Review Article

Saliva as a forensic tool

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Abstract

Forensic science is a branch that deals with a wide plethora of areas such as anthropology, migration studies and criminology. Various biological samples have been utilized to assist a scientist towards getting answers to the myriad of questions in the field. Saliva is an easily available source from victim as well as aggressors, parent-child and siblings. Various tests have been devised to aid in identifying salivary sample constituents. This paper deals with the wide utility of saliva as a forensic tool.

Key words: Criminology, forensic, saliva

Introduction

Saliva is a complex biological fluid secreted by acinar cells of the major and minor salivary glands. It is an indicator of various plasma constituents. In recent years, its role as a diagnostic and forensic tool is being increasingly researched upon and evaluated. Besides maintaining the homeostasis of oral structures such as tooth integrity, it also plays a critical role in genomics, proteomics, metabolomics, and bioinformatics. It is an important discriminating element in forensic biology, acting as an indicator of salivary gland conditions and toxicological and drug monitoring.[1]

Identification of Saliva Sample

Tests utilized for the identification of saliva are of two types: presumptive and confirmatory tests. Presumptive test can establish the possibility that a specific bodily tissue or fluid is present, whereas confirmatory test can identify a specific biological material. Screening tests are used to evaluate evidence in determining the possible presence of controlled substances, hormones, and genetic material. Both of these tests can be applied in the salivary samples.

Salivary amylase and saliva detection tools

α-Amylase is a salivary constituent. Its physiological role is in starch digestion. It exists in two isoenzymatic forms – salivary and pancreatic. The RSID™ saliva flow is a lateral flow immunochromatographic strip designed to detect the salivary α-amylase in a field sample, hence confirming the presence of saliva in a sample. However, due to limited production, SALIgAE® test (ABACUS Diagnostics Incorporated) has been introduced as a screening test for saliva. Unlike the old Spotty paper test, the SALIgAE® spray test requires no incubation or additional equipment. Polilight® (PL500 model wavelength) can be used to identify areas of contamination that can interfere with saliva detection using 470–555 nm interference filters. Saliva fluorescence using Polilight® is of weak nature; hence, a grading system proposed by Casey and Price *et al.* (2004) can be used: 0 – no fluorescence detectable, 1 – very weak fluorescence, barely visible, and 2 – weak fluorescence.[2] Biodegradable starch microspheres can also be used for saliva detection. These microspheres are covalently bound to a blue dye that is released when α-amylase is present in the sample. However, this test lacks specificity as it cannot differentiate between α-amylase 1 (present in the saliva) and α-amylase 2 (present in the semen and vaginal secretions).

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Another significant disadvantage is the loss of sample for subsequent DNA analysis. Mass spectrophotometry is a technique that can identify pure as well as mixtures of biological matrices of different species origin without destroying any DNA. Hence, the complete sample is available for DNA extraction.[3]

Saliva in Addiction Biology

The detection of psychotropic drugs and ethanol levels in the saliva as a road-side procedure is a major boost in aiming toward better control of road and traffic safety. Cannabis has been found to be the most prevalent narcotic (78%) through saliva testing. Corresponding serum samples have also been found to be positive for tetrahydrocannabinol (THC, 70%). The overall sensitivity and specificity in narcotic analysis are in the range of 91%–98%. Other narcotic agents that can be detected either as a metabolite or the drug itself include amphetamine, cocaine, opiates, and cannabinoids.[4] Established tests for THC acid (11-nor-9-carboxy-9-THC) antibodies in urine samples do not test positive in oral fluids. THC is only detectable in the saliva samples after long hours of smoking cocaine (approximately 10 h) [Table 1]. Hence, salivary detection is due to contamination due to smoking rather than by it being secreted in the oral cavity by saliva.[5,6]

Thiocyanate (SCN) is usually present in the serum, saliva, and urine in low concentrations. It is the principal metabolite product of cyanide (CN) metabolism. High CN concentration can arise from tobacco smoking and metabolic conversion to hydrogen CN. SCN concentration in the saliva can be analyzed by gas spectrometry. It is an important parameter in classifying patients as smokers and nonsmokers, in the determination of certain clinical conditions, and in forensic drug testing.[5] Saliva is not an ideal specimen for postmortem testing for ethanol due to contamination or dry oral cavity conditions postmortem. Vitreous humor is considered to be the ideal specimen for postmortem ethanol analysis since it does not contain glucose or any microorganisms for postmortem metabolism to ethanol.[6]

Extracellular Nucleic Acid Detection

Extracellular nucleic acids serve as cancer biomarkers and are detectable in a variety of biological fluids including breast milk, semen, saliva, urine, and plasma as well as supernatants in cell cultures.[7] Juusola and Ballantyne used real-time polymerase chain reaction (RT-PCR) and gel electrophoresis techniques for the detection of mRNA specific from saliva stains as old as 10 weeks. Statherin and histatin 3 are the indicators used for salivary detection.[8] Salivary RNA detection is an emerging field in forensic molecular diagnostics. RNAs in the saliva are protected by specific mechanisms such as salivary mucins, adenine and uridine-rich element binding protein, salivary chaperone

Table 1: Number of cases with detection of tetrahydrocannabinol and tetrahydrocannabinolic acid in serum and oral fluid[5]

	Number of cases
THC and in serum	97
THC in oral fluid	89
Oral fluid negative	8
THCA in serum only	40
THC in oral fluid	6
Oral fluid negative	34
Serum negative	40
THC in oral fluid	1
Oral fluid negative	39

