Biological Sources of DNA: The Target Materials for Forensic DNA Typing

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Abstract

Among physical evidences encountered at the crime scene, biological evidences, viz., blood, semen, vaginal secretion, saliva, urine, and sweat, are the most ubiquitous in nature, and their presence aids in linking perpetrator to the victim as well as crime scene. Recognition of biological fluids as substantive evidences

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is endorsed by presence of DNA in them. Advancing forensic DNA typing techniques have a great potential in characterization and individualization of biological evidences encountered during criminal investigation, but their applicability on each biological fluid for human identification varies in a great deal due to variation in the amount of nucleic acid available within the fluid. DNA concentration is relatively high in fluids such as blood that contains large number of cells while fluids such as urine and sweat possess low amount of DNA. Besides reference biological samples, forensic DNA typing can be implemented for detecting presence of traces of biological fluids on physical surfaces. The concept of "Touch DNA" or "Transfer DNA" involves analysis of low amount of DNA deposited on the surfaces that have come in human contact and can constructively help in associating evidences with perpetrator and/or victim. However, identification of sources of such materials, their collection methods, and preservation conditions can influence the quality of result. Further to this, degradation, impurity, contamination, and presence of inhibitors in such evidences demand purification and isolation of high-quality DNA. This chapter deals with various biological sources of DNA commonly encountered at the scene of crime and their evidential value, along with various factors and conditions affecting forensic DNA typing of such samples. This chapter deals with various biological samples used for forensic DNA typing along with various factors and conditions that affect forensic DNA typing.

Keywords

Biological fluids · Forensic DNA typing · Touch DNA · Crime Scene · Perpetrator

Introduction

Among physical evidences encountered at the crime scene, biological evidences, viz., blood, semen, vaginal secretion, saliva, urine, and sweat, are the most ubiquitous in nature, and their presence aids in linking perpetrator to the victim as well as crime scene. Recognition of biological fluids as substantive evidences is endorsed by presence of DNA in them. Advancing forensic DNA typing techniques have a great potential in characterization and individualization of biological evidences encountered during criminal investigation, but their applicability on each biological fluid for human identification varies in a great deal due to variation in the amount of nucleic acid available within the fluid. DNA concentration is relatively high in fluids such as blood that contains large number of cells while fluids such as urine and sweat possess low amount of DNA. Besides reference biological samples, forensic DNA typing can be implemented for detecting presence of traces of biological fluids on physical surfaces. The concept of "Touch DNA" or "Transfer DNA" involves analysis of low amount of DNA deposited on the surfaces that have come in human contact and can constructively help in associating evidences with perpetrator and/or victim.

However, identification of sources of such materials, their collection methods, and preservation conditions can influence the quality of result. Further to this, degradation, impurity, contamination, and presence of inhibitors in such evidences demand purification and isolation of high-quality DNA. This chapter deals with various biological sources of DNA commonly encountered at the scene of crime and their evidential value, along with various factors and conditions affecting forensic DNA typing of such samples.

Blood

Blood is one of the preeminent evidences that are alighted on at various crime scenes including cases of sexual assault, homicide, suicide, accidents, and burglary in disparate forms of blood-pool and blood stains adhered to the surfaces such as floor, walls, clothes, and the weapons involved in the crime. Whole blood is a composite of various blood components that are classified into liquid element, plasma, and cellular (or formed) elements, erythrocytes (or red blood cells), leukocytes (or white blood cells), and thrombocytes (or platelets).

Discovery of ABO blood typing in 1900 instigated the scope of human blood identification that further strode toward individualization in the 1980s with the advancements of molecular techniques. Existence of individual-specific minisatellites in the human genome that can aid in human identification cases was affirmed by analyzing blood samples of 20 unrelated individuals using southern blot hybridization technique (Jeffreys et al. 1985). Thereafter several protocols and their modifications with varied incubating reagents, time frame, and techniques were designed and reported for extraction and purification of human genomic DNA (Table 1).

