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(REVIEW ARTICLE)

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Salivary forensics: Investigating the role of saliva

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Abstract

Saliva is emerging as a crucial biological fluid in forensic science due to its abundant DNA and biological markers. This review delves into the diverse applications of saliva in forensic investigations, emphasizing its role in criminal identification, paternity testing and disease diagnosis. The research underscores a critical need for forensic laboratories to develop reliable and repeatable automated procedures for saliva analysis, similar to established protocols for blood and urine. By establishing saliva as a primary investigative tool in the absence of other bodily fluids, forensic odontology can significantly enhance its diagnostic and investigative capabilities.

The primary objective of this review is to comprehensively examine and highlight the multifaceted applications of saliva in forensic scientific methodologies, demonstrating its potential as a versatile and valuable biological specimen.

Keywords: Saliva; Forensics; Amylase; Biomarkers

1. Introduction

Whole saliva comprises secretions from the salivary glands, GCF, and exfoliated oral epithelial cells along with microbes. Saliva is frequently found at crime scenes, often accompanied by marks and lip prints, where the oral cavity has been involved. Additionally, saliva is also an analytical tool in cases of heavy metal poisoning by reflecting the ionic imbalances and elimination of specific toxins through this route. Despite its forensic relevance, there is a lack of compiled research on role of saliva in forensic odontology, necessitating a comprehensive review of existing literature to provide a thorough update on the subject. (Megha Upadhyay, 2023)

2. Methods of saliva collection

The retrieval of saliva is a challenging process due to its invisible nature; it is typically conducted using two swabbing techniques. The choice of method depends on type of surface where evidence is found. (Table 1)

In a single swab approach, a wet, sterile cotton swab is gently rolled over the collection site without applying additional pressure. The double swab technique involves first swabbing the area with a damp cotton swab, followed by second pass with a dry cotton swab to maximize liquid absorption.

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Table 1 The swab methods for collection of saliva from different surfaces

Method	Procedure	Suitable for
Single Swab Technique	Wet, sterile cotton swab is gently rolled over suspected the surface.	Non- absorbent surfaces (Eg. plastic, glass)
Double Swab Technique	A moist cotton swab is used first, followed by a dry swab to absorb saliva.	Absorbent surfaces (Eg.food, fabric, skin)

Once saliva is collected, it undergoes a series of analytical techniques, which are broadly classified into two—destructive and non-destructive methods. (Figure 1)

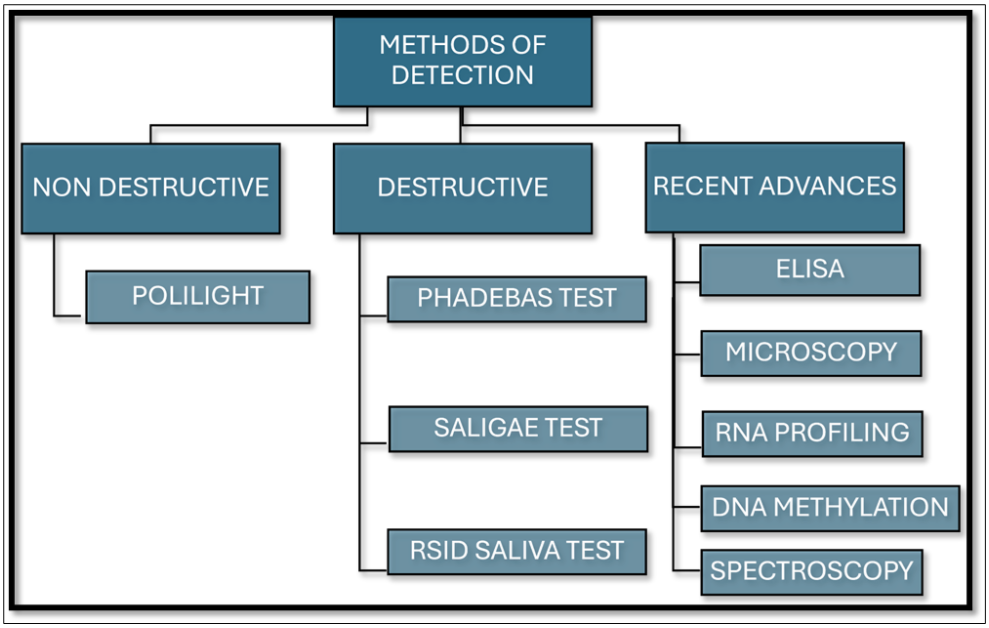

Figure 1 Methods of saliva detection

2.1.1. Non -Destructive Methods

POLILIGHT /forensic light source- Polilight is a portable, high-intensity light source used to locate biological fluids, including saliva.it is the only non-destructive type of presumptive technique for the detection of saliva. This method emits a focused spectrum of light within a wavelength range of 310-650 nm, enabling the visualization of saliva stains without altering the sample. [2] (Vandenberg, 2006)

2.1.2. Destructive methods

- PHADEBAS test- this test is used to detect alpha amylase activity in the saliva. It consists of variations [3] (Danielle J. Wornes, 2018)

  - Press test – applied directly onto a surface to localize amylase positive regions. (Press test)
  - Tube test – more sensitive than the press test, performed in a test tube to provide semi -quantitative results.

The test relies on Phadebas Starch Microspheres, which are cross- linked with blue dye. When exposed to amylase, the starch is hydrolyzed, releasing the dye and producing the blue color that confirms the presence of saliva The tube test provides both qualitative (color change) and quantitative (spectrophotometry based) analysis.

