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Overview of the Methods for Saliva Identification

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Abstract

Body fluids, such as blood, semen and saliva, are frequently encountered at a crime scene. Therefore, it's important to identify the body fluids unambiguously for criminal investigation. In addition to the individual identification, determination of the type of body fluid can also provide important clues. Forensic scientists have spent many years dedicating to developing methods for identifying the specific body fluid. However, most of the studies focused on blood and semen identification. In this paper, we review the methods for saliva identification, including traditional color tests, immunoassays, microbiological assays, molecular biological assays, spectroscopy-related assays and alternative light source (ALS) screening.

Keywords: forensic science, body fluid, saliva identification, criminal investigation, biological assays

Introduction

Evidence of body fluid is readily deposited at most crime scenes due to Locard's principle [1]. Saliva is one of the body fluids frequently encountered at the crime scenes. In sexual assault cases, investigators must rapidly identify what body fluid is left at the scene or connect the likely perpetrator to the crime. To discriminate whether oral sodomy or intercourse has occurred, the identification of semen and saliva must be adopted. Presumptive and confirmatory tests for semen identification have already been well-developed, such as the ACP test, microscopic examination, p30 assay and zinc test, etc.[2]; nonetheless, confirmatory test for saliva identification is still under development.

As for presumptive tests, based on α-amylase, a color test is often performed to screen whether saliva is present or not. While immunoassay and molecular biological assays are usually used in developing confirmatory tests, these methods are not mature enough to be implemented in forensic casework due to the cross-reactivity under certain circumstances. To have a better understanding of saliva identification, in this paper, methods including presumptive and potential confirmatory tests for saliva identification were reviewed.

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Color tests

α-amylase is the key enzyme to preliminarily identify the existence of saliva by color tests. It is widely used to either localize or identify the body fluid of saliva at crime scenes or in laboratories due to its convenience. The color test is easy to be implemented, however, it is merely considered to be a presumptive test instead of a confirmatory test due to the lack of specificity and sensitivity. It is reported that saliva is not the only body fluid where the α-amylase can be detected. A limited amount of α-amylase is discovered in serum, urine and semen, etc. [3]. Nevertheless, the amount of α-amylase in saliva has been reported to be 10–100 times more than that in other body fluids [4], whereby its high amylase activity is still a distinctive index of saliva. Some color tests for salivary amylase screening are shown as the followings.

1. Starch-iodine test

Starch is a polysaccharide consisting of linear polymer amylose and branched polymer amylopectin [5]. When iodine is added to the starch, starch and iodine will then combine to form complexes and show the color purple/blue. Upon the presence of α-amylase, it cleaves internal α-1,4-glycosidic linkages and subsequently produces several oligosaccharides and other byproducts; the color thus declines. Therefore, this starch-iodine test is applied to detect the existence of salivary amylase. Plate diffusion was used in the process to recognize the disappearance or attenuation of such color as starch-iodine–based agarose gel is prepared in a petri-dish and starch is digested by salivary amylase, which causes a fading of the color [6]. Normally, the starch-iodine assay shows the fade of color immediately after the presence of α-amylase, but the assay is found to lack sensitivity when saliva is diluted [7].

2. Dyed-starch test

Phadebas® (Magle Life Sciences, Cambridge, MA, USA)

Phadebas Amylase Test is a chemical reagent using Phadebas, sometimes made in tablets, for the detection of saliva. This reagent includes starch covalently bonded with blue dye, and thus it's water-insoluble. When α-amylase is present, it breaks down the glycosidic bonds in amylose or amylopectin to release the dye, cleaving starch into pieces and becoming water-soluble. After centrifugation, pellets precipitate at the bottom of the tube, and the color of supernatant is detected under the absorbance at 620 nm or observed with naked eyes [7, 8]. Phadebas® Forensic Press test was developed to detect stains deposited at crime scenes. A white filter paper is treated with Phadebas® reagent in advance. The paper is white on one side and blue on the other. In the presence of salivary amylase, pressing the blue side down toward the suspected stain and then the white side demonstrates blue. Furthermore, Hedman et al. showed that the following DNA profile analysis will not be interfered with the Phadebas® and that 1:100 diluted saliva with saline solution is still detectable on the filter paper [8].