A number of considerable pre-analytical factors influence the quantity and quality of genomic DNA extracted from the whole blood or clotted blood sample. Sample collection with zero or minimal contamination is an important consideration that affects the stability of blood sample. Use of sterilized syringe is preferred for collection of blood pools. Wet blood soaked objects are air-dried and collected; if object is inflexible, stains can be collected on sterilized cotton swabs and air-dried. Scraping or tape-lifting is employed for collection of dried blood stains. Stability of blood is influenced by other factors such as use of stabilizing agents such as heparin or anticoagulant agents such as EDTA, time difference between collection, and storage of sample (Vaught 2006). Storage period, storage conditions such as temperature of blood, as well as isolated DNA are shown in Table 2. Exposure to ultraviolet radiations, heat, light, humidity, and soil contaminations (McNally et al. 1989) have adverse effect on extraction of good quality of DNA.

Blood evidence in various conditions, viz., frozen blood, clotted blood, or dried blood spots, can also be found during forensic investigation. Such samples require additional pre-analytical treatment or modified protocols for extraction of DNA. Modification in conventional proteinase K/phenol chloroform isoamyl alcohol (PCIA) protocol with pre and post-trypsination of frozen blood samples with the

Table 1 Protocols and their modifications for DNA extraction from blood

S. no	Protocol	Material used	Technique used	Result	Reference
1	Rapid method of DNA isolation from human leukocyte	SDS for lysis, potassium acetate for precipitation	Diethylaminoethyl (DEAE) cellulose chromatography	50–70 μg of DNA in 10 ml of blood sample	Potter et al. (1985)
2	Rapid method for the purification of DNA from blood	Guanidine hydrochloride, ammonium acetate, sodium sarkosyl, and proteinase K	Southern blotting	20 μg of DNA in 1 mL of blood	Jeanpierre (1987)
3	Salting out procedure for extracting DNA from human nucleated cells	1 ml of saturated NaCl after digestion	Centrifugation at 2500 rpm for 15 min	DNA quantity comparable with that of phenol-chloroform protocol	Miller et al. (1988)
4	Non-organic procedure for the isolation of genomic DNA from blood	Ice cold CLB (0.32 M sucrose, 10 mM Tris-HCl pH 7.6, 5 mM $MgCl_2$, 1% Triton X-100)	Southern blotting	Isolation period less than 4 h	Grimberg et al. (1989)
5	Direct PCR from whole blood, without DNA extraction	Blood introduced directly to the PCR reaction of 50 mM KCl, 10 mM Tris-HCl pH 8.0, 1.5 Mm $MgCl_2$, 0.1 mg/ml gelatin, 200 uM each dNTP	3 PCR cycle of 3 min at 94 °C then cooled for 3 min at 55 °C	Convenient alternative to the tedious DNA extraction process	Mercier et al. (1990)
6	Isolation of fetal DNA from nucleated erythrocytes in maternal blood	Phosphate buffered saline (PBS: 0.137 M NaCl/0.002 M KCl/0.008 M Na_2HPO_4/ 0.0015 M KH_2PO_4, pH 7.4), 2% fetal calf serum, and 0.1% sodium azide on ice	TfR Analysis	0.1–1 ng of fetal DNA present in maternal blood at 15–16 weeks of gestation	Bianchi et al. (1990)

(continued)

Table 1 (continued)

S. no	Protocol	Material used	Technique used	Result	Reference
7	Alkaline extraction of Human Genomic DNA	5 μL of sample incubated with 20 μL 0.2 M NaOH at room temperature for 5 min in case of blood and at 75 °C in case of stain	Centrifugation at 12000xg for 5 min	30 ng of nuclear DNA per μL of blood	Dissing et al. (1996)
8	Modified salting out method using laundry detergent	Additional treatment with laundry powder solution, glass beads, and NaCl prior to DNA precipitation	Centrifugation at 15000 rpm for 5 min	30 mg/ml of powder yielded sufficient DNA 56.3 μg/mL	Nasiri et al. (2005)

