

## Application of Saliva Samples in Forensics: a Brief Update

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### Abstract

Salivary detection and analysis is playing an increasingly important role in the field of forensic sciences. Its benefits include ease of collection based on non-invasive methods, safety in handling and high similarity of constituents with those of plasma. It has a wide range of applications in forensics and this update is aimed to highlight the same.

**Keywords:** Saliva; DNA; Forensics.

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### Introduction

Teeth and saliva are considered as the primary biologic materials of significance in forensic dentistry. Over the recent years, growing interest has been shown in diagnostic utility of saliva as an alternative to blood or urine. Serological and cellular analysis of collected saliva is of immense use in identification of criminals. The main benefits of salivary analysis include noninvasive method of collection and avoidance of intrusion of private functions which can be performed even by novice investigators [1].

However, certain drawbacks do exist in salivary analysis including difficulty in collecting dried stains from skin, clothing, paper or other inanimate objects due to limited visibility. In addition, substrates with saliva deposits mainly such as skin cannot be submitted directly to examination procedures [2]. The importance of saliva in forensic odontology and its related research are highlighted in this brief update.

### Methods of Detecting Dried Saliva

Saliva can be collected from potential sources such

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as bite marks, cigarette butts, postage stamps, envelopes and other objects. The dried salivary stains on the skin are reasonably stable and, if the environmental conditions are not too extreme, can be detected and collected months or years after the deposition. However, the stains of the dried saliva are invisible, making its identification and collection laborious. Several methods can be employed in detecting saliva stains: -

1. *Chemicals:* Commonly used enzymes for saliva detection include alkaline phosphatase and amylase. Particularly, amylase detection has been used as a presumptive test for locating crime scene saliva stains. Salts like nitrate and thiocyanate can also be used for the detection by methods such as gas chromatography-mass spectrometry. All these methods have limitations and variable sensitivity depending upon the age of the saliva stain and quantity of deposit [2,3].
2. *Light Sources:* Lasers and ultraviolet light, quartz arc tube and argon ion laser can be utilized for saliva stain detection. Stains generally appear bluish-white when being viewed under an ultraviolet light, although it cannot be clearly differentiated from other bodily fluids. High intensity quartz arc tube was observed to be superior to argon ion laser based on portability, cost, sensitivity, and power output. However, they had a limited lifetime compared to other light sources [4,5].

3. *Fluorescent spectroscopy*: The aromatic amino acid, tryptophan, in  $\alpha$ -salivary amylase gives a characteristic emission spectrum on fluorescent spectroscopy, thus possessing a good sensitivity in detecting dried saliva stains on the skin [6].
4. *Scanning electron microscopy with energy dispersive X-ray analyzer*: This method can be used as for screening purposes by identifying the relative concentrations of metal trace elements in the questioned sample. In the saliva samples tests, potassium was the largest peak and can be used as a basis of identification [7].

### Saliva Collection

Swabbing is the preferred over other methods of saliva sample collection in forensics. Swabs are convenient for collecting and releasing samples without altering their integrity. The classical technique for saliva collection is using a single wet cotton swab or section of wet filter paper laid passively on the skin [7].

To improve the quality of DNA collection a double swab technique can be employed wherein the first swab is moistened with water, buffered saline, or lysis buffers thereby allowing dried samples to become rehydrated. This is followed by a dry cotton swab to collect the remainder of the sample [8].

### Application of Saliva Samples in Forensics

#### *DNA Profiling/Fingerprinting*

Every individual has a unique DNA profile that can help in identifying perpetrator of the crime. Particularly, cells derived from saliva contain mitochondrial DNA (mtDNA) which can assist in identification. mtDNA can be easily profiled owing to a high copy number due to abundance of mitochondria in most cells. Chromosomal DNA is inherited from both the mother and father whereas mtDNA is strictly maternally inherited. Hence, mtDNA testing may be successful when nuclear DNA testing fails or when genomic DNA cannot be analyzed, possibly because it is too degraded [9].

Preservation of collected sample is of paramount importance as 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. Thus, even if immediate families are not available, distant maternal relatives can be used as a reference source to support identification [10].

### Drugs of Abuse Analysis

Saliva is preferable over urine samples for drugs of abuse determination due to its advantages of easy, rapid and noninvasive sampling methods. Amphetamines, barbiturates, benzodiazepines, phencyclidine, cocaine and opioids, etc. can be detected in samples. Saliva can also be used to detect marijuana use by means of radioimmunoassay techniques [11].

Most of the drugs enter saliva by simple passive diffusion based on transfer of drug molecules through a concentration gradient. Saliva is currently being used in the measurement of steroid hormones such as cortisol, cortisone and testosterone based on the fact that salivary levels of these steroids reflect the free, unbound circulating fraction [12].

### Age and Sex Determination

DNA methylation is a major control program of gene expression based on gene silencing through the activity of DNA methyltransferases. Particularly, DNA strands demonstrate age-related methylation changes including genome wide hypomethylation (non-CpG islands) and regions of hypermethylation (primarily CpG islands). Methylation is strongly correlated with aging process and epigenetic alterations have been directly linked to longevity in several species [13].

Recently, researchers at University of California developed a method based on DNA methylation in saliva samples that can estimate age of an individual within a range of five years.

Vilain et al analyzed DNA from saliva samples of 34 pairs of male identical twins between 21 and 55 years of age as compared to 31 men and 29 women aged 18 to 70. Results showed age estimation of an accuracy within 5.2 years with 73% of the variance in age. They concluded that this procedure helps in determining the biological age more accurately rather than the actual age [14].

