the "victimization debilitation" approach as a conclusion in itself, as a methodology that creates a distinction of the formal chairmen of social control plans to the wrongdoing issue. Magnanimous penologists who advance the "social disorganization and social control" approach see the "victimization crippling" strategy as an allocation of the more prominent procedure of inhabitants playing a parcel which is working for essential taught insides their community to address the causes of wrongdoing.

Be that fundamental penologists that may see through ongoings, sometimes, recently two models as steps inside the planning of building community, from the responsive to the proactive organization. The above mentioned

organization is one in which it got ended up through the fight of inhabitants to address the fundamental highlights of the social orchestrate which limits community progression and limits the capacity to address the wrongdoing issue itself. In conclusion, in looking into the unmistakable approaches to utilizing the community to control wrongdoing, one must be careful of the unmistakable doubts which each of these approaches is making with regard to the conception of the community, the cause of wrongdoing, and the inadequacies of the approach [31, 38–44].

Along these lines, wrongdoing is not a result of mental affliction in the standard sense, despise of the truth that there is a little rate which certainly is so, but a cognate appearance of social adjustment. There are, for the occasion, rougher and self-evident causes, such as deficient family environment and arrangement, besides the more individual and less cognizant components, which join concealment and corruption of intuitive plans.

The impact of information advancement and its amplifying estimations need to be recognized by the true blue society, particularly those who are concerned with the organization of criminal value. The courts, advocates, academicians, the law teaches, and undoubtedly the prosecutors, everyone got to familiarize themselves with the utilization in the making of disobedient and developments that meet the demands hurled up by different statutes and case blasts [31, 38–44].

Digitization of courts, painting environments, courts and libraries, and disclosure of cases and judgments have enabled rapid access to primary data. Therefore, it generally facilitates the elimination of escape clauses from common fraudulent toll gadgets, revealing and eliminating inefficiencies, horrifying tastes, and horrifying visibility that permeate criminally important organizations. The endeavors that are being made afterwards to switch over to e-courts with e-governance for e-justice would certainly go a long way in reestablishing the certainty of the people inside the criminal value system which lost its legitimacy being as well exorbitant and moderate. The progression of ADR instrument Lok Adalat's plea bargaining and setting up of the Quick-Track Courts are some of the measures which certainly offer help to fortify the cause of the criminal value system in India [31, 38–44, 46].

15.33 Conclusion

Our dialogue was at the opportunity that today's prison doctrine, a more advanced way to understand the skills of fraudsters, contributed to the subsequent growth of the prison population. In spite of the fact that we accept that it has, we moreover recognize that the unused penology is both the cause and impact of increments. We recognize that those conditions we alluded to at the start as "outside" have set weights on criminal equity teaching that, in turn, have caused them to adjust in a wave of ways. The point of our research, in any case, has been to show how this exhaustive adjustment has been fair. It has driven to a noteworthy reconceptualization of penology, a move that institutionalizes those versatile behaviors. It grasps the modern shapes that have emerged as a result of this adjustment. As such, the unused dialect, the modern conceptualization, guarantees that these unused shapes will

endure autonomously of the weights. They show up to be changeless highlights of the criminal equity framework [45].

Victims of fraud have a variety of rights, including hearing, protection from retaliation, processing of court records, instruction to experts, collection of witnesses, reviews, enhancements, and digitization. Ideally, Digicam technology is in the midst of investigating and preparing for unique cases such as ambush and violence in the country, suitable for maintaining identity verification thrillers and dealing with personal injury proceedings. Is now making an unpleasant claim to clarify all the claims of a hobby that have passed the court for a long time, to clarify accusations or convictions for fewer insults or lack of compensation limits. About instances where corrupt people are ambushed to stop [45, 46].

Supporting methodologies alone cannot succeed since person variety and frequency-dependent payoffs for distinctive behavioral methodologies guarantee that there continuously are a few individuals who are spurred to utilize constrain, extortion, or stealth to get assets: either to create the finest out of a terrible circumstance or to require advantage of a great situation. That is, some individuals have competitive advantages over others within a particular niche because

I. Personal characteristics make them more able to compete employing a specific behavioral methodology (i.e., coordinate, work alone, take).
II. They esteem the asset more profoundly and in this way will compete harder.
III. The behavioral technique they utilize is predominant to that utilized by others endeavoring to misuse the specialty [45].