Table 2 Storage conditions for blood samples

Storage condition	Maximum time	Reference
45 °C	6–7 weeks	Madisen et al. (1987)
23 °C	1 week	
−30 °C	12 years	Chen et al. (2018)
Dried blood spots on FTA cards	16 years	Rahikainen et al. (2016)
Blood stains at room temperature	15 years	Barbaro and Cormaci (2006)

inference that trypsination before cell lysis yielded DNA with 88.17% purity that declined to 63.23% in case of untrypsinized frozen blood (Ahmad et al. 1995). Another rapid protocol with a modified composition of cell lysis buffer and extraction buffer substituting the toxic reagents yielded DNA from frozen blood that was comparable with fresh blood (Guha et al. 2018). In case of clotted blood, mechanical shearing of clot by homogenization, scraping, or slicing (Xu et al. 2010) raised the quality of DNA. Use of nylon mesh and serum separator in various studies reports a good yield of DNA from clotted blood.

The texture, quality, and absorptivity of the surface acting as blood-stain carriers also influence the yield of DNA. Prinz and Berghaus (1990) successfully isolated sufficient amount of DNA from two days old dried blood stains on eleven different stain-carrier surfaces including variety of fabrics stores at room temperature. DNA isolated from surfaces such as wool, denim, suede, and carpet was chemically contaminated possibly due to surface-specific challenges in extracting leukocyte from the carrier.

A commonly encountered forensic situation is the deliberate removal of visible blood stains with cleaning agents that does not possibly obstruct the visualization of the stains but generate contamination of stain and degradation of genomic DNA (Tas 1990) (Thabet et al. 2018). Bleach has the most detrimental impact on the yield of

DNA as compared to other chlorinated and non-chlorinated detergents (Harris et al. 2006).

Bloodstains treated with fingerprint-enhancing and/or blood-enhancing reagents are another set of challenges for recovery of DNA. Fingerprint enhancement reagents such as Cyanoacrylate Fuming (Newall et al. 1996; Mutter et al. 2018), silver nitrate (Lee et al. 1989), and other bloody fingerprint enhancement chemicals such as luminol (3-aminophthalhyrazide) (Manna et al. 2000), benzidine, leucomalachite green (LMG), phenolphthalein KM, Amido Black (methanol based), Crowle's Double Stain, and Hungarian Red (fuchsin acid) (De Almeida et al. 2011; Frégeau et al. 2000; Everson et al. 1993; Tobe et al. 2007) have deteriorating impact on the quality and quantity of DNA. Yield of DNA is also reduced by exposure to short ultraviolet rays that are used for enhancing fingerprints found in the blood (Andersen and Bramble 1997).

DNA fingerprinting technique is influenced by diverse range of factors including ecological factors, improper collection techniques or mishandling during the chain of custody, as well as analytical procedure. The inference drawn from the well-known O. J. Simpson trial (1985) (Thompson 1996) elicited the necessity of improving the diligence of evidence collection and analysis by the forensic DNA laboratories as well as upgrading the presentation of DNA evidence in the courtroom. The defense alleged on the collection and preservation of evidence more willingly than raising questions on the validity of DNA evidence by presenting evidence of negligence by Los Angeles Police Department (LAPD) in form of cross-contamination, switching of dried swatches, and premeditated planting of blood onto the evidences (Butler 2005).

Semen

Sexual assault cases contribute nearly 50% of the total cases received in the Forensic DNA Laboratories. The importance of DNA in sexual assault cases was known to be long ago since the "Pitchfork Case" of Leicestershire (1988), the first trial involving DNA analysis of semen for conviction of rape and murder. Semen, a viscous, slightly yellowish or grayish fluid mainly comprises of seminal fluid (made up of water, proteins, sugars, minerals, and vitamins) and sperm cells (spermatozoa). A typical ejaculation releases 2–5 mL of semen of which spermatozoa (50 μM in length) make up approximately 5% of the total volume of semen. Swabs, clothing, vaginal slides, and bedding items are generally collected for DNA analysis. For a successful STR (Short Tandem Repeats) analysis, nucleated cells with a sufficient amount of DNA are required, and once seminal fluid is detected on samples by applying different preliminary and confirmatory tests, the next step is DNA extraction.