Red-starch assay(Red-Starch, Megazyme, Ireland)

Another product was also developed at the very same time when blue-dyed starch (Phadebas®) was developed. The progress of the Red-starch method is similar to that of the blue starch, while the red dye, Procion Red MX-5B, is cross-linked to starch, showing red color. A filter paper is also used in the process; the paper should be evenly dipped or sprayed with Red-starch solution and let dry. Afterward, a sample with saliva is dropped or a swab containing saliva is rubbed on a dried Red-starch paper. Rinse the paper with tap water and a positive result is characterized by fainting the color from dark red/pink to pale white, which α-amylase hydrolyzes the covalent bond between the starch and the dye [9].

3. Nitrophenol derivative test

CNPG3 (Sclavo Diagnostics International S.p.A, Siena, Italy)

In addition to covalently bonded starch, 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3) is used to detect the presence of α-amylase by measuring its activity. Since CNPG3 can be catalyzed by α-amylase on the α-glycosidic bond, it generates 2-chloro-4-nitrophenyl-α-D-maltoside (CNPG2), 2-chloro-4-nitrophenol (CNP), and maltotriose (G3), and the color shifts from transparency to yellow attributed to CNP. CNP concentration can be measured using absorbance at 405 nm, and the increase in absorption of CNP is directly proportional to the amount of α-amylase. This method is originally used to detect the potential existence of acute pancreatitis as well as to diagnose and monitor pancreatic

diseases. Though it focuses on the pancreatic amylase (p-type amylase), in forensic science salivary amylase (s-type amylase) is also available to be quantified by this method [10, 11].

E- pNPG7 (Randox, Kearneysville, WV, US)

E-pNPG7, ethylidene-p-nitrophenyl-maltoheptaoside, is a chemical compound for the detection of α-amylase. Seven glucoses are attached to para-nitrophenol on one end and blocked with ethylidene on the other end. When α-amylase is present, it cleaves the glycosidic bonds between two glucoses, releasing para-nitrophenol after the indicator enzyme glucosidase is employed. Later on, the color yellow is observed or the absorbance of para-nitrophenol at 405 nm can be detected [12].

4. SALIgAE® test (Abacus Diagnostic, West Hills, CA, US)

SALIgAE® test is an amylase test to preliminarily detect the existence of saliva. The mechanism of SALIgAE® test has not yet been disclosed. The result interpretation follows the rule that when saliva is added to the tube with SALIgAE® solution and then produces yellow color, indicating a positive result. For the sensitivity test, it shows that solution diluted up to 1:1000 is still in the range of detection but the color change is hard to be observed with naked eyes; fortunately, the color could also be detected by spectrophotometer at 402 nm [13]. For the specificity test, it shows that other body fluids, including semen, vaginal secretions, blood and sweat, along with animal saliva, including dog's and cat's saliva, produce negative results. Other than the tube test, a spraying method to localize possible saliva stains has been developed as well [14].

Immunoassay

Immunoassay is one of the approaches to detect specific body fluid. The basic idea behind this assay is based on the binding of an analyte (protein or other macromolecules) to a specific antibody. Different immunoassay formats result in different interpretations of a positive result. Two variable detections are mentioned in this paper.

One of the immunoassays is ELISA (Enzyme-Linked Immunosorbent Assay). It is an examination that utilizes a fixed antibody/antigen to specifically bind to the corresponding antigen/antibody and detect it by adding an enzyme-linked antibody to appear colors after the substrate is subsequently added [15]. Additionally, this method can also be used for quantification by detecting the intensity of absorbance from the product. Statherin is a specific protein marker for saliva; it has been successfully tested using ELISA and its specificity has been compared to α-amylase detected using ELISA and the Phadebas® amylase test [16]. The results showed that when saliva and five other body fluids (blood, urine, vaginal fluid, sweat, and semen) were analyzed, cross-reactivity was observed for all of these other body fluids when α-amylase was detected by ELISA, and for four of these other body fluids (excluding semen) when the Phadebas® amylase test was used. However, when statherin was detected by ELISA, it showed no cross-reactivity, indicating statherin is an effective marker for saliva identification.

The other immunoassay mentioned in this paper is immunochromatography. RSID-SalivaTM Rapid Stain Identification (Sigma, St. Louis, MO) and SERATEC® Amylase Test (Goettingen, Germany) are two commercial products developed by the immunoassay approach-immunochromatography. It only takes a few minutes to complete a run. When a human salivary amylase antigen is present, salivary α-amylase antibody attached to colloidal gold binds the antigen and diffuses on the membrane creating a line at the "test line" after the complexes are bound to immobilized monoclonal antibody [17, 18].