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Chain of Custody: Scaling the Investigation to the Event

16

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Abstract

The chain of custody is described as the procedure for preserving and recording facts. The chain of custody is the most thorough and evaluative method of documenting data. In a legal proceeding, it aids in testing the validity and permissibility of facts relevant to a crime. The validity of the evidence can be determined by comparing the evidence gathered to that found at the crime scene. Evidence, whether tangible or intangible, plays an important role in criminal investigations. It aids in the recovery of a crime, as well as the recognition of the likely assailant or culprit, ensuring their authenticity which is necessary to maintain superiority. The chain of custody is a set of measures that begins when an investigator takes custody of evidence at a crime scene and is followed by each inspector general officer throughout the investigation. The chain of evidence report provides a detailed list of the individuals who gathered the evidence, processed the evidence, transported the evidence, and when and by whom the evidence was examined. The officials' ethical and professional responsibilities include maintaining the chain of custody. Evidence mislabeling, evidence modification, unauthorized personal access, and inadequate storage are all examples of these circumstances of the evidence that results in breakdown of chain of custody. If the custody chain is broken during the investigation, the evidence becomes tainted with the crime, which may make it more difficult to convict.

Keywords

Chain of custody · Criminal investigations · Evidence · Legal proceedings

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16.1 Introduction

The technical examination of physical evidence relevant to the case to suspect illegal behavior or criminal motives is referred to as a crime scene investigation [1]. The barricading of the crime scene by investigators or forensic scientists not only is the primary objective of the investigative process of the crime scene but also includes the thorough interpreting and documenting of the forensic evidence at each stage of the crime scene. The investigation of the scene is a combined effect of the diagnosis of crime scenes, scientific assessments, and physical evidence analyses, as well as recognizing links between victim suspect and crime [2]. This chain is a component of an investigation into the criminal scene. The creation of chain of evidence is a reliable way for the genuineness of true evidence to be described. The chain of custody is also known as chain of safety, chain of proof, or continuity of proof [3]. The documentation of the evidence from its possession until their presentation in the court, the custody chain maintains the integrity of the evidences. The chain of custody is defined as a process by which the continuity of possessions of evidence can be maintained, tracking the evidence path from origin to recovery and transporting it to the laboratory for analysis until it is admissible before the courts [4]. Using the custody chain, one can know who collected the evidence and handled the proof, to whom and from whom the evidence is transported and analyzed, through whom the analysis is carried out, and which type of analysis is carried out. The chain of custody is a set of notes that starts with a detailed description of the crime scene, including descriptions of the types of evidences gathered or received by the Court of Justice. The methods used to collect, preserve, pack, transport, and store evidence are incorporated into the chain of custody process.

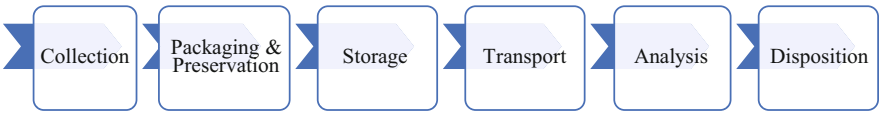
The information on the evidence on the scene includes the descriptions of the evidence, unique identification mark of the evidence, the personnel identification details, the time and date when the evidence was collected, as well as the location where it was discovered. A chain of custody is a computer-generated record that offers continuous recording of data or a sample from collection until disposal. In order to sustain the durability of the related results, crime labs including chemical, biological, serological, and numerous other laboratories needed chain. The chain of custody ensures that the proof is capable of being submitted to the court. The conventional, paper-based custody chain, which includes a full description, evidence and examination, and hash code of the investigators, was initially implemented. The chain of custody form is used by many evidence collections kits, but it was ineffective to depend on it due to discrepancies in forensic as well as in legal standards [5]. The automated chain of custody is currently used to accomplish this objective. Many forensic software contributes to the production as well as authentication of copies or images of electronic devices. The accuracy of the description of evidence, personal identification, numerical signature, and computerized assessment process is increased with such software [6]. The chronology and maintenance of the documents relating to the collection, examination, and analyzation is also termed as custody chain. The chain of custody is a controlled event in which the personnel name involved in the collection and custody of evidence, the date and description of

objects retrieved, the description of the suspect or victim profile, and the full description of the case are all monitored [7]. Many people are involved in a criminal investigation, each with their own goals, so there should be a clear distinction between the men who are in charge of the crime scene, the investigators, the criminal investigations operator, the crime scene protection, the photographer, the sketch maker, and so on.