DNA fingerprinting has two basic utilities in sexual assault cases. Firstly, the individualization of semen (when spermatozoa is less in number) and secondly is to differentiate mixed stains when the number of perpetrator is more than one. The situation becomes complex when contaminated samples are there or semen is mixed with other body fluids like blood. And here, the hemoglobin acts as an inhibitor in

the PCR (Polymerase Chain Reaction) process. Generally, semen is found to be mixed with vaginal secretion which cartons the presence of spermatozoa as the amount of vaginal secretion is much higher. Male DNA can be separated from the DNA of other cells using differential extraction method which was firstly detailed in the publication by Gill et al. (1985) and later by Wiegand et al. (1992) and Yoshida et al. (1995) (Table 3). Another difficulty which DNA experts face is when multiple males are contributors and two or more alleles may be present for one marker and in this situation chances of allele dropout can't be ruled out. If spermatozoa is absent in

Table 3 Differential extraction protocols and their modifications

Protocol for differential extraction	Reference
Sperm nuclei lysis with SDS, proteinase K, and DTT mixture	Gill et al. (1985)
Sample treated with Lysis buffer I (10 mM Tris-HCl, 0.4 M NaCl, 2 mM EDTA) with proteinase K and SDS and incubated at 37 °C for 40 min followed by centrifugation at 13000 rpm for 5 min. Supernatant lysed with lysis buffer II (proteinase K, SDS, and DTT), incubated at 56 °C for 1 h, centrifuged at 13000 rpm for 5 min, and DNA isolated using organic extraction protocol	Wiegand et al. (1992)
First incubation with TNE buffer, 1% SDS and proteinase K at 70 °C for 3 h, followed by centrifugation at 15000 rpm for 5 min. Second incubation with TNE buffer with 1% SDS, 100 ml proteinase K, and 0.04 M dithiothreitol (DTT)) for more than 8 h at 56 °C in a shaking water bath	Yoshida et al. (1995)
Tris(2-carboxyethyl)phosphine (TCEP) in Triton X-100 as lysis agent to lyse sperm cells and collect DNA on-chip, incubated for 15 min followed by addition of 40 μL of proteinase K solution (1 μg mL − 1) and incubation for 4 h at 55 °C. 100 μL of Buffer AL and 100 μL of ethanol were added to the samples, mixed by vortexing and run through gDNA extraction using a Qiagen spin column protocol	Inci et al. (2018)
Sample treated with extraction buffer (10 mM Tris-HCl (pH 8.0), 100 mM sodium chloride, 1 mM EDTA (pH 8.0), TNE, 1% SDS, and 0.2 mg/ml proteinase K) and incubated for 2 h at 37 °C followed by centrifugation of samples in spin baskets at 18000 g for 5 min, separation of supernatant, and multiple washing of pallet. Pallet lysed with sperm extraction buffer (10 mM Tris-HCl (pH 8.0), 100 mM sodium chloride, 1 mM EDTA (pH 8.0), 2.5% sarkosyl, 0.39 M dithiothreitol, and 0.5 mg/ml proteinase K) and incubated at 37 °C for 2 h. DNA purified from epithelial and sperm portion using Qiagen EZ1 Advanced XL system	Alderson et al. (2018)
Sample lysis with stain extraction buffer (1 mol/L Tris–HCl, ddH2O, 5 mol/L NaCl, 0.5 mol/L EDTA, 10% SDS, pH ¼8.0) and 15 mL of proteinase K (20 mg/mL) (Fisher Scientific, Pittsburgh, PA, USA) followed by an overnight 56 C incubation. Isolation of lysate (non-sperm fraction) using DNA IQTM spin baskets (Promega Corporation, Madison, WI, USA) at 7500 g spin for 5 min. The sperm pellet was subsequently resuspended in 200 mL of phosphate buffered saline solution (Fisher Scientific), 20 mL of Qiagen proteinase K stock solution, and 20 mL of 1 mol/L DTT (Fisher Scientific), vortexed, 200 mL of Buffer AL (Qiagen) was added, and samples were incubated at 56 °C isolation with QIAamp DNA Investigator kit. DNA was eluted in final volumes of 100 mL (nonsperm fractions) or 60 mL (sperm fractions) of Buffer ATE (Qiagen)	Goldstein et al. (2019)

semen (oligospermic, azoospermic, or normospermic), Positive Semelogenin (Sg) samples may be suitable. Y-chromosome identification and Sg biomarker should be thoroughly examined in the laboratories (Martínez et al. 2015).