Presence of exfoliated buccal epithelial cells in saliva samples has increased the possibility of sex determination of the suspect. Gender identification can be based on detecting F body of Y chromosome (specific to male) and sex chromatin (specific to female) in oral epithelial cells. Also, detection of sex hormone levels specific to males (testosterone) and females (estrogen) in saliva can help in confirmation of gender. Muro et al. used Raman spectroscopy and multivariate data analysis in developing a technique for sex determination. They could correctly identify sex in 44 (92%) of the 48 donors used [15].

### Animal Bite Mark Analysis

DNA isolated from saliva swab samples of bite marks can be used for species identification. This method requires a high copy number of mitochondrial DNA to be present in the sample which is analyzed based on polymerase chain reaction (PCR) and species-specific primers. Bite mark analysis can be utilized for identifying the predator in attacks on cattle and human beings [16].

### Conclusion

Saliva is gaining popularity as an important aid in forensic sciences owing to its ease of collection and analysis. Newer techniques are being applied that can be used to thoroughly study salivary genetic and chemical composition for obtaining detailed and accurate information thus helping in solving cases.

### References

1. Bhandari R, Kakkar T, Bhullar RK, Bansal H, Sandhu SV, Jassar S. Saliva- An adjunct in forensic odontology. *BFUDJ* 2011;2:144-7.
2. Hedman J, Dalin E, Rasmusson B, Ansell R. Evaluation of amylase testing as a tool for saliva screening of crime scene trace swabs. *Forensic Sci Int Genet.* 2011 Jun;5:194-8.
3. Paul BD, Smith ML. Cyanide and thiocyanate in human saliva by gas chromatography-mass spectrometry. *J Anal Toxicol.* 2006 Oct;30:511-5.
4. Auvdel MJ. Comparison of laser and ultraviolet techniques used in the detection of body secretions. *J Forensic Sci* 1987;32:326-45.
5. Auvdel MJ. Comparison of laser and high-intensity quartz arc tubes in the detection of body secretions. *J Forensic Sci* 1988;33:929-45.
6. Nanda KD, Ranganathan K, Umadevi K, Joshua E. A rapid and noninvasive method to detect dried saliva stains from human skin using fluorescent spectroscopy. *J Oral Maxillofac Pathol* 2011;15:22-5.
7. Mittleman RE, Stuver WC, Souviron R. Obtaining saliva samples from bite mark evidence. In: *Law Enforcement Bulletin*. Washington, DC: Federal Bureau of Investigation; 1980.
8. Sweet D, Lorente M, Lorente JA, Valenzuela A, Villanueva E. An improved method to recover saliva from human skin: The double swab technique. *J Forensic Sci* 1997;42:320-2.
9. Hutchison CA 3rd, Newbold JE, Potter SS, Edgell MH. Maternal inheritance of mammalian mitochondrial DNA. *Nature* 1974;251:536-8.
10. Stavrianos C, Eliades A, Kokkas A. The role of DNA in forensic odontology (Part 1) DNA analysis methods. *Res J Med Sci* 2010;4:334-9.
11. Vindenes V, Lund HM, Andresen W, Gjerde H, Ikdahl SE, Christophersen AS, et al. Detection of drugs of abuse in simultaneously collected oral fluid, urine and blood from Norwegian drug drivers. *Forensic Sci Int* 2012;219:165-71.
12. Cone EJ. Saliva testing for drugs of abuse. *Ann N Y Acad Sci* 1993;694:91-127.
13. Johnson AA, Akman K, Calimport SR, Wuttke D, Stolzing A, de Magalhaes JP. The role of DNA methylation in aging, rejuvenation, and age-related disease. *Rejuvenation Res.* 2012;15:483-94.
14. Daniel J. DeNoon. Hiding Your Age? Don't Spit. <<https://www.webmd.com/healthy-aging/news/20110622/hiding-your-age-dont-spit>>.
15. Muro CK, de Souza Fernandes L, Lednev IK. Sex Determination Based on Raman Spectroscopy of Saliva Traces for Forensic Purposes. *Anal Chem.* 2016 Dec 20;88:12489-12493.
16. Christen LW, Karen B, John J; Michael MK. A coyote in sheep's clothing: predator identification from saliva. *USDA National Wildlife Research Center - Staff Publications.* 2003.p.288.

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Indian Journal of Ancient Medicine and Yoga	Quarterly	8000	7500	625	586
Indian Journal of Anesthesia and Analgesia	Monthly	7500	7000	586	547
Indian Journal of Biology	Semiannual	5500	5000	430	391
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Indian Journal of Forensic Medicine and Pathology	Quarterly	16000	15500	1250	1211
Indian Journal of Forensic Odontology	Semiannual	5500	5000	430	391
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Indian Journal of Trauma & Emergency Pediatrics	Quarterly	9500	9000	742	703
Indian Journal of Waste Management	Semiannual	9500	8500	742	664
International Journal of Food, Nutrition & Dietetics	Triannual	5500	5000	430	391
International Journal of Neurology and Neurosurgery	Quarterly	10500	10000	820	781
International Journal of Pediatric Nursing	Triannual	5500	5000	430	391
International Journal of Political Science	Semiannual	6000	5500	450	413
International Journal of Practical Nursing	Triannual	5500	5000	430	391
International Physiology	Triannual	7500	7000	586	547
Journal of Animal Feed Science and Technology	Semiannual	78500	78000	6133	6094
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