RNA- and DNA-Based Identification of Body Fluids

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Abstract

The most common form of evidence to forensic investigators is the body fluids collected at crime scenes. They ascertain the suspect or victim, they provide valuable DNA evidences and can play a vital role in acquit an innocent individual. The determination of a specific bodily fluid is predominantly the initial step as the body fluid composition is very relevant to the further investigation process. The ability to identify and report an unexplained stain at the scene of crime without waiting for the laboratory results is another very important phase in the forensic body fluids analysis. Many forms of detection methods for body fluids have been known for over a century, such as alternative light source, immunological tests, spectroscopic techniques, chemical methods, catalytic tests, and microscopic methods. Although these modern forms of detection of body fluids are often definitive, these are done at a time with only one body fluid. Currently the usage of molecular genetic based approaches using DNA methylation detection or RNA-based profiling methods has recently conquered to replace the traditional body fluids identification methods.

Keywords

Blood · Saliva · Semen · Forensic · RNA · DNA

5.1 Introduction

Individual's involvement in a crime can be detected and identified by the body fluids traced at the crime scene event. These types of evidences found at the scene of crime are among the most ubiquitous in nature especially to forensic investigation

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purposes. Body fluids identification and detection is a vital part in forensic practices; however, many biological fluids are invisibly present in the crime scene or present in very small quantity due to which the easily identification is not possible. The presence of biological fluids not only provides type and origin of the samples but also provides clues in reconstructing crime scene and linkages between innocent and actual criminalists.

Biological fluids like blood (including menstrual stains), semen, saliva, urine and vaginal stains, skin, and tissues are the prime interest of forensic scientists in various crime cases. In a crime scene approach the identity of these biological fluids are not straightforward, due to their invisible presence in very minute quantities. For the identification of sequence of events at the crime scene, these biological fluids can be used as crucial indicators. Such as blood stains are a strong indicator of murder, some form of assault, or struggle, whereas upon the detection of semen or vaginal stains able to specify physical and or sexual assault.

After potential identification of body fluids at scene of crime, a specific form of light is required to visualize these stains or the spot chemical examination is required. The biological identification of these stains previously based on some limited chemical methods which were often inadequate in sensitivity and specificity, whereas the confirmatory tests based on some microcrystal tests for the identification of body fluids in forensic-related cases. The techniques apply were based on chemical tests, enzyme catalytic reactions, immunological tests, spectroscopic based tests, and microscopic examinations. The presumptive/preliminary tests which are also used for screening the biological fluids even at the crime scene or at the forensic laboratories setup; some of them are, Phenolphthalein (Kastle–Meyer test), and Luminol test (for minute traces) for blood identification, Phadebas test for saliva identification, Jaffe's test for urine identification, Acid Phosphatase test for semen identification. The other tests are based on microscopic examination (confirmatory tests) which will convincingly identify the presence and the nature of such biological fluids. These gold standard evidences contain valuable genetic information to identify a suspect or victim as well as either acquit an innocent individual. Technology has rapidly evolved to reveal the individual identity of an unidentified stain at the scene of crime scene, without much waiting for the laboratory results to arrive is a crucial breakthrough in the advancement of body fluids analysis in forensic scenario. An evident of the last decade is the progressive development of the laser technology and novel light detectors which have either gave birth to spectroscopic methods and/or molecular-based approaches for the detection of biological fluids.

In the field of body fluids detection, the latest developments must culminated in a range of novel methods and approaches which allows (1) first time identification of some body fluids, (2) experiments enabling nondestructive research at the scene of crime for more study via efficient DNA profiling, and (3) research lab-based protein and RNA techniques allowing for the requirements.

5.2 The Drawbacks and Benefits of Chemical Tests

For several years, preliminary chemical studies have been utilized and are still playing a significant role to identify a region of significance for DNA study and forensic investigation. Ample of reviews and reports for their accuracy and precision are available (Virkler and Lednev 2009; Gaensslen 1983; Tobe et al. 2007). These chemical measures are not unique to humans and usually are administered sequentially where there may be a mixed body fluid. Most depend on body fluids enzyme properties, and many reagents are sample harmful and/or obstruct downstream procedures.