The condition of the exhibit before examination remains to be a censorious aspect for successfully detecting and analyzing semen, and for this appropriate handling parameters during drafting, collecting, packaging, storing, and transportation of samples are the fundamental strides.

Vaginal Secretion

Another important evidence found in cases of sexual assault is vaginal secretion. In these cases, the identification of vaginal secretion is crucial as it can support in verifying the allegations of sexual assault. For instance, a stain is observed during investigation of a sexual assault case, and forensic DNA analysis affirms that the stain is originated from the victim, thus creating a link among the suspect and the victim. But, the litigant may contradict any criminal act by claiming that the stain is originated from sweat due to spontaneous contact. So, the evidence would have a significant value if vaginal secretion stain was found. A mixture of vaginal secretion and semen stain is generally found, and the presence of vaginal secretion confirms the incidence of sexual assault.

Human vagina is composed of squamous mucosa (comprises of stratified squamous epithelial tissue), submucosa, and muscularis. The vaginal secretion basically consists of epithelial debris, tissue fluid, leukocytes, electrolytes, lactic acid, and proteins which is generally derived from the glands of the uterus, cervix, transudation of the vaginal epithelium, and Bartholin's glands. Forensic laboratories use various methods for identification of vaginal secretion, and once it is identified, then DNA analysis is done. The main problem experts face is the ominous proportion of male to female DNA, with a surplus of the victim's material. Differential lysis is applied in this situation to isolate male DNA from epithelial cells (Gill et al. 1985).

Some other factors which should be kept in mind in order to get better results are the type of material used for collection and storage must be selected correctly. The contamination of genetic material from other sources (e.g., from the examiner and other biological evidence) should also be avoided (Butler 2005). Contamination may occur during the sexual contact (e.g., if there is more than one executioner), during collection and packaging, during transportation, during the medical examination, and in the laboratory. Proper care must be taken to restrict cross-contamination between sexual assault evidences.

Oral Fluids

Oral fluids or whole saliva is a mixture of secretion produced from major specific salivary glands, numerous minor salivary glands along with secretion from non-salivary sources such as nasal secretion, gingival crevicular fluid, bronchial

mucus, buccal cells, bacterial products, and food remains. Non-invasiveness, easy collection, and less contamination are some of the beneficial features of saliva over blood for DNA typing. Saliva are generally recovered from the scene of crime in conjunction with bite mark evidence on the skin as in cases of violent crimes, on eatables, clothing, cigarette butts, chewing gums, chewed betel quid stains, documents, postage stamps, and other objects (Anzai-Kanto et al. 2005; de Oliveira Musse et al. 2019). Despite frequent occurrence, quick drying of saliva stains makes them indiscernible, hampering their recognition and collection.

The earliest DNA isolation from the saliva on cigarette butts involved PCR amplification at HLA-DQ alpha and D1S80 markers and analysis by reverse dot-blot technique and polyacrylamide gel electrophoresis (Hochmeister et al. 1991). Similar study of DNA isolation from saliva and saliva-stained samples such as buccal swabs, gags, envelopes, and cigarettes stored at different conditions demonstrated identical DNA banding patterns as obtained from blood, hair, semen, or mixed saliva (Khare et al. 2014; Walsh et al. 1992) (Table 4). Watanabe et al. (2003) reported inhibitory impact of certain dyes present in the cigarette butts on PCR amplification. Sweet et al. (1996) were constantly involved in the studies related to DNA extraction from saliva in various conditions. In 1996, they proposed modified Chelex method involving pre-analytical use of proteinase K, incubation at 56 °C for 60 min, and 100 °C for 8 min and subsequent microconcentration of solution (Sweet et al. 1996). Saliva deposited on the skin is present in limited amount. Double swab technique ensures maximum collection of saliva stains with