- SALIGAE test-The SALIGAE test is used to determine the trace amounts of saliva. In this procedure, a small amount of sample is introduced into a colorless solution. If saliva is present in the specimen, the solution will turn yellow. If saliva is absent in the sample or highly diluted, the solution will remain colorless. It is a colorimetric approach to eliminate any possible false-positive reactions given by its counterpart's methods-

Phadebas and Polilight. Hence, it is a more sensitive approach for preliminary testing of saliva. [4] (chatterjee, 2019)

- Rapid Stain Identification of Human Saliva (RSID) -Saliva is a lateral flow immunochromatographic strip test designed to detect the presence of human salivary -amylase, an enzyme found in human saliva; the enzyme's physiological role is to aid in the digestion of dietary starches. Unlike the PHADEBAS test, which measures enzyme activity, RSID-Saliva specifically detects monoclonal antibodies against salivary amylase, reducing false positives and improving accuracy. [5] (Turrina, 2008)

2.1.3. Advanced screening and confirmatory tests

- Immunological tests (ELISA)
- Microscopy tests (for detecting specific metals)
- RNA profiling (STAT6 and HTN3)
- DNA Methylation analysis. [6] (Sant, 2012)
- Spectroscopy techniques:
  - Fluorescent spectroscopy identifies tryptophan in salivary amylase.
  - Raman spectroscopy uses phenylalanine to detect amylase and lipase. [7] (Methods in Molecular Biology, 2025)

2.2. Uses of detection of Saliva in Forensics

2.2.1. Identification of Drug Abuse

Various drugs including amphetamines, barbiturates, benzodiazepines, phencyclidine, cocaine and opioids can be detected in saliva. Saliva testing is a reliable method for identifying recent marijuana use through radioimmunoassay techniques. A primary psychoactive component of marijuana can be detected in saliva for at least 4 h post consumption.

Advanced drug detection techniques: In recent advancements, SECURETEC (OTTOBRUNN, Germany) introduced DRUGWIPE, a compact, non-instrumental immunodiagnostic test for the detection of detecting illicit substances on different surfaces. The application of DRUGWIPE for saliva and sweat analysis is under evaluation in multiple countries. This pen sized, immunochemical-based test strip, used for the detection of drugs of abuse on surfaces, offering a convenient efficient screening method.

2.3. Sex determination via Saliva Analysis

The ability to analyze exfoliated buccal epithelial cells from saliva on bite marks has significantly improved the possibility of sex determination in forensic investigations. Emerging research suggests that advanced DNA profiling techniques are further refining accuracy in identifying perpetrators based on saliva samples. Two parameters have been proposed, both based on successful efforts to identify the sex using blood stains:

- The presence and detection of sex chromatin (Barr bodies in females and F bodies in males)
- sex hormone level determinations based on detectable quantities and ratios of testosterone and 17B-estradiol by RIA.[8] (Saxena, 2015)

3. Oral Forensomics: Salivary Biomarkers for Criminal Profiling

Saliva contains a diverse range of proteomic and genomic biomarkers in saliva that can serve as indicators of systemic illnesses, oral diseases, detection of oral cancers, and malignant tumors. Emerging research highlights the role of extrachromosomal RNA, microRNA (miRNAs)and metabolomic profiles in refining the diagnostic potential of saliva. Studies have also demonstrated that salivary cortisol levels serve as a chronic stress and mental disorders, while immunoglobulin levels in saliva can assist in identifying autoimmune diseases like Sjogren' syndrome. The use of machine learning and AI- driven analysis in salivary biomarker research is further enhancing the precision of disease detection.[9] (Mithun Rajshekar, 2013)

4. Postmortem salivary biochemical alterations

After death, saliva undergoes several biochemical and microbial changes due to the cessation of physiological functions and the onset of decomposition. Some key changes include:

- Loss of Secretion – Salivary glands stop producing saliva due to the absence of neural and hormonal stimulation.

- pH Shift – Saliva initially becomes more acidic due to the accumulation of carbon dioxide and lactic acid from anaerobic metabolism. Over time, as decomposition progresses, bacterial activity may cause an alkaline shift.
- Increased Microbial Growth – The oral cavity becomes a breeding ground for bacteria, leading to rapid degradation of proteins and other organic molecules.
- Breakdown of Proteins and Enzymes – Salivary enzymes such as amylase and lysozyme degrade, leading to changes in viscosity and composition.
- Changes in Electrolyte Concentration – As cellular integrity breaks down, there may be fluctuations in sodium, potassium, and chloride levels.
- Putrefaction Compounds – Decomposition produces volatile compounds such as ammonia, hydrogen sulfide, and cadaverine, contributing to the characteristic odor of decay.

These changes can be useful in forensic investigations, particularly in estimating the postmortem interval (PMI). [10] (Bardale, 2013)

5. Conclusion

This review highlights the expanding role of saliva in forensic and medical diagnostics. Saliva has emerged as a crucial biological fluid in forensic science and medical diagnostics, offering a non-invasive and accessibility for biomarker analysis. Advances in saliva collection, preservation and analytical techniques have significantly improved the reliability and accuracy of saliva-based diagnostics. However, challenges such as contamination, environmental degradation and the need for standardization in methodologies remain. Ongoing research and technological advancements, including nanotechnology-based biosensors and lab-on-a-chip devices, are addressing these issues making saliva a promising alternative to traditional diagnostic methods in both forensic investigations and clinical medicine.

The integration of saliva-based evidence with other forensic tools and the development of automated, standardized protocols will further solidify its role in forensic science ensuring more accurate and efficient investigations.

Compliance with ethical standards

Disclosure of conflict of interest

There is no conflict of interest related to this review article.

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