Microbiological assays

The distinct bacterial community can be found in some tissues and body fluids, such as saliva, skin, vaginal fluid, menstrual blood and semen [19], and most of the bacteria can be detected by genetic method through PCR (Polymerase Chain Reaction) or NGS (Next Generation Sequencing).

Streptococcus salivarius and *Streptococcus mutans*, two of the oral bacteria, were used as targets of human saliva. Their DNA markers, GTFK (glucosyltransferase-K) and GTFD (glucosyltransferase-D) respectively, were detected in saliva but not in semen, urine, vaginal fluid and skin surfaces, indicating *Streptococcus salivarius*

and *Streptococcus mutans* were specific targets for human saliva [20, 21]. According to the establishment of Human Microbiome Project, NGS for amplified V4–V5 of bacterial 16S rRNA can be used not only for saliva but also for other body fluid identification [22]. Furthermore, based on the results of NGS, the composition of the microbiome among body fluids can be compared.

Molecular biological assays

Genetic biomarker is a promising target to identify body fluids. According to the tissue specificity, body fluids can be defined. A large number of studies reveal that methylated DNA, mRNA and miRNA are potential markers for saliva identification.

1. DNA methylation

DNA methylation is an epigenetic modification that occurs frequently on C-5 of cytosines, usually coming with guanine, resulting in a high density of CpG dinucleotides and typically shutting down gene expression. In general, there are two approaches used for detecting DNA methylation: bisulfite conversion methods and non-bisulfite conversion methods. The former uses sodium bisulfite to convert un-methylated cytosines to uracils while leaving methylated cytosines unaffected; to detect the change, Methylation-specific polymerase chain reaction (MSP) [23], Single base extension (SBE) [24] or pyrosequencing [25], among others, can be used. The latter does not use sodium bisulfite to treat DNA, but it does require a methylation sensitive restriction enzyme (MSRE). MSRE digests the specific sequences including the un-methylated CpG sites but does not cleave the sequences where cytosines in CpG are methylated. Therefore, methylation sensitive amplification polymorphism (MSAP) [26] or methylation sensitive restriction enzyme-PCR (MSRE-PCR) [27] is used to determine the status of methylation in a target.

DNA chip, like Illumina Infinium Human Methylation 450 BeadChip array, is usually used to select body fluid-specific CpG sites. Lee et al. focused on 64 sites and filtered those having low discrepancy in DNA methylation status as well as those might cross-react with other body fluids, they eventually uncovered a specific CpG site, cg09652652, on FAM43A for saliva [28]. According to the data analyzed by multiplex methylation SNaPshot, cg09652652 showed a relative high methylation signal in saliva but low or no signal in other body fluids.

The study from Silva et al. showed that regarding the use of the array mentioned above and bisulfite treatment along with Single-base-extension (SBE), a good DNA methylation biomarker for saliva, BCAS4, was found, and its sensitivity assessment showed that it needed at least 10 ng of DNA to have a clear-cut result [29].

2. RNA-based assay

Tissue specific mRNA expression

Since 1999, mRNA markers for body fluid identification have been proposed [30]. STATH (Statherin) and HTN3 (Histatin 3) are found to be stable mRNA markers in saliva [31]. Body fluid identification workflow includes RNA extraction (or DNA/RNA co-extraction), reverse transcription, PCR and then followed by capillary electrophoresis as an end-point detection or qPCR (quantitative polymerase chain reaction) approach as a real-time detection [32, 33].

Besides the thermal cycling amplification method, isothermal amplification has also been successfully used to amplify mRNA targets. Tsai et al. demonstrate that RT-LAMP (reverse transcription-loop-mediated isothermal amplification) is a robust method for amplifying STATH [34]. Features of this method include 4–5 primers needed to be designed, single temperature (such as 65℃) during amplification and the products can be either observed with naked eyes, detected by a turbidimeter or analyzed by agarose gel electrophoresis**.**

Tissue specific miRNA expression

miRNA is another target for RNA-based assay. It generally binds to the 3'UTR and is involved in post-transcriptional regulation of mRNA. Since miRNA has only 18–25 nt long, it is more stable than mRNA. To detect miRNA, stem-loop primers are designed and implemented in qRT-PCR (quantitative real-time PCR) based on its short sequence [35]. Due to the characteristic of its short length, miRNA is not prone to be degraded by complicated environments or hazardous conditions. As a result, when saliva (or any other body fluids) is deposited in an open area where peptides, DNA and mRNA, may be degraded, resulting in a failed test on these targets, miRNAs become the best candidate for saliva identification. It is reported that miR-203 [36], miR-223 and miR-145 markers [37] are specific for saliva.