Stains on dark surfaces or non-visible stains face challenges to detect and similarly been deciphered with alternative light sources using the auto fluorescence provided by some body fluids (Kobus et al. 2002; Vandenberg and van Oorshot 2006). The effectiveness of such approaches may be influenced by differences between body fluids and various surfaces, and application to these light sources may induce DNA damage in the stain. The super reactive luminol method which is used in the darker areas to identify the blood stains, but with a broad variety of chemicals, it provides false positives and devalues any stain that might be needed for further study (Webb et al. 2006; Quinones et al. 2006).

5.3 Limitations and Drawbacks of Antibody-Antigens Studies

The reasonable degree of accuracy and sensitivity has been found while using immuno chromatographic and enzyme-linked immunosorbent assay (ELISA) for the detection of body fluids (Akutsu et al. 2012a, b; Simich et al. 1999; Casey and Price 2010). Such measures determine the identity of the antigen in question instead of the antigen behavior. Environmental conditions may influence antigen–antibody interaction contributing to false positive or negative outcomes, and the elevated-dose hook effect affects both of these studies. These experiments are not intended to identify staining areas or in a systematic fashion, so a part of the sample is extracted so solubilized in both of these methods prior to the study.

5.4 Blood

Chemical blood tests are susceptible but lacks precision and tend to rely onto the catalytically activity of the hemoglobin groups and the oxidant, including plant peroxidases, which can produce false positive results (Virkler and Lednev 2009; Tobe et al. 2007; Cox 1991). Assays include Leucomalachite Green, named as less sensitive but also more specified tests; Tetramethylbenzidine (TMB), Ortho-tolidine, and Phenolphthalein (Kastle–Meyer test), some form of high specificity sensitive testes for the identification of blood (Gaensslen 1983; Webb et al. 2006; Cox 1991). Confirmatory yet often cumbersome testing requires a microscopy of red and white blood cells and the formation of crystals with hemochromogenic crystals

(Takayama) or hematinic crystals (Teichman) that indicate the blood existence (Gaensslen 1983).

Some tests are based on the monoclonal antihuman antihemoglobin antibodies which shows cross reaction with primates blood and mustelidase, presumably triggered by a specific sequence of amino acid in hemoglobin alpha chain, in the ABAcard® Hematrace® (Misencik and Laux 2007; Johnston et al. 2003). RSID™ blood tests (Independent Forensics, Hillside, IL, U.S.) screening test detects the rich and precisely red blood cell membrane of glycophorin A protein without any cross reactions (Schweers et al. 2008).

5.5 Saliva

An α-amylase-based detection The Phadebas® test is the regular standardized test for the identification of presence of saliva. Due to the small traces of the α-amylase enzyme are known to found in different other body fluids such as breast milk, blood, semen, urine, fecal urine, and other mammals as salivary and pancreatic sources this test is not confirmatory for the human saliva identification (Kipps and Whitehead 1975; Auvdel 1986; Keating and Higgs 1994). The SALIgAE® test from Abacus Diagnostics provides an substitute test for saliva based on calorimetric approach, which is known to be more sensitive target reagent than the Phadebas® test (West Hills, CA, USA) (Pang and Cheung 2008; Liang and Roy 2014).

False positive values have been traced in breast milk, rat saliva, clean urine and fecal/feces and semen samples. The RSID™ test for saliva is focused on anti-amylase antibodies of antihuman salivary (Old et al. 2009). The above test has been found to be more flexible and more accurate than the two tests Phadebas® and SALIgAE® tests, respectively (Casey and Price 2010; Pang and Cheung 2008).

5.6 Semen

In the lack of microscopically detected spermatozoa, semen usually takes the presumptive examination that detects phosphatase of seminal acids, an enzyme isolated from the prostate gland, although that not directly specifically connected with the fluid of seminal substances (Gaensslen 1983; Kind 1957). While no longer commonly used, confirmatory crystal tests for semen detection are present, along with the Florence test based on choline crystal formation (Gaensslen 1983).

Semen is detected for a certain time using glycoprotein, the prostate-based antigen (Kallikrein 3 and PSA or P30) (Gaensslen 1983; Graves 1995). The widely used immunological tests nowadays which includes semiquant PSA test SERATEC, p30 ABA card® and PSA testing Biosign®, while some of these test kits shows false positive reactions against vaginal stains, urine, breast milk, postmortem semen samples (Graves 1995; Yu and Diamandis 1995; Lunetta and Sippel 2009).

