10.5005/jp-journals-10037-1136

REVIEW ARTICLE

Application of Saliva in Forensics

[1]Shanly J John, [2]Divya Rajaji, [3]Dafniya Jaleel, [4]Arun Mohan, [5]Neethu Kadar, [6]Vidya Venugopal

ABSTRACT

Introduction: Saliva is a complex body fluid, which is emerging as a popular source of forensic evidence. They act as a source of valuable deoxyribonucleic acid (DNA) samples. This, along with its ease of collection and handling, has increased its popularity as a forensic tool for crime detection, in cases of poisoning, animal bites, drug and alcohol abuse, and hormone identification.

Objectives: To assess the different methods used for detection and recovery of saliva, and to analyze the various applications of saliva in forensics.

Materials and methods: In this review, PubMed was searched for relevant English papers from 1981 to 2017.

Conclusion: Saliva has recently attracted much attention among researchers, particularly in the branch of forensic sciences. More research should be dedicated for this innocuous body fluid for acquiring comprehensive information regarding its application in forensic sciences.

Keywords: Forensic odontology, Forensic tool, Saliva.

How to cite this article: John SJ, Rajaji D, Jaleel D, Mohan A, Kadar N, Venugopal V. Application of Saliva in Forensics. Oral Maxillofac Pathol J 2018;9(2):85-87.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Recovery of traces of body fluids is one of the most important types of forensic evidence.

They contain valuable DNA samples which may help in identifying an accused or a victim. Saliva is a complex body fluid which plays a critical role in forensic evidence.[1] Saliva may get deposited on skin in instances of biting, sucking, or licking and can act as a significant source of forensic evidence. In recent years, saliva is attaining popularity because of the easiness in collection, safety in handling, and its close similarity with plasma.[2]

The current article is intended at emphasizing the application of saliva in forensic odontology.

[1,3,6]Postgraduate Student, [2]Lecturer, [4]Senior Lecturer, [5]Reader

[1-6]Department of Oral Pathology and Microbiology, Royal Dental College, Palakkad, Kerala, India

Corresponding Author: Shanly J John, Postgraduate Student Department of Oral Pathology and Microbiology, Royal Dental College, Palakkad, Kerala, India, Phone: +918547490171 e-mail: shanlyjjohn@gmail.com

Advantages of Use of Salivary Sample in Forensics

Saliva shows some benefits over blood as source of DNA as follows: (1) Noninvasive, (2) can be collected easily, and (3) comparatively safer to blood which has higher potential risks of contamination, especially from hepatitis and acquired immunodeficiency syndrome which require needles for its collection.[3]

Methods of Detection of Saliva

It is not easy to detect and recover saliva stains from the crime scenes because dried saliva stains are undetectable to the bare eye. Saliva stains can be present on skin, clothing, paper, or other inanimate object. The DNA contained in the saliva is difficult to collect and extract from the skin, as the substrate on which saliva is present cannot be directly submitted to extraction processes. An advanced collection method is needed to discover the undetectable saliva stains on human skin.[4]

There are diverse methods for detecting saliva stains.[1] Various chemicals and enzymes have been tested to identify dried saliva stains. Saliva detection from envelopes and stamps can be done by chemicals that react with reducing sugars giving a red insoluble precipitate. Most frequently used enzymes are alkaline phosphatase, starch, and amylase. Use of these chemicals in saliva detection has limitations.

Alkaline phosphatase test is not very specific; it can give false-positive result. Iodine test or starch test for detecting salivary amylase enzyme gives a negative reaction, leading to false-positive result if excess starch is present. Nitrate and thiocyanate salts have also been used for the same purpose but the main drawback of this test is that it is applicable for samples only up to 2 days.

Thiocyanate is not commonly present in saliva. Thus, all these methods have variable sensitivity and limitations depending on the age and amount of the saliva.[2] Lasers, ultraviolet light, quartz arch tube are used as a screening technique in identifying dried saliva stains.[3] Fluorescence spectroscopy is a technique which is widely used to analyze structure, dynamics, and functional interactions of proteins.

