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FORENSIC SIGNIFICANCE OF SALIVA: A COMPARATIVE STUDY OF INDOOR AND OUTDOOR CASES

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ABSTRACT

Saliva is an increasingly valuable form of biological evidence in forensic investigation, offering critical insights through its rich composition of enzymes, epithelial cells, DNA, and biomarkers. This Review Explores the forensic relevance of saliva by comparing its detectability and DNA integrity in indoor versus outdoor crime scenes. Saliva has become a crucial non-invasive biological fluid in forensic science, offering valuable genetic and biochemical information. Its simple collection process, epithelial cells containing DNA, and applicability across various forensic contexts such as identifying suspects, detecting drug use, and analysing health markers make it a versatile tool. Despite these advantages, environmental factors like temperature, humidity, and exposure time significantly influence the stability and integrity of salivary DNA, particularly in outdoor crime scenes. The study employs a Nanodrop spectrophotometer for DNA quantification and evaluates how environmental degradation influences sample viability, and it highlights the biochemical and physiological properties of saliva that enhance its role in forensic science, while discussing challenges associated with sample collection, preservation, and analysis in variable environments. It examines the forensic significance of saliva, focusing on its composition, diagnostic potential, and susceptibility to environmental factors. Emphasis is placed on comparative studies involving indoor and outdoor saliva samples, utilizing DNA extraction and quantification techniques.

KEYWORDS: Saliva, biomarkers, non-invasive, DNA, Environmental conditions.

INTRODUCTION

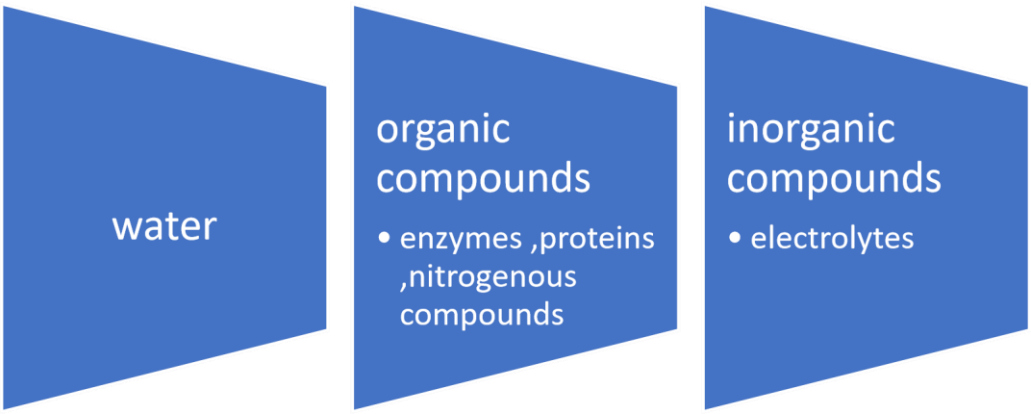
Forensic science is the application of scientific principles and techniques to investigate and analyse evidence in criminal or civil court of law; it entails the investigation and establishment of facts through the application of science and technology in relation to law thus playing a crucial role in legal proceedings by utilizing advanced scientific techniques to establish facts to identify suspects and support the judicial system. Through the integration of disciplines such as biology, chemistry, physics, and digital forensics, ensures the objective and accurate examination of evidence, aiding law enforcement and the courts in the pursuit of justice. It involves the observation, documentation, collection, analysis, assessment and Scientific Interpretation of evidence during the course of investigation required for the different fields of law, including criminal, civil, work, family, and administrative. By analysing items or materials we can connect evidence to a crime using a scientific approach. Saliva is one of the most important types of biological evidence that is frequently found at crime scenes along with blood, semen, and hair. It is becoming more recognized for its forensic diagnostic value because saliva is widely available and may be easily deposited at crime scenes through acts like biting, licking, speaking, or spitting. It is a vital resource despite historically being disregarded in Favour of more noticeable biological samples.

Saliva is a complex biological fluid composed primarily of water (approximately 99%), but enriched with organic and inorganic substances. These include numerous electrolytes (such as sodium, potassium, calcium, and chloride), enzymes (like amylase and lipase), proteins, epithelial cells, and DNA. These components make saliva a rich source of information for identifying individuals, determining physiological conditions, and detecting the presence of drugs or diseases. Saliva is often encountered on everyday items at crime scenes, including cigarette butts, drinking glasses, and bite marks. Due to its frequent and often inadvertent transfer during criminal activities, saliva is now widely recognized as a valuable and underutilized form of biological trace evidence. Its relatively invisible nature compared to blood or semen can make it less susceptible to tampering, and it is easily collected using non-invasive methods. However, saliva's forensic utility can be compromised by environmental exposure, particularly in outdoor settings. Factors such as temperature fluctuations, humidity, and exposure to pollutants may accelerate the degradation of DNA, affecting the viability of saliva as evidence. This review aims to compare saliva from indoor and outdoor parameters, evaluating the impact of environmental exposure on DNA purity and concentration. The use of advanced analytical techniques, such as DNA extraction and a Nanodrop spectrophotometer, enhances the assessment of these variations. By synthesizing current research, case studies, and analytical methodologies, this study aims to highlight both the opportunities and limitations associated with the forensic examination of salivary evidence, ultimately contributing to more informed practices in forensic science and criminal investigations. This comprehensive review seeks to inform forensic practice by identifying optimal conditions for saliva collection and preservation and by highlighting areas that warrant further investigation. The findings aim to assist forensic practitioners in optimizing their evidence-handling techniques and encourage further research into saliva as a dynamic and informative biological substrate.