Semenogelin is often used to identify semen via immunochromatographic tests (Sato et al. 2004; Pang and Cheung 2007; Old et al. 2012). The RSID™-semen test

has been noticed less susceptible while comparing with the other test kits like ABAcard®, P30 and SERATEC® PSA, and some test results are occupied with inaccurate results (Boward and Wilson 2013).

5.7 Vaginal Secretions and Menstrual Blood

The differentiating feature between menstrual blood and vaginal secretions is found to be very difficult in classification. The identification of the vaginal wall glycogenated squamous epithelial cells through Lugol's staining, the endometrial cells microscopic identification and tracing the lactate dehydrogenase isoenzymes 4 and 5 are not the definitive methods for tracing the vaginal cells (Jones and Leon 2004; Stombaugh and Kearney 1987).

Immunochromatography examinations are known as potential menstrual blood checks for D-dimers, fibrin which is soluble and degradation drug medically identified for thrombosis diagnosis (Baker et al. 2011; Holtkoetter et al. 2015). One different method ELISA is utilized which focuses on estrogen receptor α and fibrinogen to differentiate the peripheral blood from menstrual blood, whereas none of the other body fluids showed any cross reactions (Gray et al. 2012).

5.8 Urine

Urine stain localization is complicated because it is usually translucent, light, and ubiquitous. Usually, the presumptive tests are focused on urea, urease and uric acid analysis. Such tests are not unique since sweat and other urea-substances often respond favorably (Gaensslen 1983; Huang et al. 2002). Creatinine checks were also used for urine detection (Gaensslen 1983). Tamm–Horsfall Glycoprotein (TMP) protein has also been found in animals' urine and has already been documented and included in the RSID™ urine study of humans. TMP tends to be sufficient as a special urine test, but the existence of vaginal fluid may complicate the test results and it could be difficult to read the test if the blood is in the specimen (Akutsu et al. 2012a, b).

5.9 Sweat

No realistic sweat recognition test is available to date. While DNA is often recuperated and profiled in clothing areas which may contain sweat, there is little study. ELISA-based testing of G-81 and dermicidin has developed but have not been commonly used to detect sweat-specific proteins (Sagawa et al. 2003; Sakurada et al. 2010).

5.10 Discovery of New (Protein) Markers

The identification and characterization of common markers are the basis for ELISA and immunochromatographic studies. 2-DHPLC, MS, and QMS were all inured to establish protein profiles typical of all six primary forensically important body fluids i.e., blood, menstrual blood, saliva, semen, vaginal substance, and skin and also acquire new candidate protein markers for instance osteopontin and uromodulin to identify urine (Legg et al. 2014). Statherin in Saliva and Semenogelin 1 and 2 in Seminal fluids are used for the examination of mRNA as markers.

5.11 Identifications Based on RNA and DNA Technologies

Ribo Nucleic Acid (RNA) and Deoxyribo Nucleic Acid (DNA) are being used progressively for so many new forensic applications, including: body fluid detection, assessing postmortem and age decay of RNA; the estimation of injury age through the analysis of reactive gene expression changes; and determining the cause of death (Bauer et al. 2003; Anderson et al. 2010; Cecchi 2010; Palagummi et al. 2014). These approaches including the utilization of messenger RNA, micro RNA, other forms DNA methylation, and microbial characterization of each and everybody fluid.

The list of potent markers in various body fluids identified using RNA- and DNA-based methods are given in Table 5.1.

5.12 mRNA-Based Methods

Most of the body fluids contain several types of cells, each expressing an individual mRNA transcript pattern. The layout and carrying out of mRNA profiling in research associated to forensic science is focused on using these multicellular transcriptomes. mRNA in body fluids deposited on a range of surfaces is now generally understood to be stable and can be collected in an appropriate quantity and consistency for analysis of various samples (Bauer et al. 1999; Setzer et al. 2008; Visser et al. 2011; Kohlmeier and Schneider 2012; Fox et al. 2014; Alvarez et al. 2004).

A benefit of mRNA profiling is that the recovery of RNA from tissues, with a variety of specific RNA extraction procedures, can be incorporated into a standard workflow of DNA profiling.