The chain of custody is comprised of the comprehensive management, description, and inventory of individual professionals, along with the evidence and their photographs. The evidence will not be manipulated or altered with the help of chain of custody throughout the stages of a criminal investigation, and protocol the documents in terms of how evidence is gathered, transferred, analyzed, and generated in a court of law. The chain of custody is made up of five Ws, who, what, where, where, and why, as well as one H, how [6]. Various parameters are used for determining the consistency and competence of chain of custody, such as minimum human contact with evidence, managing a complete overview of the case and documenting every detail in order to ensure that all evidence is packed, sealed, and delivered appropriately, and acquisition of evidence ensuring safety with each transfer of evidence to staff. Oral evidence is generally given in combination with the chain of custody. The type and importance of the case will decide the specificity of the chain of proof. The chain of custody documents the monitor who had access to the data, when, and for what reason. The chain of custody lifespan begins as soon as the first piece of evidence is taken from the crime scene and continues until it is presented in a court of law. Prior to the administrative arrest of evidence, the chain of custody is generally established. The chain of custody usually stops when the proof is introduced in a court of law, although it may be terminated earlier depending upon the nature of the evidence and the type of case involved. Over the course of the investigation, each person may be called upon to provide assurance that the evidence is properly secured and maintained during his or her custody. The recording of evidence and staff from the end of this review for each of the time period is required to maintain the chain of custody [8]. To ensure an efficient chain of custody, each sub-item should be properly logged, stored, and protected before being held with the main evidence to avoid uncertainty about its sources. To prevent confusion about the authenticity of the evidence obtained from the crime scene, all staff involved in processing and storing real evidence work together to maintain the chain of custody [9].

16.2 Steps of Chain of Custody

The steps of chain of custody ensure the reliability of evidence and rule out any possibility of falseness and spurious. The following steps are included in the chain of custody:



16.3 Evidence Collection

The collection of evidence depends on the nature of evidence encountered and its examination schemes used for its analysis. Evidence should be collected in such a way as to avoid the possibility of contamination, destruction, or damage [7]. In order to establish the chain of custody and authenticity of the evidence in the proceedings, proper packaging and labelling of the evidence must be carried out. The size and sample collected for each piece of evidence varied according to the crime but should be more than small size for their full analysis. Control samples are also collected for comparison while collecting the samples in question. The collection of control samples aims to strengthen the analysis and interpretation of laboratory results. For example, in the homicide investigation, besides the suspected blood pool, a blood control sample is collected from the crime scene. The sequence of the collection of evidence is, according to the available information in the crime scene, the location of the scene, whether it is inside the boundary, outside the boundary or mobile scene, the condition of the evidence whether it is in a fragile or stable condition, the environmental conditions and how these factors affect the crime scene or the evidence, the management of the scene or the handling of certain evidence, and so on.

The biological specimen is so fragile that it requires special treatment and packaging. Biological evidence includes all biological fluids, bones, tissues, and teeth [10]. All biological evidence or stains are air-dried to prevent bacterial growth prior to packaging. They all are stored in individual airtight containers, such as paper bags or envelopes. While collecting the evidence, the personnel should also take care to avoid contamination or cross-contamination of the evidence. To avoid cross-contamination, disposable tweezers and pipes should be used as a collection device. The collection of the liquid blood sample is done on disinfected cotton swab or white cloth and dried under the shade throughout natural ventilation. In the case of dried blood stain, it can be collected either by cutting the area and transported to the laboratory or on a sterile swab soaked with saline solution [11]. It is then air-dried and collected in individual bags or envelopes. In addition to biological evidence, there has been a broad range of trace evidence during the criminal investigation. Trace evidence exists in a small amount, either in a chemical form or in a biological form. Proper handling and preservation of evidence is done as it cannot be detected by macroscopic examination.