Table 4 Salivary DNA extracted from different surfaces

Substrates	Number/type of samples	Conclusion	Reference
Betel quid	50	92% success rate for DNA isolation from 4 years old forensic BQ samples	Chiou et al. (2001)
Food	20 cheese pieces	Collection of saliva from the center instead of peripheral surface yielded better results	de Oliveira Musse et al. (2019)
	2 surfaces of cheese	Variation in DNA concentration recovered from upper and lower surface	Sweet and Hildebrand (1999)
Skin	5	Lower DNA recovered from skin probably due to degradation during saliva deposition, collection, and extraction	Anzai-Kanto et al. (2005)
	15	Double swab technique yielded better DNA quantity than filter paper and single swab technique	Sweet et al. (1997)
	A body submerged in water	Mixed DNA profile with minor component correlating with DNA profile of suspect	Sweet and Shutler (1999)
Cigarette butts	200	Deterioration in concentration of DNA with storage time, but all DNA were PCR amplifiable	Hochmeister et al. (1991)
	100	Inhibitory effects of dyes present in cigarette	Watanabe et al. (2003)

minimal contamination (Cherian et al. 2015). It involves swabbing of the skin surface with first swab immersed in sterile water in circular motion followed by second swabbing with dry swab using same pressure and motion (Sweet et al. 1997). In a drowning case, the bite mark present on the victim's body submerged for 5.5 h in water served as the source of salivary DNA, and DNA profile of the suspect was identified and distinguished at HUMTH01 and HUMvWA loci (Sweet and Shutler 1999).

Storage of saliva at −70 °C yields fair quality of DNA up to 1 month. Storage at 4 °C led to bacterial growth but yields sufficient PCR product (Ng et al. 2004). Quantity and quality of DNA from saliva stored at 4 °C and −20 °C for up to 3 months was comparable to that of fresh saliva samples. However, gradual deterioration was observed when the storage period was extended to 5 months (Kim and Kim 2006).

Trace quantity of saliva can also be transferred to the surfaces during speaking, coughing, and flipping pages of documents. Double swab technique for collection of such samples is preferred. Deposition of trace samples can also be the result of contamination at the crime scene by adventitious transfer or during investigation and analysis. Use of face mask during investigation, collection, and analysis of samples is recommended to minimize such contamination.

Sweat

Sweat became unfailing evidence that are deposited unconsciously at various touched surfaces or handled objects and due to their transparent and evaporative nature, rarely engages one's attention during deliberate cleaning of other evidences. Biologically, sweat is a watery fluid secreted from eccrine and apocrine sweat glands present throughout the body along with dissolved mineral, metabolites, and epithelial cells. Around 650 sweat glands are present in average square inch of skin resulting in primary transfer, i.e., deposition of trace amount of sweat on surfaces that come in contact with the skin. The concept of "Touch DNA" by primary transfer and "Transfer DNA" by secondary transfer of DNA relating to Locard's Principle of exchange has gained much attention over the last few years (Kisilevsky et al. 1999; Ladd et al. 1999). Touch DNA or trace DNA are described as low levels of DNA deposited on handled, touched, or worn object without presence of detectable body fluid. Minute traces of epidermal cells along with sweat generally result in deposition of touch DNA. Transfer DNA, on the other hand, are resulted from secondary transfer and include foreign DNA present on individual's hand from previous contact that are subsequently deposited on other surfaces (Wickenheiser 2002). This may include skin-skin-object mode of transfer or skin-object-skin mode of transfer (Burrill et al. 2019).