Generalizations between different commercial RNA removal methods have revealed different yields and diagnose of DNA and RNA, of no better solution than the other; collective studies have confirmed this finding. The enormous amount of transcript and consistency by means of substitute markers designed for the identical fluid with unlike susceptibilities in the same body are attributed to the capability to detect a messenger RNA transcript that is of significance (Zubakov et al. 2008; Haas et al. 2014, 2015, 2011; Van den Berge et al. 2014; Fleming and

Table 5.1 List of potential markers in various body fluids with their chromosomal location

Body fluid	Markers	Official symbol	Chromosomal location
Blood	Ankyrin 1	ANK 1	8p11.21
	Glycophorin A	GYPA	4q31.21
	Beta-spectrin	SPTBN1	2p16.2
	Alpha hemoglobulin	HBA	16p13.3
	Beta hemoglobulin	HBB	11p15.4
	Porphobilinogen deaminase	HMBS	11q23.3
	Amino-levulinate synthase 2	ALAS2	Xp11.21
Seminal fluid	Protamine 1	PRM 1	16p13.13
	Protamine 2	PRM 2	16p13.13
Saliva	Histatin	HTN	4q13.3
	Statherin	STATH	4q13.3
Vaginal fluid	Beta-defensin 1	DEFB1	8p23.1
	Mucin 4	MUC4	3q29
	Cytochrome P450	CYP2B7P1	19q13.2
	Myozenin 1	MYOZ1	10q22.2
Menstrual blood	Matrix metallopeptidase 7	MMP7	11q22.2
	Matrix metallopeptidase 11	MMP11	22q11.23
Epidermal cells	Late cornified envelope 1 C	LCE1C	1q21.3
	Loricrin	LOR	1q21.3

Harbison 2010a, b; Lindenbergh et al. 2012, 2013a, b). In presumptive tests where these comparisons are made, mRNA profiling is comparable (Haas et al. 2011).

The creation of a robust mRNA quantification method will boost RNA profiling; excessive template leads to target amplification and an increased incidence of “nonspecific artifact” (Haas et al. 2011). The present steps are applied to the entire nucleic acid or Ribo Nucleic Acid which happen to be not human specific and quantified mostly by utilizing techniques like UV-Visible spectrometry, fluorometry, Nanodrop ND-1000, Agilent 2100 Bioanalyzer System, or Quant-iTMRiboGreen® RNA kits (Visser et al. 2011; Grabmuller et al. 2015; Fleming and Harbison 2010a, b; Juusola and Ballantyne 2005).

A flexible method of analysing low-abundance mRNA extracted from minute amount of samples is by reverse transcriptase polymerase chain reaction (RT-PCR) (Kohlmeier and Schneider 2012; Zubakov et al. 2008; Fleming and Harbison 2010a, b; Hanson et al. 2012; Haas et al. 2009; Bustin 2000; Juusola and Ballantyne 2007). The most reasonable approach in forensic work is the endpoint reverse transcriptase PCR in conjunction with capillary electrophoresis, which allows the concurrent finding of multiple body fluids and thereby minimizes sample utility and background impacts. Juusola and Ballantyne first introduced this approach in 2005, followed by others (Fleming and Harbison 2010a, b; Lindenbergh et al. 2012, 2013a, b; Juusola and Ballantyne 2005; Richard et al. 2012; Roeder and Haas 2013). The quantitative RT-PCR (qRT-PCR) measures the variation in between the focused transcript and a household gene called the ΔCT (Zubakov et al. 2008;

Bustin 2000; Juusola and Ballantyne 2007; Nussbaumer et al. 2006). The benefit of this very reactive method is the possibility of setting numerical thresholds. The existing dyes limitation, which limits the sum of markers that will be able to reach in a solo reaction, represents a disadvantage. In order to address this constraint, enhancements were suggested, such as a high-resolution melting study (Hanson and Ballantyne 2013a, b).

Findings between RT-PCR and qRT-PCR indicate that perhaps the detected abundance of mRNA transcript to a large or small DNA portion (Van den Berge et al. 2014; Harteveld et al. 2013) can still not be allocated with any of these methods. New techniques have recently been developed such as isothermal amplification mediated by real-time RT loops, offering the same sensitivity and precision but then much simpler and faster studies (Su et al. 2015). The housekeeping genes constitutively expressed make available a reference point and determine the efficiency of a reaction by means of capillary electrophoresis approaches, and they are important in quantitative techniques (Bustin 2000; Moreno et al. 2012).