They are examined either by microscopic or instrumental methods for accuracy and authenticity. Hair, fiber, soil, glass particles, paint chips or flakes, gunshot residues, organic and inorganic materials, and other biological materials are common traces. The collection and packaging of trace evidence will therefore be done in such

a way so as to prevent the loss of trace information. All trace evidence are collected either by means of vacuum, forceps, cello tape, or swapping along with their original clothes. Liquid evidence such as inflammable liquids must be collected in spill-proof containers such as metal cans or buckets [12]. Except for ashes, the semi-solids such as dirt, pastes, electrical wires, or electrical equipment are collected through forceps. The ash is collected by sliding the paper underneath it. The collection of hazardous materials or weapons is generally carried out by trained personnel with complete protection. Bullets, cartridges (fired or unfired), shells, pellets, and wads are collected with the help of tape-end forceps. The marking of bullets and cartridges is done on the base or the nose of the bullets. Electronic data is often analyzed by trained personnel, including a computer forensic examiner, who can accurately manage, collect, and package these objects without sacrificing the content of forensic significance [13]. Fingerprints on smooth surfaces are collected with the aid of fingerprint lifting tape. With the aid of tweezers, fingerprints on the work places are obtained. Forceps are often used to capture printed materials, notes, and checks while retaining the latent fingerprints on them.

Footprints, tire marks, and tool marks are recorded first and then cast using plaster of Paris. Pills, powders, and particulates are collected in a vial with a label on the outside. Soil samples are taken on a clean sheet of paper, while maggot samples are taken on cardboard sheets [24]. For identification purposes, marking is done at each stage of collection and packaging. Evidence is marked on the outside of the bottle, plastic bag, or envelopes with information such as the department case number, item number, receiving or retrieving date, and investigators' or personnel's information. The investigative agencies transfer the scientific proof to the property room before sending it to the laboratory for testing. The details of the case number, types of evidence, investigating personnel details, administrative details involved in transporting the package, signatures of all officers involved in exchanging and transporting the evidences from another individual or location, and details of the crime scene all are included into the documentation of the property room.

16.4 Preservation and Packaging of Evidence

The inspectors or officers in the chain of custody use proper handling and storage requirements to ensure the integrity of the evidence. Photography can be also be used to preserve evidence such as fingerprints, notes, impressions, and crime scenes. Paper packets, cardboard boxes (gun boxes, knife boxes, etc.), plastic envelopes, airtight containers, butcher papers, metal cans, adhesive tapes, syringe and safety tubes, Kraft papers, warning labels, and other items are essential for packing the evidences [14]. Since inappropriate packaging can harm or spoil evidence, therefore, the selection of packaging materials depends upon the type of evidence collected from the scene. For example, organic materials such as plant or animal products are packed in paper bags or cardboard boxes. To prevent bacterial growth, plastic containers are avoided during packing of biological or serological products [11]. Drying is done before packing the bloodstained clothing in a paper sheet and maintained in a paper cloth. Anticoagulants are used to preserve the liquid blood, or

the evidence is held in a cold environment. Hard materials such as weapons or tools, cardboard boxes, or plastic tubes are used for packaging. Liquid evidence like hazardous chemicals is always packed in containers such as wooden boxes or buckets to prevent them from fluctuating or moving. Arson-related debris materials are packed in aluminum with a size that is proportional to the size of the sample collected.

Each trace evidence, such as fibers, hairs, paint chips, or flakes, is packed in an individual druggist folding method kept in a separate envelope. Weapons such as handguns or rifle guns and their parts such as shotguns and cartridges (fired or unfired) are stored in soft paper tissue placed in a pillbox or wooden box and prevent friction, displacement, or contact during transport to the lab. The shot rounds, bullets, and wads are stored in paper envelopes or match boxes. If the evidence is wet, avoid plastic containers. The remnants of gunshot found on the fabric are collected without bare hands and packed in paper bags, with one fabric in one bag. Tiny glass particles are packed in pill boxes, while glass fragments are packed in cotton or soft tissue. The collected fingerprints from the polished and unpolished surfaces are transferred on a non-porous card, and the card is packed and sealed in plastic envelopes. Impressions such as footprints, tire marks, and tool marks are packed in wooden boxes after taking photographs of the impressions. Liquid samples, such as poison or drugs and acids, are packed in a wooden box or even in a sawdust, and the drugs which are collected in the form of tablets or powders are packed in a cotton wool container. The tools gathered are packed in plastic envelopes, and the tool marks are packed in a wooden box.