Quality and quantity of touch DNA recovered from any surface is affected by a wide variety of factors. Shedding status is one of the factors that influences the yield of touch DNA and is described as the tendency of an individual to lose skin cells. It is reported to be higher in women compared to men due to the presence of

thicker stratum corneum in men, making it more stable (Faleeva et al. 2018). Shedding rate also depends upon the individual's age due to high proliferation rate and less degraded DNA in children as compared to elderly people (Poetsch et al. 2013). The yield of trace DNA is affected by the number of deposited DNA-bearing cells, nature of the surface carrying the deposited DNA, lapse of time between deposition and recovery coupled with exposure to environmental conditions, method of sample collection and DNA extraction employed and is independent of timeframe for which skin remains in contact with the surface (Alketbi 2018).

Worn clothing, footwear, beddings, wallets, and bags and door handles are some of the common substrate bearing sweat stains. A study on potential transfer of touch DNA revealed that samples of sweat collected from the beddings after one night of sleep provide good DNA profile. DNA profiles of former individual in contact with the bedding can also be generated. Similar study on sweats of foot and soles of footwear inferred higher DNA amount from the top of foot than the soles. Microbial impacts, cell compressions, and presence of certain PCR inhibitors at the underside of foot and sole justify the loss of DNA on the sole. Synthetic sport shoes yielded better amount of DNA than the leather shoes. The areas as well as techniques of sample collection were also found to affect DNA recovery (Bright and Petricevic 2004). Adhesive tape lifting, dry swab, and cutting out are some of the commonly employed techniques of sample collection. Double swabbing technique for collection of sweat stains, with first wet swab and second dry swab, yields greater DNA recovery from dry swabs than the wet swabs.

Minimal amount of available touch DNA necessitates maximum sample collection and extraction of DNA in shortest possible time period. Zhou et al. (2016) described use of 96-well centrifugal filtration plate and automated DNA extraction on liquid workstation from swabs from door handles, gloves, beverage bottles, cigarette butts, tools, etc. resulting in 54.43% successful profile rate. Recovery of touch DNA from metal surfaces such as ammunition, door handles, and furniture is affected by interactive nature of DNA with metal cations as well enzymatic actions of certain metals on degradation of DNA. Combination of collection method and buffer specific to the metal surface carrying DNA resulted in improved recovery of DNA (Tucker 2015).

Latent fingerprints resulting from deposition of sweat, skin cells, and particulate matters, frequently occurring in any crime scene, are also an efficient source of samples for DNA profiling. Recovery of DNA from fingerprints depends on the substrate, standard pressure, frequency of hand washing, and exposure to fingerprint-enhancing chemicals (Table 5).

Urine

The importance of bodily fluids as sources of DNA for identification purpose has been known since a longer period of time (Hilhorst et al. 2013). Urine, being a useful tool as a source of genetic material, has not been densely applied as a

Table 5 Effect of fingerprint-enhancement chemicals on DNA profiling

Fingerprint-enhancement techniques	Surface	Effect of DNA profiling	Reference
White powders	Glass and wood	Better DNA yield from wood surface than glass. BVDA white, Faurot white, magnetic black, special black fingerprint powders had no impact on DNA and yielded a good profile	Van Hoofstat et al. (1999)
Black powders			
Metal powders			
Black magnetic powders			
UV light, DFO, ninhydrin	Paper	Increased inhibitory effects observed	Raymond et al. (2008)
White powders, black powder, magnetic powder	Glass	No effect on DNA yield	
Cyanoacrylate fuming, Cyanoacrylate + rhodamine 6G, Cyanoacrylate + VMD	Plastic	Rhodamine stained latent fingerprint resulted in better DNA profile	
Ninhydrin	Paper	Less amount of DNA but sufficient for DNA profiling without any inhibitory effects	Schulz et al. (2004)
Vacuum metal deposition (VMD)	Plastic	No effect on DNA quality and quantity	Bhoelai et al. (2011)
Cyanoacrylate fuming	Plastic	No effect on DNA quality and quantity	
Ninhydrin	Paper	Degradation and contamination of DNA	
1,8-diaza-9-fluorenone (DFO)	Paper	Degradation and contamination of DNA	
Black fingerprint powder	Glass	Acceptable decline of DNA quality and quantity	Alem et al. (2017)
Magnetic latent print powder	Glass	Less efficient genetic analysis	
Luminescent cyanoacrylate fuming	Plastic	Observable extent of DNA degradation	Khuu et al. (2018)

potential source of DNA for identification purposes in Forensic Sciences (Junge et al. 2002). Urine may be submitted as forensic evidence in violent crimes, hanging, and illicit drug screening tests (Ng et al. 2018).