The desired housekeeping genes expressing themselves across all tissues, need not be different substantially between people and is not greatly influenced by physiological/pathologic problems. A variety of housekeeping genes, including ACTB, GAPDH, B2M, S15, UCE, TEF, UBC, 18S RNA and G6PD, are being used in forensic research (Zubakov et al. 2008; Haas et al. 2014; Fleming and Harbison 2010a, b; Lindenbergh et al. 2012, 2013a, b; Juusola and Ballantyne 2007; Roeder and Haas 2013; Moreno et al. 2012; Jakubowska et al. 2013; Donfack and Wiley 2015).

Even between similar samples, RT-PCR can show significant variation. This may be caused by many factors including different secondary structures of RNA transcripts, stochastic variations in samples, and inhibition and consistency of RNA. Housekeeping genes is no exemption and there is overall conformity that the quantity of transcripts will differ from individual to individual in different fluid (Fleming and Harbison 2010a, b; Roeder and Haas 2013; Bustin et al. 2005; Park et al. 2007) e.g., the prevalence of the household genes relative toward the body fluid as specific genes in buccal cells and semen is quite small and there is probably hardly any appropriate housekeeping gene meant for every single body fluid. The messenger RNA (mRNA) markers are chosen from a candidate gene method for which the gene function is normally known (Fleming and Harbison 2010a, b; Lindenbergh et al. 2012, 2013a, b; Juusola and Ballantyne 2005). Instead, a discovery technique such as a comparative multicellular transcriptome analysis based on the microarray or RNA sequencing (transcriptome) analysis can be employed (Zubakov et al. 2008; Park et al. 2013a, b; Hanson and Ballantyne 2013a, b). Candidate markers dependent on the specific role of a body fluid, such as hemoglobin, are often more prone to be precisely expressed.

Furthermost, commonly suggested blood RNA markers may typically classified proteins involved including the erythrocyte membrane (such as glycophorin A, beta-spectrin, and ankyrin 1) and the proteins allied with hgb and hemic biosynthesis (such as α and β hemoglobins, porphobilinogen deaminase and (ALAS 2) aminolevulinate synthase 2). In the afore-mentioned immunological tests, some proteins

(e.g., Glycophorin A) are being used. The tissues, for instance menstrual blood and saliva, can be classified as being nonspecific due to trace levels of blood markers in them (Kohlmeier and Schneider 2012; Zubakov et al. 2008; Fleming and Harbison 2010a, b; Lindenbergh et al. 2012; Juusola and Ballantyne 2005; Haas et al. 2009; Richard et al. 2012; Roeder and Haas 2013; Jakubowska et al. 2013; Donfack and Wiley 2015; Park et al. 2013a, b).

Protamines—PRM1 and PRM2—are most frequently used for sperm identification, and transglutaminases 4 and semenogelines 1 and 2 for seminal fluids include seminal fluid and spermatozoa markers. Semenogelin is a substratum used by some for PSA/P30/kallikrein 3. Specific markers also show little, or no, cross-reactivity (Kohlmeier and Schneider 2012; Fleming and Harbison 2010a, b; Lindenbergh et al. 2012; Juusola and Ballantyne 2005; Haas et al. 2009; Richard et al. 2012; Roeder and Haas 2013; Nussbaumer et al. 2006; Donfack and Wiley 2015; Park et al. 2013a, b). Among the four peptide groups, statherin and histatin are preferred as Ribo Nucleic Acid markers for saliva, divided by the salivary glands into saliva. A significant proportion of the overall salivary peptides contain three proline-rich proteins. Some keratins were also used (Zubakov et al. 2008; Fleming and Harbison 2010a, b; Lindenbergh et al. 2012; Juusola and Ballantyne 2005, 2007; Haas et al. 2009; Richard et al. 2012; Roeder and Haas 2013; Donfack and Wiley 2015; Park et al. 2013a, b; Sakurada et al. 2011). In factors such as the daily routine and personal circumstances of the person are affected by both the amount and content of such peptides and the complete length and partly damaged mRNA transcripts observed in saliva and many authors also reported that there can be a wide variability, along with small amounts of RNA in samples of saliva (Fleming and Harbison 2010a, b; Haas et al. 2009). Statherin allows to be observed at high levels in nasal secretions and at low levels in vaginal secretions often.

A specific problem is the differentiation of stratified squamous cells in the skin, vagina, and mouth, since they are strongly connected structurally. Because of the similar functionality (protection and secretion) of these cell types, it is difficult to find observable distinctions between them, especially in non-keratinized oral and vaginal cells.