All the entomological evidences collected are preserved in formalin or in alcohol. The digital evidences encountered on the scene of the crime are packed individually in a case or a sheath and sealed in a labelled paper envelope or a paper bag. Evidence found at the time of the accident or received in the criminal investigation is packed to prevent loss or cross-contamination. Labeling is done on the outer side of the packaged containers with all the case and evidence-related information, such as case number, serial number, make and model, clothing material description, investigator details, etc. At the opening site of the package, the container is sealed with the government department seal or adhesive tape to prevent contamination or cross-contamination. A biohazard label is used for the evidence container that will contain the bio-hazard material for careful handling of the container [11]. Packaging should not be reused on the used products to avoid cross-contamination. The seal or the adhesive tape contains the information about the investigators' details and the date of the seal applied on the containers. The presence of the sealing ensured that the evidences' authenticity was preserved. After marking the package, the package generally is sealed with the seal or proof tape of the Government department prior to acceptance and release into the custody of the Agency. In order to allow multiple entries of evidence in the packages, the seal must not be broken while opening the package, and the package should be resealed from this same side whenever the evidence is returned to the pack. Packaging should be done in such a way as to protect the evidence from the damage, adulteration, and deterioration that may occur when the evidence is transported from the time of the accident to the laboratory for

analysis [15]. The evidences are sent for storing quickly as possible following proper packaging and labelling. Any substance that escapes to the outside can be prevented by a properly sealed container. A properly sealed box contains identifying marks that show who actually sealed it [15].

16.5 Storage of Evidences

There is little evidence that is not provided on the same day as the crime, and therefore special storage facilities are required. Improper management and storage facilities have a serious impact on the condition of the physical evidence, particularly the biological or serological evidence. This storage provides temporary shelter with limited access with proper security facilities [10]. Access to these storages generally is limited to authorized personnel or investigators. Personnel outside the staff must be registered and logged in and accompanied by the observation staff. The Agency involved in the processing of crime should have a policy and procedure for storing physical evidence. These rules would specify the personnel's or officers' responsibilities when presenting evidence to the storage room. These policies will make it feasible to satisfy the documentation standards and help in the proper testing of evidence. The selection of the storage area depends on the size of the packaging evidence and its temperature requirements. The areas commonly used by staff for storage are freezer or refrigerator, bulk storage, and high-security, useful, secure, or highly secure storage [12]. The storage area or location is secured by an alarm or CCTV camera. Storing of evidences whether small or large is properly documented with labels of case information on the storage area.

While storing evidences, consideration is given to proper equipment, safety, training, and management of the personnel handling the evidences in storage locations [11]. Alarms or video surveillance monitors the storage area or locations. Evidence storage, whether small or large, is properly documented with criminal record labels on the storage room. The appropriate equipment, protection, training, and management of the staff handling the evidences in the storage locations are all taken into account when storing the evidences [25]. The storage area should be organized in a systematic manner. Storage facilities can be either temporary or permanent. Evidence lockers or cages, repurposed lockers, offices, bins, freezers or refrigerators, and other temporary holding facilities are available. In most of the biological samples, a short-term storage in the refrigerator with temperature of 4 degrees Celsius is recommended [11]. On the basis of the availability of the evidence examination and the seriousness of the case, the evidence may be transferred to a long-term warehouse. Long-term storage facilities include hanging folders, cardboard or paper box shelves, racks and cabinets, and rooms. Mostly, in the case of biological samples, a long-term storage, especially for 2 weeks or more, is needed at −20 degrees Celsius or lower [11]. Security measures relating to the warehouse or location are written, and government individuals handling the evidence are asked to sign the chain of custody form.

