

BIOLOGICAL ANALYSIS OF TRACES FOUND ON CIGARETTE BUTTS – A PROSPECTIVE STUDY

Forensic Medicine

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ABSTRACT

Cigarette butts serve as additional evidence in cases of sexual assault, homicide, and even suicide, aiding in the investigation of burglary and dacoity. Cigarette butts may be a breakthrough for investigation in those circumstances since they may include remnants of saliva and epithelial cells of lips. Cigarette butts can identify a person through DNA only when there is a presence of saliva or epithelial tissue. The possibility of contamination or loss of biological evidence from cigarette butts arises when the samples are subjected to undergo different tests, including presumptive, preliminary, and confirmatory examinations. The primary focus of this study was to examination of saliva in 100 samples of cigarette butts collected from the vicinity of tea shops. The conclusion drawn from the three distinct analyses highlights the necessity of conducting DNA testing before the biological evidence found in cigarette butts becomes susceptible to contamination. The techniques employed for saliva detection included UV light examination, Starch-Iodine, and Gel diffusion. The amylase in cigarette butts is present in an insufficient amount which makes it difficult to detect, hence giving unfavourable results.

KEYWORDS

UV light, Starch-Iodine test, Amylase, Radial gel diffusion, Preliminary examination

INTRODUCTION

Saliva is an aqueous fluid that is present in the buccal cavity. It is made up of a complex mixture of substances that come from the oropharynx, upper airway, gastrointestinal reflux, gingival sulcus fluid, food deposits, and blood-derived compounds, in addition to secretory products, that is, organic and inorganic products from the salivary glands. One of the most complicated, adaptable, and vital body fluids, saliva satisfies a wide variety of physiological requirements. Saliva is essential to the physiology of the oesophagus, digestion, and gastric cell protection in the digestive tract.⁽¹⁾ With 99% of its makeup made up of water, saliva is primarily water. The organic and inorganic molecules that make up the solid components, which are dissolved in the aqueous component, differ greatly from person to person and sometimes even within the same person during the day.^(2,3)

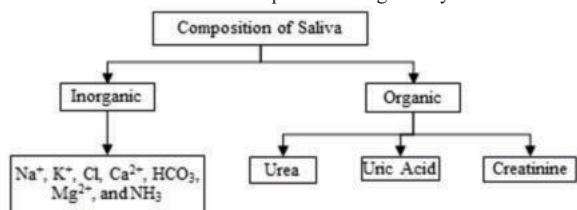


Figure 1: Composition of Saliva

Saliva As Biological Evidence

Saliva may be found on many objects at a crime scene, such as cigarette butts, food leftovers, or bite marks. However, most saliva stains are undetectable to the human eye after drying. One of the most important biological fluids that humans secrete is saliva, which can be found on the skin through various actions including biting, sucking, or others. In suicides, biological fluids like saliva and urine are commonly discovered. Any inanimate item that comes in contact with either the victim or the perpetrator may have biological fluids. Gingival crevicular fluid exfoliated oral epithelial cells, and bacteria are all components of salivary gland secretions, which are combined to form saliva.^(4,5)

Source Of Saliva In A Crime Scene

In contrast to blood, saliva may be collected easily, safely, and non-invasively with a low chance of contamination, especially from infectious diseases like hepatitis. This gives saliva a variety of benefits over blood. Since saliva is transparent, it is typically disregarded as evidence and is less likely to be falsified by criminals. Amylase saliva screening has been used as a presumptive test in crime investigation,

mainly to look for saliva stains on various surfaces. The starch-digesting enzyme amylase is present in almost all living things, albeit specificity, activity, and needs differ from tissue to tissue and species to species. Amylase is a substance that is abundant in human saliva. Due to the difficulty in identifying particular saliva places on the body; the face, neck, chest, shoulder, belly, forearm, and thighs are crucial saliva recovery areas.^(6,7,8)

Personal Identification Through Saliva

Short tandem repeat (STR) DNA profiling technology is known for its high accuracy in obtaining DNA. The Crime Scene Investigation (CSI) Team plays a pivotal role by collecting various types of evidence that may contain DNA, including blood, sperm, tissue, and saliva. Saliva, being a non-invasive and easily collectible bodily fluid, holds particular significance as a viable source for identifying individuals. Leveraging this advanced scientific method, forensic experts can match DNA profiles to individuals, aiding in criminal investigations, victim identification, and establishing crucial links in various legal and investigative proceedings.^(9,10)

Methodology

Analysis was carried out in accordance with the steps outlined in the DFSS manual, 2019. The presumptive test for saliva is the starch-iodine test based on a colour shift, while the confirmatory test involved a gel diffusion test with starch and iodine. In addition, UV examination is predicated on the idea that biological fluid would turn white when exposed to UV light. Cotton swabs dipped in distilled water were used to extract saliva, although Phosphate Buffer Saline (PBS) can also be used.

