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Forensic Implications of Saliva: An Overview

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Abstract

Over past years, investigators and researchers have been repeatedly attracted by possible applications of saliva related evidence in forensics. It is specifically looked for in cases of sexual assault, drug abuse, driving while intoxicated, and in cases involving animal bites. Oral fluid has a role in linking suspects or victims to a crime scene, as well as in profiling of the unknown individual. Collection of salivary evidence should thus be given deserved importance and should be carried out using scientific methods, considering the progression of time, type of case, sequence and type of analytical methods to be performed, nature of surface etc. This review emphasizes the comprehensive use of saliva in forensics.

Key words*: Identification, Investigation, Forensics, Analysis, Oral Fluid, Saliva*

Introduction

Mandel in 1990 quoted that "saliva lacks the drama of blood, the sincerity of sweat and the emotional appeal of tears''. [1] An exocrine secretion from the salivary glands, saliva is the predominant secretion in the oral cavity. With an average secretion of about a litre per day, and a slightly alkaline pH, it has a multifunctional role. [2] Having a range of molecules in content, saliva has an indispensable role in forensic odontology. [3]

Although with few solutes, saliva is has a varied scope in drug detection, identification, DNA fingerprinting, cases of abuse, analysis of psyche, and so on. Saliva can be collected from bite-marks on skin and edible items, from stains in surfaces, and from various items like utensils, straws, phones, cigarette butts, stamps etc. [4]

In the past, forensic investigations have revealed saliva in lip-prints, bite-marks, and other objects, helping in identification to a greater extent. [5] As saliva enters the oral cavity, it coalesces with blood cells, oral microflora, food, and upper airway secretions. This increases the chance of saliva containing information about a person. [6]

The current article will summarise on the use of saliva in various context of forensics.

Presence of Saliva at the Crime Scene

In numerous crimes, saliva is stored in 'nibble marks'. [7] The advantages of using saliva its ease of access, ease of handling, its non-invasive collection, and economical analysis. [3] On the other hand, oral fluid deposited on substrate can't be specifically submitted to extraction procedures. [8]

Recovery and Detection:

- Visual Examination: As dry colourless stains, saliva is difficult to identify in a crime scene. However with the use of alternate light sources and ultraviolet lights, discovery becomes easier. Under ALS, ranging 415–490nm (using orange/red goggles) [9], quartz arch tube or argon ion laser, saliva presents soft edged white spots, less intense than other stains. [4] Saliva stains will appear bluish-white under an ultraviolet light. However, it degrades the DNA in the sample. [10]

- ***Presumptive tests:*** Due to lack of solutes, there are no confirmatory tests for saliva. [8] The presence of saliva stains can be detected through test for amylase, present in high concentrations in saliva, pancreatic

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fluid, and faeces. Detection of amylase is particularly important in cases involving fellatio. [11]

Test for saliva involves starch and Florence iodine solution (~1:100), with the sample. Starch and iodine form a deep blue complex. Amylase hydrolyses starch and the colour fades (time: 15 mins, temperature: 37°C). However, albumin, gamma-globulin, semen, etc. may give a false positive result. [10] Elevated amylase levels may also be from oral sex/fellatio or from vaginal cavity. [9]

The method has limitations and variable sensitivity depending upon the age of the stain and quantity of deposit. [9]

- ***Collection:*** Collection involves three aspects: the crime scene, the victim and from suspect(s). The classical technique is using a single wet cotton swab/ filter paper laid passively on the surfaces. [4]

Sweet et al developed the double swab technique, to provide a better yield through rehydration of the saliva traces by swabbing with a sterile swab, wetted in nuclease free water with moderate pressure in a rolling motion for about ten seconds, followed by a second dry swab. [4, 12]

Stimulated saliva can be collected from the suspect using paraffin or citric acid crystals, and non-stimulated saliva by simple rinse with mouthwash. While these samplings are considered as diluted saliva, whole saliva is directly milked from the opening of Stenson's duct. Commercial kits like Oragene are also available, when salivary DNA is required.

