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A GENERAL REVIEW ON THE IDENTIFICATION AND DETECTION OF SALIVA FOR FORENSIC TESTING

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ABSTRACT

Saliva is the biological evidence that is most frequently discovered at crime scenes. Because it is clear, there will be no indication that the liquid has been altered by the offender. Saliva is often utilized as biological evidence in forensics and is particularly useful in identifying a person's sex, ABO blood type, uniqueness, biomarkers, microbiological signature, or habits. The importance of saliva as essential forensic evidence and the numerous ways it can appear at a crime scene are covered in this review article. Over past few decades, various collecting and detection techniques have been discussed. The data that is now accessible have been attempted to be gathered, showing the benefits and drawbacks of various identification methods. All of the published and reported literature from 1980s through 2023 has been consulted to gather the pertinent facts. Forensic casework's main objective is to identify evidence to lay the foundation for additional inquiry. Saliva can be found as a puddle or stained substance, however, because to its transparency, its identification is difficult. The discovery and description of numerous proposed approaches or techniques over the years has aided in detecting and recognizing saliva as confirmation.

Keywords: Saliva, Detection, Identification, Crime scene, Forensic evidence.

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INTRODUCTION

The proofs at crime scenes (blood, urine, saliva, vaginal secretions and semen) can be trusted to connect crimes to offenders and to recreate the crime scene. Each of them has unique identity screening procedures. In contrast to other types of cases, blood, saliva, and semen are frequently gathered as evidence in homicide, murder, and sexual assault cases. Urine and saliva are two biological fluids that are frequently found in suicide cases. Blood has a disadvantage over saliva, a clear liquid. In situations of murder, saliva is frequently left behind marks. Additionally, an attacker (or attackers) frequently causes saliva to be deposited on the victim or surroundings. Half-eaten fruits or other delicacies can occasionally serve as sources of salivary evidence in burglary cases, and unconscious trickling of saliva is frequently noticed during hangings. As numerous factors affect the quality of evidence, gathering these fragments of data is difficult as well [**1**]. The entire saliva is made up of the secretions from the salivary glands, which also include bacteria, exfoliated oral epithelial cells, and gingival crevicular fluid. Saliva is helpful in numerous ways, including sex determination from bite marks, drug abuse analysis, animal bite marks analysis, and personal identification by DNA profiling. Only saliva exhibits properties comparable to plasma [**2**]. In addition to 99% water and a variety of electrolytes (sodium, potassium, calcium, chloride, magnesium, bicarbonate, phosphate), salivary fluid also contains proteins that are important for oral health [**3**]. In addition, there is glucose, leucocytes, desquamated epithelial cells, nitrogenous substances such urea and ammonia, and

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oral bacteria [**4**]. Because saliva collection is non-invasive, simple, and safer than blood collection and carries a lower risk of contamination, particularly from contagious diseases like hepatitis, saliva offers numerous advantages over blood collection. Since saliva is a transparent liquid, it is frequently disregarded as evidence and is less likely to be altered by criminals [**5**]. It could be on any surface in stained form or in the pool. Various actions, such as biting, licking, spitting, and sucking, deposit saliva on human skin. Clearly visible bite marks on food items can also be used to evaluate it [**6**].

The current research concentrated on the many assays or detection and identification methods used to confirm the identity of saliva as significant forensic evidence during an investigation. Additionally, it provides an overview of current developments in efficient methods for identifying saliva. Additionally, a comparison of the significance of various methodologies has been presented [**7**]. The structure of this article is as follows: 1. Saliva samples collection methods; 2. Preliminary tests were done to identify the saliva. This comprises the immunochromatographic strip tests, Phadebas test, Polilight test, and SALIGaE test; 3.A variety of sophisticated saliva screening techniques, including DNA methylation and microscopic fluxes for the identification of saliva.

COLLECTION OF SALIVA SAMPLES

Saliva is an extremely complex operation that typically involves two swabbing methods. In the single-swab method, the collection site is covered with a wet, sterile cotton swab that has been dampened with distilled water or normal saline. To prevent the collection of substrate material, the swabbing is carried out without using additional pressure. However, the sample location is swabbed twice when using the twofold swabbing approach. A dry cotton swab is then used after rolling a moist one over the area. With this technique, the most saliva from the substrate is collected [**8**]. The investigator must be careful when swabbing to avoid contaminating area unnecessarily, which could result in inaccurate or inconsistent DNA profiling. Double swabbing is favored over single swabbing because it ensures that the most quantity of evidence is collected from the surface. The evidence is processed using a number of ways after the saliva has been collected. These methods can be roughly divided into two categories: destructive and non-destructive. Destructive methodology refers to a method that compromises the integrity of the evidence following evaluation. The evidence in this situation cannot be saved or used for further processing. However, in the case of non-destructive procedures, the integrity of the evidence is unaffected following assessment. In the following **Fig. 1**, mechanism of molecular transport from serum into salivary gland ducts. Like, A. Active transport. B. Passive diffusion. C. Simple filtration. D. Acinar cells actively pump sodium ions (Na+) into the duct. E. Pore on the cell membrane. F. Cell membrane. G. Acinar cell. H. Intracellular space. I. Duck cell pump Na+ ions back into blood.

