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Advances in Saliva Detection for Forensic Investigations: A Modern Perspective

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ABSTRACT

Saliva, an abundant and easily obtainable biological fluid, has emerged as a crucial type of forensic evidence due to its widespread presence at crime scenes and its ability to provide both biochemical and genetic information. It has a crucial importance in the justice system, as It helps to identify perpetrators, clear the wrongly accused, and assist in criminal investigations. It gives wide range of information in forensic contexts such as DNA and Genetic Information, Blood Group Typing, Microbial Signature, Lifestyle and Behavioral Clues etc. It can be transferred during numerous activities such as biting, licking, speaking, coughing, or kissing, thereby frequently appearing in cases of assault, sexual violence, and homicide. Traditionally, saliva identification relied heavily on enzymatic assays targeting alpha-amylase; however, these methods often lacked specificity and were prone to false positives. With the advancement of molecular biology, nanotechnology, and analytical instrumentation, a range of novel techniques has been developed to detect and confirm the presence of saliva with greater sensitivity and specificity. These include immunoassays, RNA and DNA profiling, spectroscopy-based techniques, biosensors, and epigenetic markers. This review highlights both conventional and cutting-edge approaches to saliva detection, discusses their forensic relevance, and evaluates their potential for integration into routine forensic workflows. The continuous innovation in saliva detection techniques not only enhances the evidentiary value of this biological fluid but also contributes significantly to the accuracy and reliability of forensic investigations.

Keywords- Saliva, Forensic evidence, Genetic information, Saliva detection techniques and Crime scene analysis.

1.INTRODUCTION

Saliva has a crucial importance in the justice system, offering DNA and biochemical evidence that helps identify perpetrators, clear the wrongly accused, and assist in criminal investigations [1].Saliva is a complex and dynamic biological fluid, primarily secreted by the major and minor salivary glands, consisting of approximately 99% water and 1% organic and inorganic constituents, including enzymes, proteins, electrolytes, mucins, and epithelial cells [2]. It plays a vital role in maintaining oral health and serves as a transport medium for numerous biochemical markers. In forensic science, saliva has gained growing importance due to its frequent occurrence at crime scenes and its capacity to provide valuable genetic and molecular information [3].Saliva is often deposited through actions such as licking, biting, speaking, coughing, or spitting, making it a common form of trace evidence, especially in cases involving sexual assault, homicide, and physical abuse [4]. Its ease of deposition on a variety of substrates like skin, fabric, and food items, coupled with its non-invasive collection, makes saliva an attractive target for forensic analysis. Moreover, it contains abundant nuclear DNA, which allows for the identification of individuals through Short Tandem Repeat (STR) profiling, and also carries fluid-specific RNA and microRNA biomarkers [5]. Conventional detection techniques primarily target salivary alpha-amylase, an enzyme present in high concentration within human saliva. Presumptive tests like the Phadebas and starch-iodine tests are simple and quick; however, they suffer from poor specificity as alpha-amylase can also be found in other bodily fluids such as semen, vaginal secretions, and perspiration [6]. Consequently, the forensic community has increasingly shifted focus toward confirmatory and more sensitive approaches. Recent advances in analytical science have introduced a range of novel saliva detection methods. These include immunological assays using monoclonal antibodies, nucleic acid-based methods such as mRNA and miRNA profiling, spectroscopy-based techniques like Raman and FTIR, and biosensor technologies offering rapid and portable detection [7], [8]. Additionally, epigenetic markers and omics-based analyses are being explored to increase the forensic value of saliva by enabling body fluid identification, aging of stains, and determination of donor characteristics [9].

2. SIGNIFICANT INFORMATION DERIVED FROM SALIVA

Saliva can provide a wide range of information in forensic contexts such as[10]-

DNA and Genetic Information

- **Identity of an individual** through DNA profiling.
- **Paternity and familial relationships**.
- **Sex determination**, using markers like the amelogenin gene.

Blood Group Typing

- Detection of **ABO blood group antigens**, especially in secretors (individuals who express these antigens in their body fluids).

Microbial Signature

- Unique patterns of oral **bacteria and microbiota**, which can help link a person to a location or object.
- May also help in determining **geographic origin** or lifestyle factors.

Lifestyle and Behavioral Clues

- Evidence of **smoking**, **alcohol use**, or **drug abuse**.
- Some studies suggest saliva can reflect **dietary habits** and **health conditions**.

3. FORENSIC DETECTION TECHNIQUES FOR SALIVARY EVIDENCE

3.1 Enzymatic Assays

Alpha-amylase, also known as salivary amylase or ptyalin, plays a crucial role in the initial digestion of starches. It is secreted in large quantities by the parotid gland and is considered a marker of salivary origin due to its high concentration relative to other body fluids [11].One of the most commonly used enzymatic assays for saliva detection is the Phadebas test, which is based on the hydrolysis of a starch–dye complex by alpha-amylase. A positive reaction results in the release of a blue dye, indicating the presence of enzymatic activity [12]. Another method is the starch-iodine test, where the breakdown of starch by saliva results in the loss of a blue-black color after exposure to iodine. While these tests are cost-effective and easy to perform, they lack specificity because alpha-amylase is also present, although in lower quantities, in other biological fluids such as vaginal secretions, sweat, and semen, which may result in false-positive results [13].

Limitations of Enzymatic Testing

The principal limitation of enzymatic tests is their inability to distinguish the source of amylase. For instance, human pancreatic amylase shares structural similarity with salivary amylase, leading to cross-reactivity. Additionally, environmental factors such as heat, pH, and substrate degradation over time can significantly reduce enzyme activity, resulting in false-negative outcomes [14]. Moreover, enzymatic tests are presumptive in nature and do not offer confirmatory results, which limits their reliability in forensic casework where the burden of proof is high.