Collection Of Samples

Forceps were used to manually pick up cigarette butts for analysis. Zip lock bags were used for the storage of samples and with the help of label stickers samples were labelled.

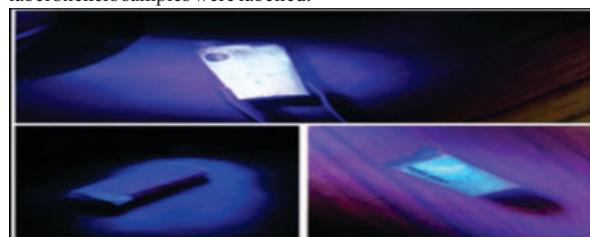


Figure 2: Examination of collected samples using UV light source.

Examination Of Samples

UV Examination

An alternative light source (ALS) was used to identify biological fluid i.e. saliva, on the collected samples.^[11,12,13,14]

Starch-iodine Test

The samples were swabbed with cotton swabs and placed in the test tubes that have been arranged in a rack. To each test tube, three drops of the soluble starch solution were then added.

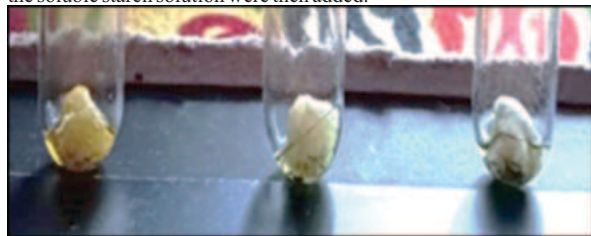


Figure 3: Starch iodine test for detection of saliva.

The test tubes had been mixed, corked, and left at room temperature for an hour, then 2 drops of Lugol's iodine were added.^[15]

Radial Gel Diffusion Test

The samples were grown on starch-restraining agarose gel to allow amylase activity to resume following the addition of iodine. The permutation of starch and iodine produces a distinctive blue color that is connected with the negative control, and hence the absence of color indicates a positive reaction.

Table 1: Reagents And Their Required Quantity For Radial Gel Diffusion Test.

NaH ₂ PO ₄ anhydrous	2.7 g
Na ₂ HPO ₄ anhydrous	3.9 g
NaCl	0.2 g
Distilled water	500 ml

Table 2: Materials And Their Required Quantity For Gel Plate Preparation

Gel test plates (2% agarose, 0.1% soluble starch)	
Agarose	0.2 g
Soluble starch	0.01 g

The samples were cultured on a starch-restraining agarose gel, allowing the amylase activity to resume. The agarose and soluble starch mixture was taken in a beaker and boiled in a water bath with continuous stirring using a glass rod until the agarose dissolve. Then, the gel mixture was poured onto a glass slide. Using a precision pipettor, the wells were made leaving 1.5 cm between each sample well and samples were loaded using different droppers. The gel plates were incubated at 32 °C for 1 hour.^[17]

RESULTS AND DISCUSSIONS

The three distinct examination tests performed on the samples produced both positive and negative results.

Table 3: Average Result Of The Samples

S. No.	Samples	Result	Examination		
			UV Test	Starch-Iodine Test	Radial Gel Diffusion
1.	Cigarette butts	Positive	39	46	0
2.	samples (n=100)	Negative	61	54	100

When the samples were examined under UV light source, 39% of the samples yielded positive results, while the remaining 61% showed negative results. However, it should be noted that this test may also detect other biological fluids like sweat, semen, blood, etc., leading to potential false positive and false negative outcomes.



Figure 4: Yellow color indicates the presence of amylase activity.

In the starch-iodine test, the absence of a dark blue color indicates that starch is not available for complex formation. Additionally, the absence of amylase activity signifies the presence of saliva, resulting in the absence of a blue color. Out of the samples tested, 46% showed positive results, and the remaining 54% showed negative results.

Due to the low levels of amylase in the samples, it was observed that 100% of the collected samples failed the radial gel diffusion test. While the radial gel diffusion technique is suitable for examining saliva from various evidence, it may not be appropriate for confirming the presence of saliva in cigarette butts.

CONCLUSION

Serological testing and salivary component analysis are increasingly being utilized in various cases, including human bite marks, drug and alcohol misuse, crime detection, and age and gender determination. Saliva, one of the vital bodily fluids secreted, offers the advantage of being collected non-invasively. For personal identification, cigarette butts can be used as a source of DNA (saliva or blood) for personal identification purposes. To prevent contamination or the loss of biological evidence, it is essential to promptly send saliva samples for DNA analysis as soon as they are discovered.

A drop of extracted cigarette butt sample on FTA cards can be used to disclose a person's identity. The majority of the samples that were gathered for the purpose of the study were contaminated with dirt and soil. In such a scenario, using preliminary examination techniques like UV light test and chemical examination techniques like starch-iodine and radial gel diffusion which has a destructive nature, might produce unfavourable results.

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