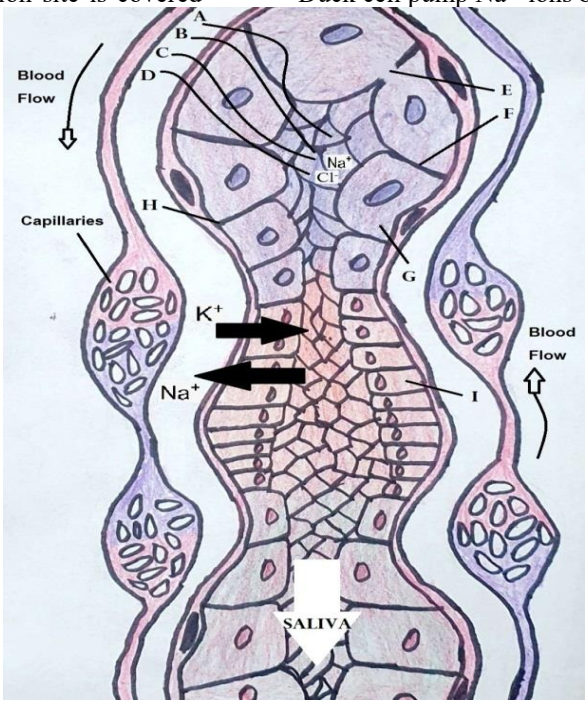


Figure 1: Mechanism of molecular transport from serum into salivary gland ducts.

PRELIMINARY TESTS

The amylase enzyme activity is the basis for the preliminary tests, also known as presumptive tests, for saliva. However, this amylase is connected to various bodily fluid productions in addition to saliva [**9**]. AMY2 is frequently detected in semen, the pancreas, and vaginal fluid while AMY1 is typically found in saliva, breast milk, and perspiration. Saliva has higher concentrations of amylase-1 than other body fluids, and radial diffusion assays make it simple to distinguish between amylase-1 and amylase-2 [**10**].

Polilight Detection

Alternating light source (ALS) is another name for it. With regard to large surfaces in particular, this is quick and labor-efficient. Locating bodily fluids is made possible with Polilight, a portable, high-intensity light source. Between 310 and 650 nm in wavelength, it emits a strong, focused band of light [**11**]. However, due to the reduced fluorescence intensity under Polilight, saliva is frequently difficult to find; in addition, surface absorbency interferes with fluorescence. There are goggles with specific filters that only let through desired wavelengths and produce odd observations. The drawback of polilight is that the stain's ability to be seen clearly is hampered by the material's color. Additionally, non-specificity results from fluorescence patterns that are comparable to those of other bodily fluids [**12**].

Phadebas Test

Presumptive assays are frequently employed, such as the Phadebas test. A DSM-P microsphere with blue dye attached to it is the active ingredient. The test is susceptible to multiple false-positive outcomes since it depends on saliva's alpha-amylase activity. The release of blue dye, which happens when starch is hydrolyzed in the presence of amylase, typically confirms a favorable reaction [**13**]. According to a recent study, the Phadebas press test can also be used to identify spit stains on particular materials and samples that have been stained for up to three months. Phadebas press test can also be used to identify spit stains on particular materials and samples that have been stained for up to three months, according to a recent study [**14**]. Its effectiveness has been proven in studies that contrast it with strategies like Polilight, starch iodine, and SALIgAE. However, the Phadebas test was unable to determine the identification of saliva. The answer will depend on the sort of evidence and the surface where saliva was discovered.

SALIgAE Test

It uses colorimetry to get rid of any potential false-positive results produced by the technologies it competes with, Polilight and Phadebas. SALIgAE's efficacy has been extensively researched using a variety of methodologies. To prevent false-positive results from other body fluids, which often take longer than 5 min, reaction time must be limited to 5 min [**15**]. SALIgAE is proven to be superior to Phadebas and more sensitive when compared with other assays.