FTA cards (Fritz Technology Associates) are useful for the collection and storage of DNA from body fluids, including saliva.

Laboratory analysis: Amylase mapping is performed for saliva stains on samples of larger area [Figure-1]. [9]

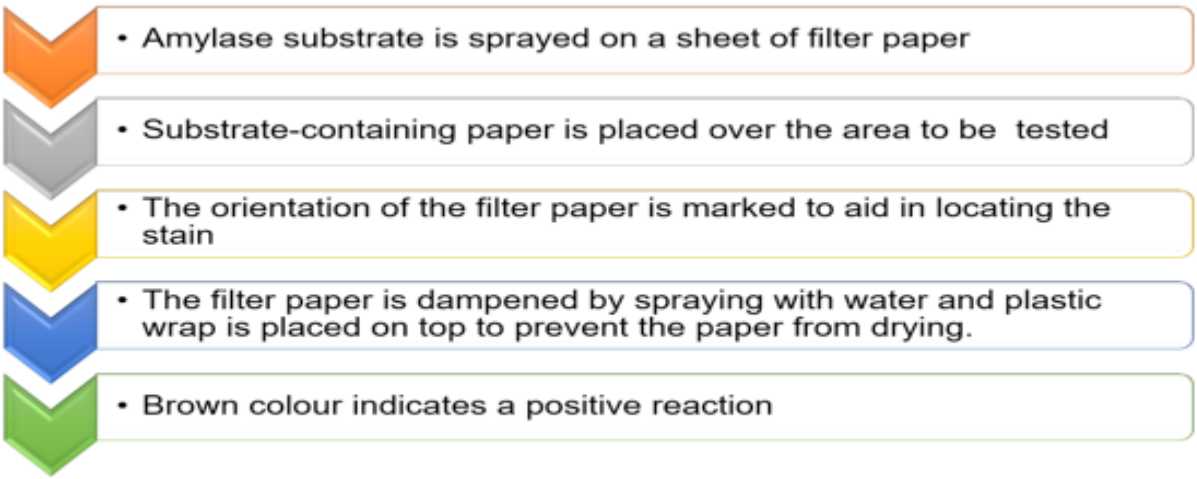

Figure 1: Procedure for amylase mapping

A radial diffusion assay has been used to distinguish sources of AMY1 (amylase found in saliva, breast milk, perspiration) and AMY2 (amylase in the pancreas, semen, and vaginal secretions) [Figure-2]

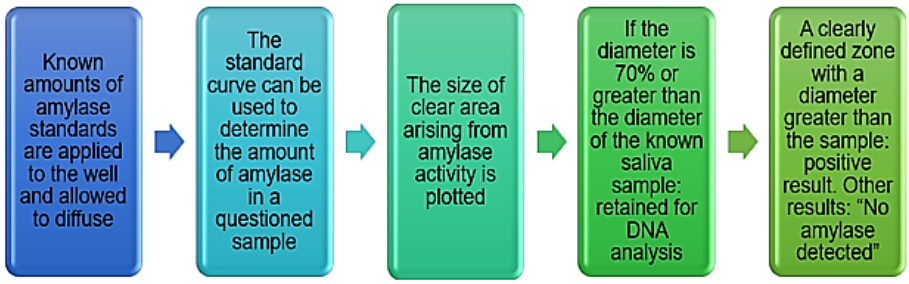

Figure 2: Radial immunodiffusion reaction of salivary amylase

Immunological methods: ELISA is not widely used. α-amylase is detected using a horseradish peroxidase conjugate combined with monoclonal antibodies. The Phadebas1 test reagent, used in tube and press tests, with sensitivity up to 1:12810, incorporates procion red amylopectin.