Its principle is that when a fluorescent material is excited using radiation of a particular wavelength, it emits back radiation of a longer wavelength which can be recorded.

Salivary amylase contains an aromatic amino acid component called tryptophan. Tryptophan, when excited with radiations of 282 nm wavelength, gives a unique emission spectrum of 345 to 355 nm. It possesses good sensitivity in identifying dried saliva stains on the skin.

Recovery of Saliva from Skin

Traces of salivary evidence should be recovered since it is useful for identity testing.[2] Only a very minute amount of saliva will be deposited on the skin with bite marks. Proper collection methods will help in the revival of the maximum attainable quantity of cells in the saliva.

Also any potential contamination of the cells from the victim's skin will be reduced. Due to exposure to air, the saliva is dried out and most of the desquamated epithelial cells and leukocytes in the saliva are desiccated.[5] Following are the techniques used for recovering saliva from the skin.

Single Swab Technique

This is the classical method. In the single swab technique, a wet cotton swab or a wet filter paper is used to retrieve saliva traces from the skin.

Double Swab Technique

Two sterile cotton swabs and 3 mL of sterile, distilled water is required for this technique. The procedure is as follows: (a) Soak the cotton swab in sterile distilled water. (b) Roll the cotton swab over the suspected area by applying slight pressure and by following a circular motion. (c) Allow this first swab to dry in a contamination-free environment for a minimum of 30 minutes. (d) Within 10 seconds after completing the first cotton swab, roll the second dry swab across the now moist area. (e) Apply moderate pressure to absorb the wetness from the skin into the second swab. (f) Allow the second swab to dry in a contamination-free environment for a minimum of 30 minutes. (g) After drying, pack and seal both swabs together, and mark with sample and case numbers.[6] This study was conducted by Sweet et al.[5]

They found that the double swab technique is a better method for yielding saliva from the skin surface. This may be because moisture from the first swab will rehydrate and loosen majority of the epithelial cells dried within the saliva which will then adhere to fibers present in the cotton swab.

On the application of the second (dry) swab, the cells in the saliva adhere to the fibers more easily as they are rehydrated. Thus, this technique allows collection of a larger amount of DNA sample than the classical method.[5]

Role of Saliva DNA Isolation

Salivary DNA can be recovered from different nonliving objects including clothing, foods, tobacco products, drink containers, oral hygiene devices, stamps, dental prostheses, envelopes, etc.

The ability to obtain forensic DNA data from salivary evidence was described in 1992, but the standard of practice for salivary DNA collection from skin was truly established when Sweet et al[5] published the double swabbing technique. Deoxyribonucleic acid is isolated from the saliva sample using phenol–chloroform method. Phenol–chloroform extraction is a liquid–liquid extraction technique used for separating nucleic acids from proteins and lipids.

Aqueous samples, homogenized tissue, or lysed cells are mixed with equal volumes of phenol–chloroform mixture. The mixture is then centrifuged. Two distinct phases are formed since the mixture is immiscible in water. Phenol–chloroform mixture, which forms the organic phase, will sediment below the aqueous phase due to their higher density. Nucleic acids will remain in the aqueous phase, while the protein and hydrophobic lipid content will settle down into organic phase.

The upper aqueous phase is separated by pipetting it out.[7]

Sex Determination

Saliva contains epithelial cells, which are exfoliated from oral mucosa, mostly buccal mucosa. These cells have increased the application of saliva for 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. Based on successful results to identify the sex using blood stains, two parameters have been proposed.

