

FORENSIC CORNER
REVIEW ARTICLE

Saliva in forensic odontology: A comprehensive update

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ABSTRACT

In recent years, saliva has attracted much interest among researchers especially in the field of forensic sciences. This complex body fluid is gaining popularity due to its ease of collection, safety in handling and its close relationship with plasma. Analysis of saliva for serological testing and cellular content has proved to be of wide use in crime detection, drug and alcohol abuse, hormone identification, cases of poisoning and animal bites. There is a need for forensic laboratories to automate the settings specific for saliva as routinely done for blood or urine in order to consider saliva as the primary investigating tool in the absence of other body fluids. This update is aimed at highlighting the many uses of saliva in the practice of forensic odontology.

Key words: Forensic odontology, investigating tool, saliva

INTRODUCTION

Over the last decade or so there has been a growing interest in saliva and its use as a diagnostic tool as an alternative to blood or urine. Whole saliva is a mixture of secretions released from salivary glands, gingival crevicular fluid and contains exfoliated oral epithelial cells and microorganisms. The added advantage of its noninvasive method of collection even by individuals with limited training and avoidance of intrusion of private functions while collection under direct supervision, makes saliva a popular fluid for forensic analysis. Saliva is often detected in scenes of crime along with bite marks or lip prints where the oral cavity may have been involved. Serological and cellular analysis of thus obtained saliva is of immense use in identification of the accused. Use of saliva in the detection of drugs of abuse and inebriated drivers has gained popularity in recent years. In cases of saliva-derived from bite marks of unknown animals, species-specific genetic profile can help in the identification of the animal in question. Saliva is also an analytical tool in cases of heavy metal poisoning by reflecting the ionic imbalance and excretion of certain poisons through this route. There is a paucity of compiled literature on the role of saliva in forensic odontology,^[1] prompting an extensive search of the existing literature to outline the vast topic into a comprehensive update.

SALIVA IN PERSONAL IDENTIFICATION

Saliva is usually deposited in bite marks found in many homicides, assault and other criminal cases.^[2] Since many problems are encountered in bite mark analysis, primarily because of elastic and distortable nature of skin and lack of good impression medium, the saliva deposited during biting has received an important alternative focus in bite mark analysis.^[3] It is difficult to collect saliva stains from skin, clothing, paper or other inanimate objects since it remains invisible and substrate on which saliva is deposited, mainly skin, cannot be submitted directly to extraction procedures.

Methods of detection of dried saliva stains

- **Chemicals:-** various chemicals and enzymes have been tried to detect dried saliva stains. Saliva detection from stamps and envelopes has been done by the chemical which acts on reducing sugars and gives a red insoluble precipitate. Most commonly used enzymes are alkaline phosphatase, starch and amylase. Salts like nitrate and thiocyanate have also been used for the detection.^[4] All these methods have limitations and variable sensitivity

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depending upon the age of the saliva stain and quantity of deposit

- Lasers and ultraviolet light, quartz arch tube and argon ion laser^[5,6]
- Fluorescent spectroscopy:- the aromatic amino acid, tryptophan, in α -salivary amylase gives a characteristic emission spectrum on fluorescent spectroscopy, thus possessing a good sensitivity in detecting dried saliva stains on the skin.^[4]

Saliva recovery from skin

Traces of salivary evidence can be recovered for identity testing. The classical technique using a single wet cotton swab or section of wet filter paper laid passively on the skin has been effectively used to collect saliva from the skin.^[7] A technique using a wet cotton swab (similar to the classical method) followed by a dry cotton swab, known as the double swab technique, was studied by Sweet *et al.*,^[8] and found to provide a better yield of saliva recovered from the skin surface. Deoxyribonucleic acid (DNA) from saliva and skin-deposited saliva samples can be extracted by the phenol-chloroform method.

Deoxyribonucleic acid profiling/fingerprinting

Except for identical siblings, DNA profiling is unique for individualization. Heat, moisture, sunlight, surface contaminants and other factors can accelerate DNA degradation. Contamination of evidence with DNA from animals or bacteria does not pose a serious problem as the probes used in DNA profiling are specific to humans or at least primates. Bacteria does have an effect on the stability of human DNA, especially soil bacteria which are rich in nucleases.^[9] The polymorphisms within the DNA molecule are the basis of all inherited polymorphisms and they do not change over the lifetime of an individual. DNA samples are amplified by polymerase chain reaction for DNA typing using short tandem repeats (STRs).^[8] The polymorphic repair of STR mainly in small fragments also makes it possible to evaluate DNA from samples with a significant grade of degradation. In addition to genomic DNA, cells derived from saliva contain mitochondrial DNA (mtDNA), the sequence of building blocks of which can be determined to assist in identification.^[10] The main advantage of mtDNA is that there is a high copy number in each cell caused by the high number of mitochondria present in most cells.^[10] Chromosomal DNA is inherited from both the mother and father whereas mtDNA is strictly maternally inherited.^[11] Thus when identifying a set of human remains, if close relatives are not available, distant maternal relatives can be used as a reference source to support identification.^[12] Therefore, mtDNA testing may be successful when nuclear DNA testing fails or when genomic DNA cannot be analyzed, possibly because it is too degraded.^[10]