Due to the colorimetric outcome, it also has certain drawbacks in addition to these advantages. The main drawback of this colorimetric test for detection of substances mixed with blood is the production of false positive reactions. Additionally, the process is time-consuming or calls for the maximum amount of sample dilution beforehand, which can result in drawbacks and the misuse of evidence [**16**].

Immunochromatographic Strip Test

Two salivary monoclonal antibodies are activated by a lateral flow immunochromatographic strip, increasing their sensitivity [**17**]. Rapid Stain Identification (RSID) kit is a typical illustration of this strip. Due to its serological approach rather than a colorimetric one, this test is distinct from Phadebas or SALIgAE and significantly more sensitive. These three methods have been evaluated in numerous researches, and RSID has consistently shown to be far more sensitive and time-efficient than its competitors. This qualitative assay produces positive or negative results. As low as 1 L can be detected by RSID with accuracy, repeatability, and sensitivity on various surfaces (such as cigarette butts, cans, bottles, etc.). This method may effectively eliminate any uncertainty that may arise and is portable and effective. The sensitivity of RSID with saliva is significantly higher since it identifies the presence of alpha-amylase, which is also present in other bodily fluids as urine and vaginal excretion [**18**].

Sensivity

The kind of substrate that the sample has been deposited on affects how effective an immunochromatographic strip is. Additionally, factors such as light, heat, air, and humidity contribute to the deterioration of saliva. Additionally, it has been documented that the RSID approach works well on materials deteriorated by wet soil, whereas Phadebas would have yielded unfavorable results [**19**]. It was also shown that this method works well for distinguishing expired blood spatters or blow artefacts from saliva. The outcomes of the other tests were either negative, flimsy or falsely affirmative, or unsuccessful [**20**]. But RSID delivered fruitful outcomes.

Table 1: Saliva detection tests

Tests	Detection	Drawback
Preliminary test	Amylase-1 present in saliva	Not detect amylase-2
Polilight detection	High intensity wavelength 310-650 detect saliva	Stain's ability to be seen clearly can be effected by material's color
Phadebas test	Saliva's alpha amylase detected and release blue color	Not specific
SALIgAE test	It uses colorimetry for confirmation of saliva presence & more sensitive than other tests.	The main drawback of this colorimetry test for detection of substance mixed with blood is the false positive reaction.

ADVANCED SCREENING/DETECTION TECHNIQUES

To identify saliva, a variety of detection methods are thought to be confirmatory. Some of these tests have been studied for many years, while others are still being developed.

Immunological Techniques

Antigen-antibody responses are the foundation of immunology assays. These tests help identify species in addition to aiding in detection. Horseradish peroxidase conjugate and monoclonal antibodies were used in an enzyme-linked immunosorbent test (ELISA) to measure the alpha-amylase activity in saliva. The ELISA results showed positive results for saliva with no cross-reactivity. Additionally, mixed samples were easily found. (PRPs), which are other proteins, are in two varieties: basic salivary PRP 2 (PRB2) and (PRH1/2). In particular, the PRH1/2, which is expressly found in salivary glands, is saliva-specific [**21**]. These PRPs were utilised in an ELISA to identify saliva and to assess the sensitivity, effectiveness, and specificity in relation to STATH. We found similarities between STATH and PRH1/2 followed by PRB2 in terms of detection rate and sensitivity. However, PRH1/2 had a better specificity for saliva than STATH, indicating that it is more important for forensic purposes.

Microscopy Techniques

Tools for microscopic analysis can pinpoint a precise metal concentration for detection. Saliva also contains various amounts of trace elements such salt, phosphorus, sulphur, chlorine, potassium, and calcium [**22**]. However, it has been demonstrated that potassium has the most pronounced peak in saliva samples, making it useful for detection.

RNA Profiling

Although RNA is fragile and prone to deterioration, numerous research has demonstrated its stability in samples and its extensive usage for forensics. Rapid identification including as micro, and tiny RNA, are now possible thanks to the accessibility of RNAseq by Massive Parallel Sequencing (MPS) [**23**]. RNA is a group of short non-coding RNA (ncRNA) molecules of 18–24 nucleotides that regulate a variety of cellular functions and are less sensitive to environmental deterioration.

A novel and specific but expensive method for detection called reverse transcription-loop-mediated isothermal amplification (RT-LAMP) amplifies a particular RNA sequence in just one step [**24**].

