Review Article

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"DIAGNOSTIC APPLICATIONS OF SALIVA IN FORENSIC ODONTOLOGY - AN UPDATED REVIEW"

Dental Science

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ABSTRACT

In the past few years saliva has gained much interest as a diagnostic tool for systemic diseases, oral diseases and in forensic investigations. The main advantages include the ease of collection and non-invasive techniques of sample collection. The analysis of salivary biomarkers acts as a boon in the forensic investigative crime scenes. The salivary analysis has wide uses in the alcohol and drug abuse, cases of poisoning, and animal bites.

KEYWORDS

Forensic Sciences, Forensic Odontology, Diagnostic tool, Saliva.

Introduction:

Forensic medicine is the scientific method of law enforcement in relation to criminal or civil liabilities. The forensic genetics a sub discipline of forensic medicine which concerns with the identification and analysis of biological material that provides probative information for law enforcement investigators [1]. A genetic analysis of such biological material by the forensic biologist may aid in the inclusion or exclusion of a particular individual associated with the crime. The different types of biological material encountered at crime scenes are body fluids such as blood, semen, saliva, vaginal secretions and a variety of other tissues such as hair, teeth, bone, muscle, and adipose tissues [1].

Forensic dentistry is the application of dental knowledge to those criminal and civil liabilities in a criminal judiciary systems. Forensic dentistry deals with proper handling, examination and evaluation of dental evidence in criminal liabilities, which will be presented in the interest of justice. Teeth and saliva are considered as the main biologic materials in forensic dentistry [2].

Saliva is usually deposited on bite marks found in many homicides, assault and other criminal scenarios. The bite mark analysis on the skin presents multiple practical because of elastic and distortable nature of skin and lack of good impression medium, the saliva deposited on bite marks has received an important alternative focus of interest [2,3]. It is difficult to collect saliva stains from skin, clothing, paper or other inanimate objects as it remains invisible and substrate on which saliva is deposited mainly the skin surfaces, cannot be submitted directly to examination procedures [3].

Methods of salivary collection from the skin:

Besides the physical evidence present on the bite marks, the saliva acts as a biological evidence that can assist the criminal investigations [4]. During the biting process, saliva will be deposited on the skin or object surface in vast amounts to allow typing of the deoxyribonucleic acid (DNA). For this purpose, the bite mark area of the skin surface is swabbed using a standard bite mark operating procedures, and DNA can be extracted and analysed during criminal investigations [5]. The classical technique of using a single wet cotton swab or a section of wet filter paper touched passively on the skin of the victim can be used to collect the saliva from the skin.

The double swab technique is very effective for DNA collection and analysis in dried salivary stains of victim's skin [4,5]. In this method, the first swab is immersed in sterile distilled water to wet the cotton tip completely. The tip is then rolled over the surface of the skin using a moderate pressure in circular motions. Rotating the swab on its long axis aids in attaining the maximum contact between the swab and the skin to wash the dried saliva from the surface. The swab is then set aside to air dry completely for more than thirty minutes.

Then, a dry second swab is taken and using similar pressure and movements as that of first swab, the dry tip is rotated over the skin to recover the remaining moisture on the skin's surface from the wet swab. The dry swab is rolled over entire area to ensure that all of the moisture is recovered and set aside to air dry completely for thirty minutes. As the swabs are collected from the same site, they are then pooled together into a single sample. After sample collection, the filter paper discs, the single and double swab samples are labelled and stored at 4°C for DNA extraction and quantitation [5].

It is possible to recover a vast number of cells using the double swab technique. It is believed that this is due to the fact that the moisture left from the first swab rehydrates and loosens the majority of the epithelial cells from the skin of dried saliva and releases them to adhere the cotton fibers of the swab [5]. When the second dry swab is applied to the specific skin, the cells from the dried saliva are able to adhere to the cotton fibers more easily because they are rehydrated after application of the moisture from the first cotton swab.[11] However, the quantity of useful DNA strands obtained in a salivary samples with the double swab technique decreases with the time, especially during the first 24 hours after deposition on the skin [4,5].