The fluid in which the menstrual fluid curves, cervix, endometrium, and fallopian tubes, and blood differ according to age and health conditions comprises the vaginal fluid. Early vaginal marker candidates, human beta-defense 1 and mucine 4, are indeed reliably represented in vaginal secretions, they were also found to be in nasal secretions, semen, and saliva. These can be used as mucosal markers better (Sijen 2015; Haas et al. 2014; Lindenbergh et al. 2012; Juusola and Ballantyne 2005; Richard et al. 2012; Roeder and Haas 2013; Donfack and Wiley 2015; Hanson and Ballantyne 2013a, b; Cossu et al. 2009). Recently, two other candidates' genes MYOZ1 and CYP2B7P1, equally appearing responsive markers were found using transcriptome profiling. CYP2B7P1 has not been identified with any cross-reactivity, although a skeletal muscle protein, MYOZ1 localized in the tongue, was noticed in saliva (Hanson et al. 2012). Most authors have been used to classify vaginal material by microflora, which is defined in the following document (Giampaoli et al. 2014).

Menstrual blood is often a diverse fluid comprised of different quantities of flowing blood, discharges from vagina, infectious species, and menstrual cycle cells. The refurbishments of the endometrium while menstruation offer ideal identification candidate markers. MMP7 and MMP11 are the most significant groups studied (Haas et al. 2014; Fleming and Harbison 2010a, b; Lindenbergh et al. 2012, 2013a, b; Juusola and Ballantyne 2005, 2007; Richard et al. 2012; Roeder and Haas 2013; Jakubowska et al. 2013). The transcription of MMP genes during the entire menstrual cycle was found to differ.

Distinctions concerning the epithelial (vaginal and mouth) and the epidermal cells are established in the detection of skin cells on a sample from which a DNA profile can be derived. For the epidermal cell recognition, a variety of cytokeratin families are suggested. Among these LCE1C and LOR showed that skin samples containing weak and inefficient identification of the other markers and housekeeping genes have the most stable detectable abundance of transcription, which is possibly representing the very low level of mRNA in the cell (Hanson et al. 2012; Schulz et al. 2010). Many of these markers appeared significantly in vaginal secretions (Hanson et al. 2012; Simons and Vintiner 2012). Regardless of the methodology used, laboratory confirmation documenting standards are needed. The suggested PCR end point reporting approaches differ. The following are weighted rating schemes focused on presence (peakingapex), dearth and sensitivity of several markers; consensus amplification; multifacet scale strategies and the use of controls. The related testing methods are used for quantitative PCR methods, yet they have trouble with mixed sample interpretation. The analysis of DNA is becoming widespread in the forensic sciences analysis and the Massive Parallel Sequencing or Next Generation Sequencing (MPS) technique has only been recently extended to Ribo Nucleic Acid (RNA) analysis. Assay of messenger RNAs in bodily fluids utilizing MPS/NGS for menstrual blood, saliva, and vaginal secretions and the simultaneous sequence of Deoxyribo Nucleic Acid and RNA out of the identical sample (Lin et al. 2015; Zubakov et al. 2015).

5.13 NanoString®

NanoString® nCotunter is a tool for recording and counting of the various mRNA transcripts, which can use color-coded molecular bar codes to measure the expression of up to 800 mRNA applicants with one reaction (Malkov et al. 2009; Brumbaugh et al. 2011). In recent times, NanoString® used as a tool for distinguishing body fluid with the utilization of messenger RNA in criminal research. In the first test, 18 mRNAs unique to body fluid and two endogenous controls were used. Throughout the study, a total RNA was used and the counts of housekeeping gene GAPDH were normalized. The use of body fluid-based markers for blood and semen were correctly identified, but the vaginal marker and the mRNA for saliva were not reliable (Park et al. 2013a, b). In a study a wide variety of different specimens, including total RRNA extracted from body fluids, were analysed using direct cell lysates with 23 messenger RNA markers and ten

housekeeping genes (Danaher et al. 2015). Samples out of a solo source of semen, blood, menstrual blood, skin, and vaginal secretions, every part of which showed gene expression specific to body fluid using an algorithm and maximum likelihood calculations. Once again, the use of this technique made saliva samples troublesome.