3.2 Microscopic Examination

In some cases, microscopic analysis is performed to identify epithelial cells shed in saliva, particularly from buccal mucosa. These cells can be stained using various techniques (e.g., Papanicolaou or Hematoxylin & Eosin staining), providing supplementary evidence of saliva deposition. However, the number of epithelial cells in a sample can vary greatly and is influenced by the individual's oral hygiene, hydration, and even the surface onto which the saliva was deposited [15].

3.3 Immunological Approaches

Immunological tests utilize the principle of antigen-antibody binding to detect salivary components. The development of immunochromatographic lateral flow devices has significantly improved the specificity of saliva identification. One of the most validated commercial tests is RSID-Saliva, which uses monoclonal antibodies specific to human salivary alpha-amylase [16].Unlike enzymatic tests, immunoassays reduce the risk of cross-reactivity and offer better accuracy. The test typically takes less than 10 minutes and can be performed at the crime scene or in the laboratory. Although more expensive than conventional assays, the benefits of immunoassays in terms of specificity and ease of interpretation outweigh the cost in most forensic settings.These tests are now being refined with multiplexing capabilities, allowing simultaneous detection of multiple fluids (e.g., blood, semen, saliva) using a single sample. This reduces consumption of limited forensic material and enhances evidentiary value.

4. MOLECULAR BIOLOGY-BASED DETECTION

4.1 DNA Profiling

Saliva contains abundant epithelial cells which provide nuclear DNA suitable for STR (Short Tandem Repeat) profiling. This method allows individual identification and has become a cornerstone in forensic casework [17]. STR profiling is universally accepted in legal systems worldwide due to its high discrimination power. Advancements in PCR (Polymerase Chain Reaction) and miniSTR techniques have made it possible to recover usable profiles from degraded, aged, or trace saliva samples. However, DNA profiling alone does not confirm the biological origin of the sample—whether it's from saliva or another fluid—thereby necessitating a combination of fluid-specific and DNA-specific assays.

4.2 RNA-Based Detection

Messenger RNA (mRNA) profiling has emerged as a powerful tool to identify the tissue origin of biological stains. Saliva-specific mRNA markers, such as STATH (Statherin) and HTN3 (Histatin-3), are expressed exclusively in salivary glands and can confirm the fluid's identity [18]. These assays can also be multiplexed with other body fluid-specific markers, creating comprehensive detection panels. MicroRNA (miRNA) profiling, due to its smaller size and greater stability in harsh environments, is gaining popularity in forensic research. These molecules resist degradation and can be detected long after sample deposition, providing an alternative to traditional DNA or mRNA-based techniques.

4.3 Epigenetic Approaches

DNA methylation, an epigenetic modification, offers another method for distinguishing biological fluids. Specific methylation patterns are associated with different body fluids, including saliva. These patterns are less prone to degradation and can be analyzed even in challenging forensic conditions [19].

5. SPECTROSCOPIC AND IMAGING TECHNIQUES

5.1 Raman and FTIR Spectroscopy

Raman spectroscopy and Fourier-transform infrared (FTIR) spectroscopy offer non-destructive analysis by identifying molecular vibrations. These techniques can differentiate saliva from other fluids based on their unique spectral signatures [20].Such tools are valuable in situations where sample integrity must be preserved for subsequent DNA testing. They also reduce sample preparation time and can be performed directly on the substrate, including fabrics and skin.

5.2 Hyperspectral Imaging

Hyperspectral imaging combines spectroscopy with high-resolution imaging, allowing investigators to visualize and localize biological fluids on complex surfaces. Saliva stains can be detected based on their reflectance and absorption characteristics [21]. Although still emerging in forensic applications, hyperspectral tools show promise for large-scale crime scene scanning, particularly in sexual assault and violent crime investigations.

6. BIOSENSORS AND POINT-OF-CARE TECHNOLOGIES

Recent innovations in biosensor technology have led to the development of paper-based and portable saliva detection kits. These systems often use microfluidics and nanomaterials, enabling rapid, real-time detection of salivary biomarkers such as amylase or specific proteins [22].Some biosensors are smartphone-compatible, offering visual results or digital readouts that can be archived or transferred for further analysis. This enhances field usability, especially in resource-limited or time-sensitive crime scenes.While these technologies are still being validated, their integration into forensic practice could transform evidence collection, especially in remote areas or mass disaster scenarios.

7. CHALLENGES AND LIMITATIONS

Despite significant advancements, saliva detection faces several challenges. Environmental factors such as heat, humidity, UV exposure, and microbial contamination can degrade salivary components, reducing test efficacy. Moreover, while advanced molecular and spectroscopic tools offer higher specificity, they often require specialized equipment, skilled personnel, and high operating costs [23].There is also a lack of standardization across forensic laboratories regarding the validation and interpretation of newer technologies. Legal admissibility of emerging tests can be questioned unless supported by peer-reviewed research and precedent case law.

8. CONCLUSION

Saliva has become an increasingly valuable biological fluid in forensic investigations due to its non-invasive collection, frequent deposition at crime scenes, and its rich content of DNA and molecular markers. It plays a crucial role in cases involving sexual assault, homicide, and physical violence. The development of novel saliva detection methods has significantly strengthened the role of saliva in forensic science. These advancements not only improve the reliability of evidence but also support faster and more accurate criminal investigations. As technology continues to evolve, the adoption of these methods will play an increasingly vital role in modern forensic analysis and justice delivery.

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