The DNA is contained in epithelial cells of human urine, such as renal tubular, squamous cells, transitional urothelial, leukocytes epithelial cells (renal tubular, squamous cells and transitional urothelial), and malignant cells. There are a lot of factors on which the quantity of extractable DNA depends such as the extraction procedure, storage condition, gender, bacterial contamination, and release of nucleases from cells. As the concentration of DNA in urine is low and the instability of DNA in urine preservation, personal identification using urine samples becomes difficult. Healthy individuals, especially males, contain very less nucleated cells and also shelter bacteria that can act as inhibitor during amplification of DNA. The major urine component, i.e., urea, also acts as an inhibitor and can affect the yield of DNA (Aoki et al. 2017).

The yield of DNA is dependent on many factors like the gender of the urine sample, temperature and storage conditions, quality, quantity, and age of the sample.

Generally, yield is greater in females than males and in fresh urine sample rather than stored urine sample (Aoki et al. 2017; Ng et al. 2018). In cases of urine stains, sample should always be collected from the largest stains that are available. The collection and storage of samples should be properly done in order to avoid degradation so that maximum DNA could be extracted (Yokota et al.1998).

Fecal Matter

Fecal matter identification is helpful in providing relevant clue during a criminal investigation, and the individual peculiarities of a fecal sample can be adequately determined by DNA analysis. Fecal as a forensic evidence can be found in many cases where crimes with cruelty are done like sexual assault, sodomy, vandalism, and burglary during which the executioner defecated at the scene of crime. In cases related to animals/wildlife species, genetic evidence from animals, plants, bacteria, and viruses has been used in criminal investigations as forensic tool for identification and individualization purpose (Forgacs et al. 2019).

Feces are a type of waste matter formed in the intestines during the last phase of digestion as a direct result of food. Its composition includes a complex mixture of undigested foodstuffs, intestinal bacteria, intestinal epithelial cells, electrolytes, bile pigments, soluble and insoluble gastrointestinal tract products, mucus, and water. Due to low number of cells and co-extraction of various unwanted substances (due to presence of several PCR inhibitors from digestive system, soil, and foodstuffs), DNA analysis from fecal matter is a challenging aspect. Although the quality and quantity of DNA from fecal matter is comparatively lower than traditional sources of DNA (such as blood, semen, saliva, etc.), few studies have suggested fecal samples as a crucial and valid source of genetic material by comparing the results from the same individuals to high-quality DNA samples (Forgacs et al. 2019). So, DNA analysis from fecal matter can be highly important in criminal investigation.

After three and half decades, due to technologocal advancements, forensic DNA technology has become a potential tool in the court of law, resulted into increasing the rate of conviction. Nowadays, reliable and sensitive techniques for DNA isolation from a variety of biological samples have been developed which attained through gradual technological advancement which shown in Fig. 1.

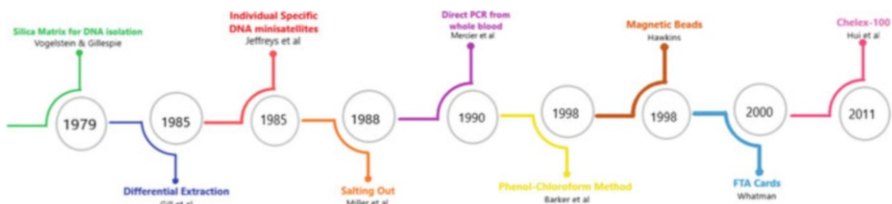

Fig. 1 Timeline of DNA extraction protocols

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