5.14 miRNA Profiling Approaches

There are a wide range of different messenger RNA markers have been projected being sensitive and specific in support of body fluids detection in criminal cases and have also proven their stability in the different stored samples over longer period of time (Zubakov et al. 2009; Setzer et al. 2008). The recently introduced micro RNA (miRNA) markers have explored to be alternate tool for body fluids detection in criminal cases (Zubakov et al. 2010). Non-coding RNA molecules are miRNAs that are of 18–25 nucleotides length which regulates expression of gene at the post transcription levels and is also reported that several miRNAs have tissue-specific expression patterns (Bartel 2004; Jost et al. 2011). Additionally, the very small strands of miRNA markers make them very less susceptible to dreadful conditions by environmental factors, which offer advantage biomarkers for body fluids identification than mRNA markers (Hanson et al. 2009).

The foremost microRNA profiling to criminalistics was reported by Hanson et al. in 2009, and assessed the miRNA expression in biological fluids relevant to forensic approach. Using qRT-PCR analysis he explored the expression of 452 human micro RNAs in saliva, blood, semen, menstrual blood and vaginal fluids. They have characterized nine microRNA markers viz. miR451, miR135b, miR16, miR10b, miR205, miR124a, miR658, miR412, and miR372—these markers are expressed distinctively in genetic experiments related to forensic cases, and have also established their competences to detect body fluids utilizing as minute as 50 pg of whole RNA. Zubakov et al. in 2010 have also reported the expression degrees of 718 microRNAs in biological fluids addressed to forensic utilizing genome-based microarrays and recognized 14 distinctive markers for possible body fluid identification. In this authentication test using qRT-PCR, merely blood and semen exclusive microRNA candidates indicated similar expression degrees, whereas fewer similarities were identified intended for vaginal secretions, menstrual blood, and saliva. Another study which was designed similarly but including skin cells was also reported. In this study they identified 14 new markers which revealed suitable body fluid exclusive expression arrays and also reported that microRNA-4761-5p can distinct skin cells and menstrual blood; microRNA-137 can make a distinction between cells of skin, peripheral blood and saliva; microRNA-4473 can individualize semen and skin cells; microRNA-585-3p can separate vaginal secretion from skin cells (Luo et al. 2015).

5.15 DNA Methylation Profiling Methods

The discovery of entire genome studies specified that the genetic material called DNA relays tissue-distinctive methylation arrays, the possibilities of tissue-specificity DNA methylation has been intended for examination of different body fluids identification in forensic fields (Frumkin et al. 2011; Lee et al. 2012). DNA methylation patterns took place at the CpG dinucleotides-5′ position of pyrimidine ring of cytosine, is largely supposed to express the genes of particular body fluids (Miranda and Jones 2007). The DNA methylation arrays observed will be depends upon the different types of cells and chromosome portions called tissue-distinctive Differentially Methylated Regions (tDMRS) are identified to spectacle unique DNA methylation profiles. Accordingly, the recognition of DNA methylation at a CpG location of Differentially Methylated Regions (tDMR) allows for the tissue or else cell-specific kind of samples of DNA (Byun et al. 2009; Ohgane et al. 2008).

Around 2011, Frumkin et al. described 15 genomic loci to identify saliva, semen, blood, skin, epidermis, vaginal secretions and urine. Within this research they applied methylation sensitive restriction enzyme PCR (MSRE-PCR) designed using an assay for certain markers which are composed up of methylation sensitive restriction enzyme. These types of restriction enzymes digest the samples of DNA subsequently multiplex PCR reactions of specified loci of genomic DNA with fluorescently marked primers, followed by capillary electrophoresis of amplified products using programmed signal detection method. These particularly designed assays can be easily incorporated in forensic science laboratories like other techniques of DNA typing viz. SniPS (SNPs), microsatellites or Short Tandem Repeats (STRs) which would positively identify the source tissues. Similarly, Wasserstrom et al. (2013) reported with the advance methodology by build-up a kit, DNA source identifier (DSI)-Semen™, which was designed to substitute the microscopic investigation of sperm cells for identification of semen in forensic DNA samples. This kit was designed on the basis to detect the semen-distinctive DNA methylation arrays at five genomic loci utilizing MSRE-PCR. The source kit was ratified with 135 samples of different body fluids and 33 case evidences from forensic laboratory, which was verified to be vigorous and consistent by presenting a positive outcome for samples of semen as minute as 31 pg of DNA template (Madi et al. 2012). In another research carried out by Lee et al. studied the tissue-distinctive Differentially Methylated Regions possibility for forensic body fluids detection by bisulfite sequencing approach, which regulates the methylation of DNA via detecting the nucleotide base alteration as a result of treatment of sodium bisulfite. Treatment Bisulfite does not affect any methylated cytosine instead alters free or else unmethylated cytosine of CpG into uracil, which converts into thymine during the PCR reaction. By means of this methodology, they generated five tDRMs profiles in combined DNA samples from saliva, blood, menstrual blood, semen, and vaginal fluids. The tDRMs for USP49 and DACT1 existed recognized as an exclusive marker for semen—by revealing semen-distinctive hypomethylation and PFN3 tDMR was preferred to characterize the vaginal fluids. In a recent approach proposed by Madi et al. illustrated the tissue-restricted DNA methylation in biological body