ANALYSIS OF DRUGS OF ABUSE IN SALIVA

The most frequently used biological specimen for the determination of drugs of abuse is urine since it is a noninvasively obtained sample and is acceptable for routine collection. Yet even the acceptability of a urine sample is being disputed in view of the potential invasion of privacy, especially if a directly observed collection is advisable to prevent adulteration or substitution of the sample.^[13] Improved analytical techniques have made it possible to analyze a large number of drugs in a small amount of oral fluid. The major advantage of oral fluid over urine is the easy, rapid and noninvasive sampling procedure.^[13]

Drugs that can be identified in saliva are amphetamines, barbiturates, benzodiazepines, phencyclidine, cocaine and opioids.^[14] Saliva can be used to detect recent marijuana use by means of radioimmunoassay. A major psychoactive component of marijuana can be detected in saliva for at least 4 h after marijuana is smoked.^[15]

Most drugs appear to enter saliva by simple passive diffusion which is characterized by the transfer of drug molecules down a concentration gradient with no expenditure of energy.^[16] Salivary drug concentrations generally reflect the free fraction of the drug in the blood.

Peel *et al.* found measurable quantities of drugs in saliva extracted with methanol and analyzed by enzyme multiple immunoassay technique and gas chromatography/mass spectrophotometry.^[17] This methodology, both specific and sensitive appears to be a useful adjunct to serological testing in bite marks for identification purposes. A number of drugs such as phenobarbital, amphetamine and morphine have been detected in saliva and saliva stains by radioimmunoassay (RIA) by a number of investigators.

Recently, Securetec (Ottobrunn, Germany) introduced Drugwipe, a non-instrumental immunodiagnostic assay for the detection of drugs on surfaces. The use of Drugwipe for saliva and sweat is currently being investigated in several countries.^[18] Drugwipe is pen sized, immunochemical-based test strip, used for the detection of drugs of abuse on surfaces. Separate tests are available for opiates, cocaine, cannabinoids and the amphetamine group. The wiping part enables the user to sample drug particles from any kind of surface.^[18]

Saliva is currently used in the measurement of steroid hormones such as cortisol, cortisone and testosterone because it is commonly accepted that the salivary level of these steroids reflects the free, unbound circulating fraction.^[1]

SALIVARY ANIMAL BITE MARK ANALYSIS

Fletcher *et al.* described an enzyme-linked immunoassay technique using monoclonal antibody based on the presence of

salivary immunoglobulin A for species identification in stains upto 16 months old.^[19]

Crossover electrophoresis and double gel diffusion techniques were used for comparison in cases with poor monoclonal antibody results. This technique would appear to have value in bitemark examinations from nonhumans where the biting animal is not known.

Evolutionary relationships among species are most directly determined by comparisons at the DNA sequence level.

SEX DETERMINATION FROM SALIVA IN BITEMARKS

The possibility of obtaining exfoliated buccal epithelial cells in saliva on bite marks has increased the possibility of sex determination of the perpetrator. The duration of this line of inquiry is apparently possible for several weeks, post deposition, depending on the materials containing the impressions and environmental factors. Two parameters have been proposed, both based on successful efforts to identify the sex using blood stains: (1) The presence and detection of sex chromatin (Barr bodies in females and F bodies in males) and (2) sex hormone level determinations based on detectable quantities and ratios of testosterone and 17 β -estradiol by RIA. The former parameter has been demonstrated successfully in saliva stains.^[9]

CONCLUSION

The importance of saliva as an investigative body fluid is increasing steadily over the years in forensic laboratories. These laboratories are required to have automated settings for saliva as is routinely done for blood or urine. Safety in its handling, the ease and noninvasive methods of saliva collection has gained popularity in the field of forensic testing for drugs of abuse. Sex determination and individualization of accused in scenes of crime with the help of salivary exfoliated cellular examination and DNA profiling is proving to be of immense help in forensic investigations. More research dedicated towards this particular innocuous body fluid should be aimed at for gaining detailed information in forensic sciences.

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Conflicts of interest

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