The first one is the detection of sex chromatin: Barr bodies in females and F bodies in males. The second one is the determination of sex hormone levels. It is done based on the detectable quantities and ratios of 17 B-estradiol and testosterone.[2]

Detection of Drug Abuse

Salivary concentrations of a particular drug can normally be directly correlated to the concentration of the same drug in blood. Drugs from the blood stream enter saliva through simple diffusion.[8]

Drug wipe technique is a method used to monitor the presence of drug in saliva. Drug Wipe is a test used to wipe surfaces for recovering traces of drug residue. It maybe used for testing both sweat and saliva of

individuals. The test is done by wiping a small wet wiping fleece on the suspected site. It can be used to detect the presence of drugs like cannabis, cocaine, crack cocaine, heroin, morphine, and benzodiazepines.

Immunoassay strips are used, which contain antibodies that bind with a specific component of a drug.[9] Studies have revealed that various drugs like amphetamine, phenobarbital, and morphine can be detected in saliva using radioimmunoassay. Saliva also helps to measure the steroid hormones levels.[3]

Saliva in Bitemark Analysis

Salivary animal bite mark analysis—Fletcher et al[10] suggested that salivary stains up to 16 months old can be used for species identification, with application of enzyme-linked immunosorbent assay technique using monoclonal antibody based on the presence of salivary immunoglobulin A. In cases with insufficient monoclonal antibody results, double gel diffusion techniques and crossover electrophoresis can be used for comparison. This technique is helpful in the examination of bitemarks from nonhumans when the biting animal is not known.[2]

Salivary Biomarkers in Forensics

Salivary biomarkers in forensics Saliva biomarkers are also used in cases involving bitemarks for general identification of people and for victim identification in the instances of mass disasters. First, the hospital records of the victims are tracked down to check for the history of any particular disease. The salivary biomarkers for those particular diseases are then used for a positive identification from the traces of saliva samples obtained. These biomarkers may also be used to identify victims whose DNA data are not available on any existing DNA database.[8]

CONCLUSION

Saliva has recently attracted much attention among researchers, particularly in the branch of forensic sciences. Serological testing and analysis of salivary cellular component are being increasingly applied in crime detection, hormone identification, drug and alcohol abuse, cases of animal bites, and poisoning.

Safe handling, along with the easy and noninvasive procedures of saliva collection, has increased its popularity in drug abuse tests. Sex determination and identification of the suspects in scenes of crime, with the help of cytological analysis and DNA profiling of saliva, are finding widespread applications in forensic investigations.

More research should be dedicated for this innocuous body fluid for acquiring comprehensive information regarding its application in forensic sciences.

REFERENCES

1. Virkler K, Lednev IK. Analysis of body fluids for forensic purposes: from laboratory testing to non-destructive rapid confirmatory identification at a crime scene. Forensic Sci Int 2009 Jul;188(1-3):1-17.
2. Saxena S, Kumar S. Saliva in forensic odontology: a comprehensive update. J Oral Maxillofac Pathol 2015 May-Aug; 19(2):263-265.
3. Mago J, Sidhu L, Kaur R, Kamal, Anurag T. Saliva in forensics. World J Pharm Med Res 2016;2(4):196-198.
4. 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 Jan;15(1):22-25.
5. 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 Mar;42(2): 320-322.
6. Stimson, PG.; Mertz, CA. Forensic dentistry. Boca Raton (FL): CRC Press, LLC.; 1997.
7. Kramvis A, Bukofzer S, Kew MC. Comparison of hepatitis B virus DNA extractions from serum by the QIAamp blood kit, GeneReleaser, and the phenol-chloroform method. J Clin Microbiol 1996 Nov;34(11):2731-2733.
8. Aps JK, Martens LC. Review: the physiology of saliva and transfer of drugs into saliva. Forensic Sci Int 2005 Jun;150(2-3):119-131.
9. Gentili S, Solimini R, Tittarelli R, Mannocchi G, Busardò FP. A study on the reliability of an on-site oral fluid drug test in a recreational context. J Anal Methods Chem 2016 Aug; 2016:1234581.
10. Fletcher SM, Dolton P, Harris-Smith PW. Species identification of blood and saliva stains by enzyme-linked immunoassay (ELISA) using the monoclonal antibody. J Forensic Sci 1984;29:67-74.