Spectroscopy-related assays

The components of body fluids are regarded as complicated. Implementing spectroscopy-related assay to identify body fluids is often related to the use of a spectrometer or spectrophotometer. The application of spectrometer or spectrophotometer on body fluid identification is still an uprising and potential approach though it has been widely adopted in other scientific fields.

1. Raman spectroscopy

Raman spectroscopy is a non-destructive method for distinguishing body fluids. It has been reported that the patterns of Raman spectra is available to differentiate between peripheral blood, semen and saliva [38]. Since each body fluid contains different components, each has its distinctive Raman spectrum [39]. Although the components of saliva are undoubtedly complicated, the spectrum can be used to distinguish saliva from other body fluids owing to the consistency of the constituent [40].

2. Fluorescent spectroscopy

Fluorescent spectroscopy is extremely sensitive for measuring chemical substances such as those found in saliva [41]. The use of fluorescent spectroscopy to identify saliva has been published due to its non-destructive nature [42]. In this study, Raj et al. collected 20 saliva stains from 20 different people. These samples were mixed with KCl, before being excited at a wavelength of 254 nm. They observed a consistent pattern in the fluorescent spectrum, with the emission spectrum ranging from 345 to 355 nm. Owing to the high sensitivity, high reproducibility and cost-effectiveness, fluorescent spectroscopy may play an important role in saliva identification.

3. Deep blue autofluorescence (dbAF)

Deep blue autofluorescence analysis is a novel approach to study protein structure [43]. As opposed to the detection of fluorescence from aromatic amino acid, deep blue autofluorescence is found to be resulted from hydrogen bonds and carbonyl groups in amino acid peptide bonds [44]. While saliva has a distinct protein composition, similar autofluorescence patterns were discovered when saliva was excited at 390 nm and emitted at 420 nm, and the pattern was distinct from other body fluids [45]. Aromatic groups from amino acids are easily degraded in aged or degraded forensic samples, whereas hydrogen bonds and carbonyl groups in peptide bonds do not break easily. As a result, detecting dbAF is a potential method to be applied at crime scenes.

Alternative light source (ALS) screening

Forensic alternative light source detection of body fluid is a common and effective method for screening possible spots of left stains. Examining the stains at various wavelengths plus orange/yellow goggles or filters has been developed to look for saliva stains on different fabrics and surfaces [46, 47]. Sterzik et al. revealed that some saliva stains left on 8 different materials (20 materials in total) were not visualized with the combination of light source (415/440/460 nm) and yellow/orange filter in a recent stage but 2 years later, indicating that undetectable samples may have better results after being stored for years [48]. The author mentioned the possible cause of this phenomenon is that since some of the constituents were degraded or broken down into fragments, the maximum absorption wavelength at 450 nm in a recent stage varies to a wider range of wavelengths with time.

Conclusions

Several strategies for identifying saliva stains with presumptive and potential confirmative tests were illustrated in this review to help readers understand current and potential saliva identification methods. A summary of saliva identification methods mentioned in this review is shown in Table 1. When a suspected stain is discovered at a crime scene, examiners usually inspect it with flashlights or alternative light source in order to identify or preclude the specific body fluid preliminarily. Due to the large amount of salivary α-amylase in saliva, detecting enzyme activity is an excellent preliminary test for filtering out other body fluids; regardless of it, immunochromatography is also widely applied to test the presence of human salivary α-amylase with higher sensitivity than color tests. However, there is still a slight chance to have cross-reactivity among body fluids, thus targets of mRNA, miRNA and methylated DNA markers turn out to be potentially suitable for the detection of saliva.

Table 1 Summary of the saliva identification methods.