fluids which are forensically relevant including saliva, blood, epithelial cells, and semen. They identified a limited genomic locus with bisulfite modified method and pyrosequencing technique to observe that the methylation arrays at the FGF7 and ZC3H12D loci can distinguish the semen sample from more genetic samples although locus—C20 and BCAs4 can distinguish the saliva and blood samples as of other biological fluids separately. The outcomes as well specify the DNA methylation-based methods are invaluable tool for the detection of biological fluids in criminal cases.

5.16 DNA-Compatible Cell-Specific Identification

Another method for identifying biological fluid ensues classification of specific cells by marking the cells in combination with a microscopy at a protein, DNA, and RNA level. In the case of immunohistochemistry, epidermal cells have been identified and cytokeratin's are used to differentiate between vaginal and mouth mucosal cells (Schulz et al. 2010; Paterson et al. 2006).

As compared directly to epidermal cells, cells of mucosal origin may be distinguished since each cytokeratin has a low expression of the other type of cell. In a different method, immunofluorescence has been suggested for the on-site recognition of human blood with fluorescently tagged antihuman antibodies, nuclear (CD45, myeloperoxidase, histone 1), and (glycophorin A) while several washing steps may find this method unworkable for forensic work (Thorogate et al. 2008). XY Fluorescent In Situ Hybridisation (FISH) combined with Laser Microdissection (LMD) and DNA profiling has been shown to be an efficient way of extracting the data from specific cell groups marked with fluorescent labels, i.e., chromosomes X and Y (Lynch et al. 2015).

ESR1 (Estrogen Receptor 1) with fluorescently labeled monoclonal antibodies has been shown to be able to differentiate between vaginal and buccal epithelial cells with this marker if messenger RNA profiling is out of the question. This displays the expression of messenger RNA and tissue-specific protein are not always interconnected (Fleming et al. 2013).

RNA suspension FISH are used to classify and detect epithelial cells using keratin 10 with the fluorescent label LNA sample; subsequently, the epithelial cells were isolated by LMD and profiled with DNA (Williams et al. 2014). While these labeling methods that tend to be accurate, the longer time taken to specifically gather labeled cells by LMD illustrates, instead for general applications, these methods are presumably restricted to particular cases.

5.17 Conclusion

Many forensic laboratories are still in practice with the chemical methods, antigen–antibody reaction, catalytic reactions, while the current DNA profiling methods are based on STR profiling which allows personal identity of sample donor. A recent

advance in forensics has advocated the usage of other types of evidences which add more information to the collected evidences. Particularly in recent times several markers have existed for body fluids detection in forensic community. mRNA markers usage in recent times have been utmost meticulously explored with the number of different markers which is sufficient for the body fluids identification in forensic perspective. The currently used methods have possible approaches for the quick detection of biological fluids, but then again in majority of the cases tissue-specific identification is difficult due to cross overreaction with other biological body fluids present at the crime scene. In this scenario mRNA marker appears as high sensitivity and tissue specificity for forensic analysis with a new method to over-power the boundaries of established methods. Similarly, micro RNA methods have explored to be alternative tool for body fluids identification in forensics. microRNA profiling has the capabilities to identify or to differentiate the different body fluids traced at the crime scene. In recent times, profiling through DNA methylation was recommended as a novel means in criminal cases involving body fluids detection. Like messenger and micro RNA profiling, DNA methylation profiling as well appeared highly sensitive and specific, for the parallel assessment of several markers particular for different tissues in a definite multiplex reaction. In the coming future, forensic examinations have to progress with a huge deal as the encroachments in molecular biology studies and forensic genetics.

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