Detection of Chemicals: Dried saliva stains can be identified with soluble phosphatase, starch and amylase resulting in a red precipitate. Nitrates and thiocyanates can also be detected. Tryptophan also helps recognize dried oral fluid, giving a trademark emanation range on fluorescent spectroscopy. [13]

Near-infrared (NIR) Raman spectroscopy has been used to measure spectra of pure dried human saliva samples, showing its heterogeneity. [14]

The RSID (Rapid Stain Identification) test, specific for human salivary α-amylase, has been very useful and quick to identify saliva. It is similar to a pregnancy test (parallel stream immuno-chromatography) and is the principal high sensitivity specific non-enzymatic test for amylase. [12]

Investigation of Drugs of Abuse in Saliva

Oral fluid is second only to urine for checking medications. [15] Medications enter the salivation by the passive exchange. [16] Most illicit and addictive drugs of abuse can be distinguished in saliva. [17] Radioimmunoassay (RIA) has also proved useful [1, 18]. Recently, Drug wipe, an immunochemical based test strip has been introduced to detect medications of mishandle in the fluid. [19]

Peel et al. discovered quantifiable amounts of medications in salivation removed with methanol and broke down by immunoassay and gas chromatography/ mass spectrophotometry, which can be adjunct to serological testing.[20]

Deoxyribonucleic Acid Profiling/Fingerprinting

DNA analysis is of great dependency when traditional identification methods fail. [21] In spite of affecting the security of human DNA dirt and microscopic organisms don't pose much threat as the tests utilized for profiling are particular to person only. [22]

Saliva contains leukocytes and exfoliated epithelial cells as a useful DNA source. [23] Since secretory genes are present, saliva can also be used for blood grouping. [9]

Saliva yields a very small amount of DNA. However, DNA tests are intensified by polymerase chain response for DNA writing utilizing short tandem repeats (STRs), unique, lifelong and durable markers consisting of repeated DNA sequences. STRs are suitable for maternity/paternity assurance and scientific examination, requiring only 0.5ng of DNA template. [26]

Mitochondrial DNA (mtDNA) is present in cells found in saliva, which has a high copy number proves useful when nuclear DNA testing fails, due to degradation. [27] mtDNA is maternally inherited [28], thus, distant maternal relatives can be used as a reference source for identification.[29]

Methylation and telomere shortening, has been observed in salivary DNA to estimate age. [32]

Salivary DNA lasts even after about fifteen days.

Sex Determination from Saliva

The peeled epithelial cells present in salivation have expanded the likelihood of sex assurance of the culprit. Even in degraded samples, sex chromatin like Barr bodies (in females) and F bodies (in males) can be observed; and sex hormones detected using fluorescent dyes or radioimmunoassay. [22]

Salivary Biomarkers

Salivary nucleic acids and proteins contain vital information. Saliva biomarkers have been used in diagnosis of various diseases but they have wider role in forensics. Markers for any particular disease can be matched and on tracking that disease to hospital visits, positive identification can be achieved. [6]

Determining Psychology

Different hormones in saliva helps in deciding the mind of a person. [11] Salivary level of steroid hormones reflects the free, unbound circulating fraction, [30] helps in determining mind-set of a person. High testosterone in spit has demonstrated people as rough, less consistent and related with individual mischief. [11] Low salivary cortisol could likewise be related with fierce criminal conduct. [31]

Conclusion

Rarely, people think of saliva, as a convenience,

handy for licking stamps and sealing envelopes. Practicing dentists find it's a nuisance, to be sponged, evacuated or dammed. However, over the years, the need and importance of saliva as an investigative body fluid is increasing rapidly in forensics. With constant examination, and a communitarian work in serological and odontological fields, salivation is developing as a fundamental device for scientific examination. A careful learning of what at a dentist faces each time he/she works in a mouth, may increase the significance of an odontologist in a criminal examination group by manifolds. One can only ponder what more is behind each drop of spit in the mouth.

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