Property / Method	Detection limits	False positives (FP)	Applicability	Limitations
Starch-iodine test	10 uL of 1/250 diluted saliva [49]	**α-amylase:** laundry detergents, other body fluids, etc. [53]	Preliminary test [49]	Starch hydrolyzed in acidic condition [54]
Phadebas® Press test	10 uL of 1/250 diluted saliva [49]	**α-amylase:** laundry detergents, other body fluids, etc. [53]	Preliminary test [49]	False negative for mixed body fluids [55]
SALIgAE® test	10 uL of 1/1,250 diluted saliva [49]	**α-amylase:** laundry detergents, other body fluids, etc. [53]	Preliminary test [49]	Light color
RSID™-Saliva kit	10 uL of 1/31,250 diluted saliva [49]	**α-amylase:** laundry detergents, other body fluids, etc. [53]	Preliminary test [49]	Possibility of high hook effect
Nitrophenol derivative test (CNPGn)	98 nL of 1/4,096 diluted saliva [50]	Pancreatic α-amylase [50]	Preliminary test [50]	Light color
ELISA	0.5 nL of 1/102,400 diluted saliva [16]	**Statherin:** No FP [16] **α-amylase:** laundry detergents, other body fluids, etc. [16]	Supplement of the preliminary tests [16]	Time-consuming, expensive
Microbiological assays	1 pg DNA [20]	S. salivary: No FP [20]	-	Time-consuming
DNA methylation	10 ng DNA [51]	Vaginal secretions [51]	-	Time-consuming
RT-LAMP assay	6.25 ng RNA [34]	**Statherin:** No FP [34]	-	-
Raman spectroscopy	10 uL of saliva [39]	Vaginal secretions [39]	Preliminary test [39]	Intact database required
Fluorescent spectroscopy	-	-	-	-
dbAF	-	-	-	-
Alternative light source	5 uL of 1/10 diluted saliva [52]	Fluorescent substance	Preliminary test [52]	Fluorescence interference

The symbol of "-" indicates that it is not mentioned in the references.

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References

1. Byard RW, James H, Berketa J, Heath K. Locard's principle of exchange, dental examination and fragments of skin. J Forensic Sci 2016; 61(2):545-7.
2. Baechtel FS, Brown J, Terrell LD. Presumptive screening of suspected semen stain in situ using cotton swabs and bromochloroindolyl phosphate to detect prostatic acid phosphatase activity. J Forensic Sci 1987; 32(4):880-7.
3. Whitehead PH, Kipps AE. The significance of amylase in forensic investigations of body fluids. Forensic Sci 1975; 6(3):137-44.
4. Kipps AE, Whitehead PH. A method for quantitating amylase and its use in the Investigation of various body fluids. Ann Clin Biochem 1974; 11(6):219-23.
5. Searcy RL, Wilding P, Berk JE. An appraisal of methods for serum amylase determination. Clin Chim Acta 1967; 15(2):189-97.
6. Xiao Z, Storms R, Tsang A. A quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities. Anal Biochem 2006; 351(1):146-8.
7. Sakurada K, Watanabe K, Akutsu T. Current methods for body fluid identification related to sexual crime: focusing on saliva, semen, and vaginal fluid. Diagnostics (Basel) 2020; 10(9):693.
8. Hedman J, Gustavsson K, Ansell R. Using the new Phadebas® forensic press test to find crime scene saliva stains suitable for DNA analysis. Forensic Sci Int 2008; 1:430-2.
9. Martin NC, Clayson NJ, Scrimger DG. The sensitivity and specificity of red-starch paper for the detection of saliva. Sci Justice 2006; 46(2):97-105.
10. Da Vela G, Bertino MG, Fusini F, Ricci U. Evaluation of an automated system for amylase detection in forensic samples. Int Congr S 2006; 1288:636-8.
11. Barni F, Berti A, Rapone C, Lago G. Alpha-amylase kinetic test in bodily single and mixed stains. J Forensic Sci 2006; 51(6):1389-96.
12. Hohenwallner W, Stein W, Hafkenscheid JCM, Kruse-Jarres JD, Kaiser C, Hubbuch A, Klein G. Reference- Ranges for α-Amylase in serum and urine with 4,6-Ethylidene-(G7)-1-4-nitrophenyl-(G1)-α,D-maltoheptaoside as substrate. J Clin Chem Clin Biochem 1989; 27(2):97-101.
13. Liang T, Roy R. Ultraviolet-visible spectrophotometry (UV-VIS) and SALIgAE® qualitative and semi-quantitative tools for the analysis of salivary amylase. J Forensic Res 2014; 5:1-5.
14. Chatterjee S. Saliva as a forensic tool. J Forensic Dent Sci 2019; 11(1):1-4.
15. Engvall E, Perlmann P. Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen-coated tubes. J Immunol 1972; 109(1):129-35.
16. Akutsu T, Watanabe K, Fujinami Y, Sakurada K. Applicability of ELISA detection of statherin for forensic identification of saliva. Int J Legal Med 2010; 124(5):493-8.
17. Old JB, Schweers BA, Boonlayangoor PW, Reich KA. Developmental validation of RSID™-saliva: A lateral flow immunochromatographic strip test for the forensic detection of saliva. J Forensic Sci 2009; 54(4):866-73.
18. Barbaro A, Cormaci P, Votano S, La Marca A. Evaluation study about the SERATEC® rapid tests. Forensic Sci Int Genet Suppl S 2015; 5:e63-4.
19. Dobay A, Haas C, Fucile G, Downey N, Morrison HG, Kratzer A, Arora N. Microbiome-based body fluid identification of samples exposed to indoor conditions. Forensic Sci Int Genet 2019; 40:105-13.
20. Nakanishi H, Kido A, Ohmori T, Takada A, Hara M, Adachi N, Saito K. A novel method for the identification of saliva by detecting oral streptococci using PCR. Forensic Sci Int 2009; 183(1-3):20-3.
21. Hoshino T, Kawaguchi M, Shimizu N, Hoshino N, Ooshima T, Fujiwara T. PCR detection and identification of oral streptococci in saliva samples using GTF genes. Diagn Microbiol Infect Dis 2004; 48(3):195-9.
22. Soriano-Lerma A, Pérez-Carrasco V, Sánchez-Marañón M, Ortiz-González M, Sánchez-Martín V, Gijón J, Navarro-Mari JM, García-Salcedo JA, Soriano M. Influence of 16S rRNA target region on the outcome of microbiome studies in soil and saliva samples. Sci Rep 2020; 10(1):13637.