Persistence and location of saliva on evidence:

Saliva can potentially be collected from bite marks, cigarette butts, postage stamps, envelopes and other objects. The stains of the dried saliva are invisible, making its identification and collection laborious [6]. Saliva also found commonly in sexual assault cases that involves the oral intercourse and it is often admixed with other body fluids such as vaginal secretions or semen. 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, a simple wash cycle with biological detergents can remove all the traces of the dried saliva stains from the victim's skin [7].

Analysis of salivary components:

The collected salivary samples from the victim's skin consist a variety of enzymes such as alkaline phosphatase, amylase and the inorganic anions such as thiocyanate and nitrite. In forensic investigations, mainly the amylase activity testing has been used to locate saliva stains on skin surfaces for over three decades [8]. Irrespective of technological advancements, there is no definitive test for the positive identification of saliva present till date. Presently, a screening method based on amylase activity testing is useful to lower the number of negative DNA samples from crime scenarios [4].

The presence of significant numbers of nucleated buccal epithelial cells in saliva, serves as an excellent source of DNA from which a genetic profile of the donor is easily analysed. This can be accomplished using standard DNA isolation methods followed by the

routine short tandem repeats (STR) DNA analysis of the resulting extracts. DNA typing with short tandem repeat (STR) markers is now extensively used for a variety of applications in forensic and clinical identification procedures [9]. Polymerase chain reaction (PCR) allows the study of small amounts of DNA by replication of thousands of copies of a specific DNA sequence in vitro. The polymorphic repair of short tandem repeats (STR) in small fragments also makes it possible to analyse the DNA from samples with a significant grade of degradation [9].

Salivary genomic applications in forensic investigations;

In recent years, the development of new assays that are compatible with the current polymerase chain reaction (PCR) based DNA analysis techniques has gained a vast popularity. Specifically, the development of mRNA based assays for the identification of body fluid stains has gained acceleration, with new research emerging at a steady pace every year. In addition to the current DNA analysis procedures, mRNA based approaches offer additional advantages over the conventional methods of body fluid identification and analysis [10].

The potential advantages of using RNA based approaches for body fluid staining or tissue identification includes greater specificity, time saving, reduced sample consumption, simultaneous analysis through a common assay format, and compatibility with the current DNA analysis procedures. Messenger RNA based methods show great potential for providing a reliable and sensitive approach to body fluid stain identification [11].

Sex determination from the salivary samples:

The sex determination from the salivary samples has been gaining importance in the recent years. The Norgen Biotek Corporation has developed an unique and simple method for the collection, preservation, storage and purification of DNA collected from the saliva using individual saliva DNA collection and preservation devices. The salivary samples from the suspected individuals are collected directly the collection tube and Norgen's Saliva DNA preservative will be added to the collected sample [12].

The preservative is an aqueous storage buffer specifically designed for rapid cellular lysis and subsequent preservation of saliva DNA from fresh collected specimens. This buffer system stabilizes the DNA for long-term storage at ambient temperatures. The buffer prevents the further growth of microorganisms, inactivates viruses and it also allows the samples to be handled and transferred safely. The DNA subsequently isolated from the preserved samples is of a high quality and can be used directly in diagnostic assays such as PCR. Thus, the saliva DNA isolated using Norgen's Saliva DNA collection, preservation and isolation kit is of a high quality and it can be used for as the template in nested PCR reactions for sex determination in forensic and clinical applications [12].

Analysis of abusive drugs from saliva;

The analytical applications of saliva for determination of different abusive drugs holds the advantages like easy, rapid and nonintrusive sampling procedure over the conventional urine analysis [13]. The abusive drugs that can be analysed in the salivary samples are amphetamines, barbiturates, benzodiazepines, phencyclidine, cocaine and opioids. Saliva can also be used to detect marijuana use by means of radioimmunoassay techniques [14]. The studies have proven that most of the drugs enter saliva by simple passive diffusion which is characterized by the transfer of drug molecules through a concentration gradient [13]. The salivary drug concentrations are generally the reflection of the free fraction of the drug in the blood stream [13,14].

Conclusion:

The significance of saliva as a diagnostic fluid is gaining the importance over the last few years rapidly. The advantages like ease of use, non invasive methods of collection has gained the popularity in the forensic investigative scenarios. The varied diagnostic applications like determination of abusive drugs, sex determination of individuals in the crime scenes aid an immense significance. The more evidence based research on the saliva as a diagnostic medium in crime scenes further strengthens the forensic investigative procedures.

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