16.6 Transportation of Evidences

While transporting evidence from the scene to the laboratory, precautions are taken to prevent evidence of contamination and destruction. Some sensitive evidence, such as biological or serological evidence, seems to be susceptible to optimum temperature or temperature fluctuations. Temperature fluctuations can be seen during the summer months or during the winter season. During winter period, precautionary measures are utilized to avoid the cooling of evidence. During summer season, due to high temperature, the evidence does not appear in the enclosed area inside the vehicle as it causes an increase in the temperature of an evidence that damages or contaminates the evidence [23]. The personnel handling the evidence must wear gloves while transferring the evidence. The transport of evidence should not be carried out in an open-top plastic container, because that raises the chances of seepage from the carton while transporting the evidence. During the temporary holding, adequately store testimony in a controlled setting or in optimum temperature while sustaining evidence of safety, or, if not possible, transport the evidence immediately to the laboratory. The transport of the evidence to the research lab concerned requires a cover letter or a transmittal letter. The letter normally contains all information relating to the case, such as the nature of the crime, the schedule of the crime, area of the crime scene, the suspect or convicted person, and the details of the victim's identification. The letter also contains a stock containing all the details of the documents provided in conjunction with the manner in which it was found. That letter also contains a request for the examination of the evidence as per its nature and information.

16.7 Evidence Analysis

To safeguard the credibility of materials and their acceptability in a court of law, all evidentiary materials are transported to the labs in evidence boxes that are packed, sealed, and labelled. Standard procedures and policies are adopted by the lab and scientists to reduce sources and the risk of contamination (By Hayden B. Baldwin, B.S and Cheryl Puskarich May). Items requiring any chemical analysis shall be documented in order to preserve their movement and location. The samples submitted for analysis was duly signed by the personnel request for analysis and documented and secured the area until the analysis is completed. The analysis of the evidence shall be conducted out by the forensic laboratory upon submission of evidence by agency or personnel and their request for laboratory service. Documentation of the evidence includes the details of the personnel handling the case, the type of evidence submitted, and the type of test they requested for the evidence submitted. The documentation is either done by the computer, or in some laboratory, this documentation is carried out by the laboratory information system that uses barcoding as a unique identifier for the individual samples. A written record of almost all the evidence provided during the laboratory examination shall be retained. A letter of request is also attached to the evidence submitted for analysis which states

that a written analysis is to be returned to the Agency or authority. The results of the analysis shall be referred to the investigator or the case officer by the Agency. When an analysis of the evidence is carried out, the evidence containers are resealed in the same packaging and stored in temporary storage if further analysis of the evidence is carried out at a later stage or years later. Usually, the evidence has so far been transferred to the Agency or is often kept in the laboratory for the purpose of the court.

16.8 Disposal of Evidences

The disposal of evidence relating to the case by the investigators is done when a notice has been given by the prosecutor or the court. The notice includes information such as the refusal of fees, the request for additional work by the prosecution agency, objection of the case, or final criminal trial. The retention of evidence of a crime is based on factors such as the type of offence and the disposition of a case [16]. For example, in cases where the charges are rejected by the prosecutor or no investigation is made, or the suspect is arrested, the evidence is disposed of. The disposition of evidence requires a disposal form signed by the investigators. However, where biological or serological evidence is engaged, the evidence is not able to dispose of, and it will be retained for as long as possible. Evidence disposal form includes details of evidence or items such as items that need to be reserved, items that do not have to be reserved or an item that need to be disposed of, etc. When the evidence disposal form is completed, information of the evidence or items retained, disclosed, or disposed of, is required: information on the item number, property identification number, and the reason why the item should be retained or released or disposed of. Employees who fill out the evidence disposal document are liable for activities they take on with the evidence as well as its disposal.

16.9 The Significance of the Custody Chain

The custody chain helps in protecting evidences by minimizing damages which could be caused either by the investigators or during analysis. The significance of chain of custody lies in the fact that it kept the evidence original and authentic during the criminal proceedings. The documentation establishes the authenticity of a form of evidence [17]. A paper trail is kept so that the person in control of the evidence at any one time may be identified swiftly and compelled to testify in court if necessary. When providing proof as a display, a documentation of the chain of evidence must be maintained and established in court [18]. Otherwise, the evidence may be dismissed with prejudice in court, raising serious concerns about its credibility, competence, and the evaluation conducted on it [19]. The sample gathered at the scene of crime is original and the samples received for the analysis after being analysed will remained in the original form or represent the original sample. The maintenance of custody chain is crucial for the reliability of the evidences whether