23. Ku JL, Jeon YK, Park JG. Methylation-specific PCR. Methods Mol Biol 2011;791:23-32.
24. Gonzalgo ML, Liang G. Methylation-sensitive single-nucleotide primer extension (Ms-SNuPE) for quantitative measurement of DNA methylation. Nat Protoc 2007; 2(8):1931-6.
25. Delaney C, Garg SK, Yung R. Analysis of DNA methylation by pyrosequencing. Methods Mol Biol: Springer 2015; 249-64.
26. Fulneček J, Kovařík A. How to interpret methylation sensitive amplified polymorphism (MSAP) profiles? BMC Genet 2014; 15(1):2.
27. Lin YC, Tsai LC, Lee JCI, Su CW, Tzen JTC, Linacre A, Hsieh HM. Novel identification of biofluids using a multiplex methylation sensitive restriction enzyme-PCR system. Forensic Sci Int Genet 2016; 25:157-65.
28. Lee HY, An JH, Jung SE, Oh YN, Lee EY, Choi A, Yang WI, Shin K-J. Genome-wide methylation profiling and a multiplex construction for the identification of body fluids using epigenetic markers. Forensic Sci Int Genet 2015; 17:17-24.
29. Silva DS, Antunes J, Balamurugan K, Duncan G, Alho CS, McCord B. Developmental validation studies of epigenetic DNA methylation markers for the detection of blood, semen and saliva samples. Forensic Sci Int Genet 2016; 23:55-63.
30. Bauer M, Kraus A, Patzelt D. Detection of epithelial cells in dried blood stains by reverse transcriptase-polymerase chain reaction. J Forensic Sci 1999; 44(6):1232-6.
31. Juusola J, Ballantyne J. Multiplex mRNA profiling for the identification of body fluids. Forensic Sci Int 2005; 152(1):1-12.
32. Wang Z, Zhang S, Di Z, Zhao S, Li C. Messenger RNA profiling for forensic body fluid identification: Research and applications. J Forensic Med 2013; 29:368-74.
33. Lynch C, Fleming R. RNA−based approaches for body fluid identification in forensic science. Forensic Sci 2021; 3(4):e1407.
34. Tsai LC, Su CW, Lee JC, Lu YS, Chen HC, Lin YC, Linacre A, Hsieh HM. The detection and identification of saliva in forensic samples by RT-LAMP. Forensic Sci Med Pathol 2018; 14(4):469-77.
35. Kramer MF. Stem-loop RT-qPCR for miRNAs. Curr Protoc Mol Biol 2011; Chapter 15:Unit 15.
36. Sirker M, Fimmers R, Schneider PM, Gomes I. Evaluating the forensic application of 19 target microRNAs as biomarkers in body fluid and tissue identification. Forensic Sci Int Genet 2017 Mar; 27:41-9.
37. Park JL, Park SM, Kwon OH, Lee HC, Kim JY, Seok HH, Lee WS, Lee SH, Kim YS, Woo KM, Kim SY. Microarray screening and qRT-PCR evaluation of microRNA markers for forensic body fluid identification. Electrophoresis. 2014; 35(21-22):3062-8.
38. Sikirzhytski V, Virkler K, Lednev IK. Discriminant analysis of Raman spectra for body fluid identification for forensic purposes. Sensors (Basel) 2010; 10(4):2869-84.
39. Muro CK, Doty KC, de Souza Fernandes L, Lednev IK. Forensic body fluid identification and differentiation by Raman spectroscopy. Forensic Chem 2016; 1:31-8.
40. Virkler K, Lednev IK. Forensic body fluid identification: the Raman spectroscopic signature of saliva. Analyst 2010; 135(3):512-7.
41. Nanda KDS, Ranganathan K, Umadevi K, Joshua E. A rapid and noninvasive method to detect dried saliva stains from human skin using fluorescent spectroscopy. J Oral Maxillofac Pathol: JOMFP 2011; 15(1):22-5.
42. Raj CK, Garlapati K, Karunakar P, Badam R, Soni P, Lavanya R. Saliva as forensic evidence using fluorescent spectroscopy: A pilot study. J Clin Diagn Res 2018; 12(9):ZC27 - 9.
43. Niyangoda C, Miti T, Breydo L, Uversky V, Muschol M. Carbonyl-based blue autofluorescence of proteins and amino acids. PLoS One 2017; 12(5):e0176983.
44. Pinotsi D, Grisanti L, Mahou P, Gebauer R, Kaminski CF, Hassanali A, Kaminski Schierle GS. Proton-transfer and structure-specific fluorescence in hydrogen bond-rich protein structures J Am Chem Soc 2016; 138(9):3046-57.
45. Rathore PS, Kumar S. Identification of different body fluids through novel deep blue autofluorescence. Forensic Sci Int 2021; 327:110976.
46. Vandenberg N, van Oorschot RA. The use of Polilight® in the detection of seminal fluid, saliva, and bloodstains and comparison with conventional chemical−based screening tests. J Forensic Sci 2006; 51(2):361-70.