THC: Tetrahydrocannabinol, THCA: Tetrahydrocannabinolic acid

Hsp70, and apoptotic bodies. Exosomes also play a critical role in protecting the salivary transcriptomes. These are vesicles meant for transfer of intercellular mRNA, thereby protecting salivary mRNA stability in the presence of extracellular RNAases. mRNA detection from saliva sample is made possible by heat treatment at ≥60°C at the beginning of RT-PCR preamplification procedure. Heat disrupts the hydrogen and nonpolar hydrophobic bonds between RNA molecules and proteins. Exosomes are derived from endosomal membrane compartment within a lipid bilayer structure. When exposed to high temperature, the lipid bilayer transforms to a fluidic state after which the encapsulated mRNAs can be released.[9]

DNA is an important salivary biomarker in forensic science. There is >99% homology in DNA sequences of two individuals, but there might be millions of genetic differences in some DNA segments. The most important extraneous factors responsible for these genetic variations are environmental influences and mutations. Copy number variations (CNV) are large genomic regions that may be absent or duplicated in different persons. Single nucleotide polymorphisms (SNPs) are single base differences among two organisms of the same species. In humans, SNPs are found to occur at an average of every 1/2000 base pairs. Collectively, CNV and SNP constitute the most important sources of genetic variations in the human genome.[9]

microRNAs (miRNAs) can also be used to test different biological matrices present within a sample. However, the detection of each miRNA must be performed in different wells. DNA methylation is a technique based on tissue-specific methylation patterns. This technique gives operator-independent results and can be multiplexed with existing short tandem repeats (STRs) typing protocols without additional sample loss. Loss of function of proteins can lead to false negative in the current commercial kits. The primary structure of proteins is highly stable and its sequence can be identified by mass spectrometry after hundreds of years which make it suitable for forensic

application. Other protein identification tools include fluorescence spectroscopy and Raman spectroscopy.[3]

Human genome is marked by repetitions at sequence length scales, number, and dispersion. Examples of such repetitions are homo- and di-nucleotide repeats (microsatellites) and alternative locus units (ALU) sequences (families of interspersed mobile elements which are hundreds of base pairs long). There are >1 million ALU sequences in human genome (300 bp long) which are able to copy themselves in various parts of the genome, generating mutations. Forensic DNA typing uses various techniques for identification of such genetic alterations. Variable number of tandem repeats (VNTRs) loci are composed of core units that 3, 4, or 5 nucleotides long and the number of repeated segments at each locus also varies. VNTRs are identified by slicing genomic DNA with restriction enzymes such as HaeIII, HinfI, or HindIII, electrophoretic separation of DNA fragments, and detecting various fragments by the use of DNA probes. Most recently, VNTR identification has been replaced by STR. DNA typing using STR is done by matching 13–17 of nuclear STR markers of a victim's profile to an antemortem sample of a victim or to family members (biological parents). A system of 13 STR markers constitutes the combined DNA Index System which is used in the USA and Canada, while the European countries have their own database. Severely degraded DNA contains a very small amount of very short DNA template molecules (<150 bp) making conventional STR typing (which uses 150–400 bp) unsuccessful.[9] Use of multiplex PCR involves using several sets of PCR primers to the reaction allowing higher amplification. Currently, STR multiplex systems have high sensitivity (matching probability) as compared to combination of five classical single locus DNA fingerprints. Although multiplexing techniques save time and finances, coamplifying optimum PCR conditions (annealing temperature and primer concentration), it is quite a challenge in setting the right reaction conditions in sample mixtures.

Blood Group Antigens

Bite marks are potential sources of salivary transfer. Approximately 80% of humans are secretors of ABO blood group antigens in the saliva. Ninety percent of ABO DNA profiles can be obtained from epithelial cells deposited in saliva by genetic fingerprinting. However, degradation of genetic material with time produces a hinderance in DNA extraction from bite marks residue.

Microbial Transfer

Locard's exchange principle states that "an individual, when in the form of physical contact with another, stands to transfer not only his own genetic identity but also those of the myriad organisms with whom he inevitably coexists."[10] Oral microflora, most commonly, mutans streptococci, *Actinomyces*, and *Veillonella* can also get transferred through saliva. Phenotypic methods include biotyping, serotyping, and bacteriocin typing for microbial identification. The ubiquity of *Streptococcus mutans* renders it to be the most useful species in forensic identification. As with salivary DNA degradation, the *S. mutans* cell count also decreases with time at the rate of 50%/h. However, bacterial DNA can be easily replicated by further culture techniques. *S. mutans* strains are acquired during birth and persist throughout life. Hence, subtyping of mutans streptococci species has definite implications in forensic microbiology. Species genotyping can be done by means of PCR-restriction fragment length polymorphism analysis.

Gender Determination

Amelogenin is an enamel matrix protein that comprises 90% of tooth enamel proteins. It regulates the initiation, growth, and maturation of hydroxyapatite crystals. Using primers specific toward intron 1 of amelogenin gene, the X and Y chromosomes give a 106 bp amplification product and 112 bp amplicon, respectively. Therefore, salivary samples from males (XY) exhibit two bands whereas female samples (XX) show only one band. These differences in amelogenin gene expression allow for gender differentiation.[11]

Conclusion

Saliva is an easily obtainable biological fluid by means of noninvasive methods. It is constituted by secreted products derived from plasma and salivary acini. Identification tools used for screening, presumption, and confirmation of salivary constituents aid in antemortem as well as postmortem analysis. It is a new research area which needs to be explored further for its optimal utilization as an adjunct as well as confirmatory source of personnel profiling and identification.

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Conflicts of interest
There are no conflicts of interest.

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