tangible or intangible and for their testimony. The chain of custody involves documenting every communication from the moment the information is gathered, from one person to the next, in attempt to prove that none of us could have acquired or acquired the evidence without permission. Regardless of the fact that there is no limit on the number of transmissions, it is critical to keep it as minimal as feasible. To minimize manipulation, evidence should be handled with care. The custody chain is defined as a chronological record or track that contributes for the process of digital or electronic evidence possession, management, transmission, assessment, and destruction. The purpose is to prove that the information is relevant to the alleged crime, that it was obtained from the scene, and that it was in its original state rather than having been manipulated with or "placed" deceptively to make someone appear liable. The sample's authenticity is protected by the custody chain. The accountability of the control, transmission, and evaluation of samples illustrates the procedure's accountability [20]. In forensic practice, maintaining the chain of evidence is crucial. Everything done for the examination and analysis of the evidence sample must be allowed and recorded; therefore, this process of documentation is essential. Everyone who interacts with the condition needs to be held responsible for all of it. Evidence collection situations, people who managed the evidence, period of safeguarding of evidence, security purposes conditions while managing and/or storing the evidence, and how evidence is handed over to consequent guardians every time a transfer occurs should be described in detail in the documentation (as well as the characteristics of those engaged at each step) [21]. Throughout the investigation process, the chain of custody aids in maintaining the evidences' past and chronology. It also helps in ensuring validation of the evidences in criminal proceedings. The documentation in chain of custody helps in maintaining a clear, precise, coherent, comprehensive, integral, retrievable, and protected sample chain. The chain of custody preserves the confidentiality or authenticity of the evidences or the personnel involved in the case. Due to the presence of the chain of custody, there is a minimal chance of evidence mishandling or contamination. The chain of custody decreases the amount of dismissal of criminal cases by increasing the availability of proper evidences. The chain of custody also documents the volumetrics or weight related to the sample, like how much of the sample is taken of the analysis and how much is left in the sample. The chain of custody act as an indispensable evidence in court. It inhibits law enforcement officers and other laboratories/enforcement personnel from tainting or losing evidence because it would eventually be traced back to them, and they will be held liable [22].

16.10 Case Study

One of the most common defenses in drug cases is the prosecution's failure to establish a chain of custody. The procedure of handling the evidences or objects, particularly the custody of illegal drugs, is defined in legislation by government entities and courts. Non-acceptance of the underlying procedure results in the evidence being inadmissible, which can lead to the accused's acquittal.

The police detain someone for the intent of apprehending drug dealers. During the arrest, the investigator officer can use standard operating procedures to recover illicit drugs contained at the crime scene. The officers take initial possession and control of the drugs and photograph them in the presence of the accused or the individual from whom the objects were confiscated. To assess the status of the medications and staff, an inventory list is also prepared.

The drugs are sent to a forensic science laboratory for quantitative and qualitative analysis within 24 h of being seized. The examiner is then given a certificate of the results within a day and gives it to the investigators or agency. Following the examination, the police file a complaint with that of the prosecutor, who file a criminal information in court. The court of law conducts an impartial inspection of the seized drugs 3–4 days after the complaint is filed. Within a day of the inspection, the drugs are destroyed or burned in the presence of a convicted or the person from whom these items were seized. The agency issues a certificate stating the destruction or burning of the subject items as well as representative samples.

During criminal proceedings, the prosecutor is accountable for proving the chain of custody, the duty of proving that the sample of drugs presented into evidence is indeed part of the inventory drugs at the time and place of seizure. The prosecutor must also demonstrate that the drugs used as evidence are the same as those taken from the accused's possession. The prosecutor is also required to submit a statement of each individual present in the chain for determining the situation and recognition of the evidence.

If there is a presence of significant gaps in the testimony of witnesses on the chain of custody of the seized illegal drugs, this casts doubt on the veracity of the evidence presented in court, and the accused cannot be convicted beyond a reasonable doubt.

16.11 Conclusion

Those having charge of the evidence considers maintaining the chain of custody as their professional and ethical duties. It is critical to raise awareness among those involved in such instances about the significance of proper procedures for preserving the chain of custody of evidence. As a presumably irrelevant and concise procedure, it is usually overlooked and granted little weight. However, it must be understood that it is the most serious process that eventually determines the acceptability of evidence in a court of justice.

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