47. Szeremeta M, Pepiński W, Niemcunowicz-Janica A, Skawrońska M, Sackiewicz A, Ptaszyńska-Sarosiek I, Okłota M. Evaluation of visualization of biological stains with the use of alternative light source (ALS) for the purpose of genetic identification. Part I. Blood and saliva stains analysis. Polish Society of Forensic Medicine and Criminology 2010; 60(4):248-57.
48. Sterzik V, Hinderberger P, Panzer S, Bohnert M. Visualizing old biological traces on different materials without using chemicals. Int J Legal Med 2018; 132(1):35-41.
49. Park, H. Y., Son, B. N., Seo, Y. I., & Lim, S. K. Comparison of four saliva detection methods to identify expectorated blood spatter. J Forensic Sci 2015; 60(6): 1571-76.
50. Su CH, Tsai LC, Hsieh HM. Evaluation of the saliva identification methods. Taiwan J Forensic Med 2021;13(1):25-33.
51. Park JL, Kwon OH, Kim JH, Yoo HS, Lee HC, Woo KM, Kim SY, Lee SH, Kim YS, Identification of body fluid-specific DNA methylation markers for use in forensic science. Forensic Sci Int Genet 2014; 13, 147-53.
52. Vandenberg N, Oorschot, RAH. The use of Polilight in the detection of seminal fluid, saliva, and bloodstains and comparison with conventional chemical-based screening tests. J Forensic Sci 2006; 51(2), 361–70.
53. Feia, A., Novroski, N. The evaluation of possible false positives with detergents when performing amylase serological testing on clothing. J Forensic Sci 2013; 58 Suppl 1, S183–85.
54. Hansen C, Wayment B, Klein S, Godfrey B. Iodine–Starch test for assessment of hyperhidrosis in amputees, evaluation of different methods of application. Disabil Rehabil 2018; 40:25, 3076-80.