DNA Methylation

DNA is a more stable molecule in bodily fluids than RNA. Methylation is a form of DNA modification that occurs at the 5′ position of the cytosine in the CpG dinucleotide sequence and is genetically controlled in mammals. By regulating gene expression through alterations in chromatin structure and tissue-specific patterns, it plays a crucial role in the development and differentiation of cells. DNA methylation is an epigenetic alteration that, if well studied [**25**], can reveal significant information. It exhibits similarities in cellular and extracellular DNA, indicating that they can be successfully examined in the absence of cells. The most popular method of DNA methylation is the chemical alteration of cytosine residues by sodium bisulfate [**26**]. By employing large concentrations of bisulfate at high temperatures, this alteration can be sped up. To solve the issue, the methylation-sensitive restriction enzyme PCR (MSRE-PCR) method was created, although it could only be used to identify semen. The second most popular technique is methylation SnaPshot, which offers the benefit of simultaneous analysis by creating multiplex methylation SnaPshot. The multiplex Snapshot microarray, which integrates various markers, is another introduction to this. Because it provides a qualitative, quantitative, and effective method of identifying 5-methylcytosine at single base-pair resolution, bisulfite genome sequencing is a core gold-standard methylation methodology. In order to avoid any potential false positives, next-generation sequencing has been advocated [**27**].

Spectroscopic Technique

Vibrational spectroscopy has made great strides in bodily fluid research, and recent breakthroughs in

this field have demonstrated tremendous potential for a different strategy. This method is non-destructive, non-labeling, and global [**28**]. Spectroscopy in the ultraviolet with numerous improvements and shifts from a limited range to a wider range, UV-Visible spectroscopy has evolved throughout time. The precise wavelength range of various bodily fluids has been shown in numerous investigations. As other drugs may interfere with results, this method is vulnerable to several false positives.

Fourier transform infrared spectroscopy (FTIR) is reliable method that can identify numerous salivary spectra [**29**]. However, many spectra of single body fluid are a drawback of vibrational spectroscopy. Body fluids produce inhomogeneous complicated spatial distributions because they are complex mixtures. Additionally, results are hampered by environmental sample degradation because of various contaminations.

SALIVA IDENTIFICATION BY MICROBIAL DETECTION

Proteins, carbohydrates, antimicrobial proteins, white blood cells, and a variety of other substances are found in healthy saliva. It does support a variety of microbial flora while having antibacterial immunoglobulins and WBCs as the first line of defense [**30**]. Streptococci are the most numerous oral bacteria, comprising the majority of the 750 million total microscopic cells per milliliter of saliva. Over the years, several approaches including PCR and loop-mediated isothermal amplification have been used to study the existence of these bacteria only in the saliva. Forensic scientists' interest in the detection of the microbiome in bodily fluids has significantly grown over time. There are numerous investigations that provide unambiguous positive detection but are carried out in various ways. The most prevalent bacteria are those that cause dental caries, such as Streptococcus salivarius and Streptococcus mutans [**31**].

These methods are cutting-edge, more practical, simple to apply, and economical. By comparing samples, conventional techniques produce a result that is particular to bodily fluid and species and favours microbial detection. This technique also has a good level of sensitivity in mixed samples.

Comparative to other methods, microbial profiling has demonstrated significant sensitivity for the detection of saliva in degraded samples. As they could be successfully detected in samples that had been exposed to the environment and UV rays. The amount of color present is directly related to the number of bacteria present in the sample. This technique is the most recent development in the forensic microbiome and can concurrently identify two bacteria in 20 minutes, in contrast to existing oral bacteria detection methods.

CONCLUSIONS

Similar to other bodily fluids, saliva is an important tool in forensics. It is reliable evidence due of its extensive utility, non-intrusive collection, and colorless look. Saliva testing provides both destructive and non-destructive evaluations. The Phadebas and SALIgAE tests depend on the test subjects' saliva samples having -amylase. Compared to the first three, the sensitivity of immunochromatographic kits for the detection of monoclonal antibodies is higher, but they frequently produce false-negative findings when used with other methods. In order to analyses evidence farther downstream, saliva detection must be established during advanced screening. Each method has advantages, but there are also drawbacks. For instance, because the DNA methylation method may distinguish between people and bodily fluids in a single processing. The NexGen sequencing method has proven to be the most effective among them so far, although NGS is expensive and not available everywhere. The spectroscopy techniques have a lot to offer. The sample's many spectra of a single bodily fluid are the main drawback, which may get worse if it was tainted or mixed. These methods produce more gratifying outcomes while preserving the validity of the sample. Under forensic microbiology, microbial profiling is the newest development and area of focus for detection. However, this method is pricy and skill-intensive. Microbes have a far higher chance of surviving in the environment.

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