HISTORY OF SALIVA

Saliva has played an increasingly important role in forensic science over time. Initially in the mid-20th century, saliva was identified at crime scenes using salivary amylase as a biochemical marker, which allowed investigators to detect dried saliva stains but not to identify individuals. A major breakthrough occurred in the 1980s with the discovery of DNA fingerprinting by Sir Alec Jeffreys in 1985 which revealed that saliva contains epithelial cells capable of providing reliable DNA for individual identification. From the 1990s onwards, the development of PCR and STR profiling further enhanced the forensics value of saliva, as these techniques required only small or degraded DNA. In the 21st century the use of saliva expanded beyond DNA analysis into toxicology for drug and alcohol testing, clinical diagnostics for diseases such as COVID-19 and oral cancer, and forensic microbiology, establishing saliva as a dependable, non-invasive, and versatile forensic matrix.

Composition of Saliva



These constituents contribute to its role in digestion, oral health maintenance, and systemic biomarker transport. DNA within epithelial cells can be reliably extracted and quantified using standardized protocols.

Comparative Environmental Analysis: Indoor and Outdoor

Environmental impact on DNA

Environmental conditions play a critical role in determining the integrity and usability of saliva samples in forensic investigations. The stability of DNA within a sample is highly sensitive to external physical and biological factors. Indoor environments typically offer controlled conditions such as consistent temperatures and limited exposure to UV radiation, preserving DNA better than outdoor conditions, where microbial growth, direct sunlight, wind, rain, and varying levels of humidity degrade nucleic acids. Outdoor samples are more susceptible to contamination from soil, leading to a potential reduction in DNA concentration and purity. This has implications for forensic reliability, especially in time-sensitive cases.

Therefore, Accurate interpretation of salivary DNA requires a comprehensive understanding of the interplay

between exposure duration and environmental stressors. Factors such as temperature, humidity, microbial activity, and UV exposure do not act in isolation but interact in complex ways that influence DNA stability over time.

METHODOLOGICAL INSIGHTS

The kit used for DNA extraction is the Qiagen DNA mini extraction kit, which is specially designed for DNA extraction and DNA stabilization that is suitable for long-term storage and high-yield DNA extraction. In this study, DNA extraction was performed using Proteinase K digestion followed by phenol-chloroform purification. Quantification and purity assessment were carried out using a Nanodrop spectrophotometer. Saliva samples collected indoors were stored under room temperature, While Outdoor Samples Were Exposed for Varied durations (15 days) this indicates that longer exposure reduced DNA yields and increased the chance of contamination. DNA purity was Assessed Via the A260/280 ratio, with ideal values ranging between 1.8 and 2.0. This methodological framework supports the Hypothesis that Environmental factors significantly influence DNA recovery from saliva.

Chemical Reagents

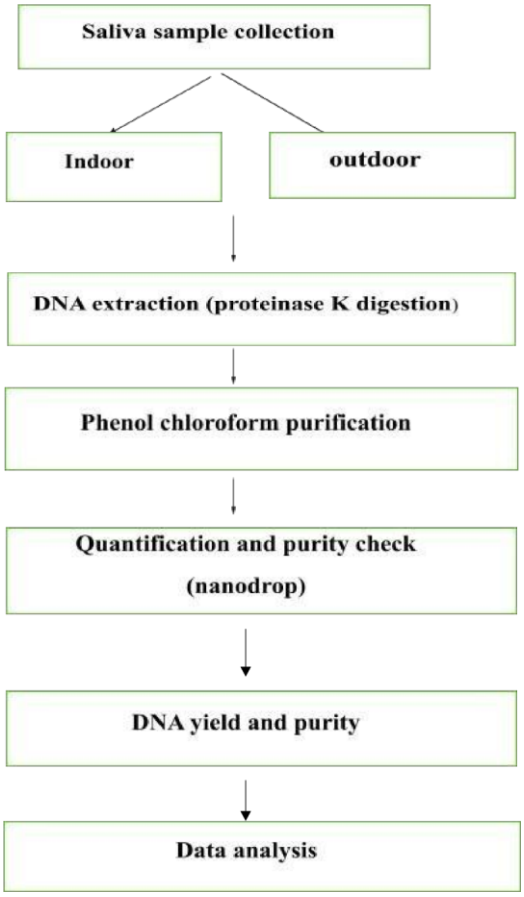
Reagent	Functions in Dna Extraction
Lysis buffer	Breaks, open cells and releases DNA
Proteinase K	Digests proteins and nucleases
Ethanol	Precipitates DNA
Wash buffer	Removes impurities
TE buffer	Preserves DNA in final solution

METHOD USED

Volunteers were asked to rinse their mouth with distilled water prior to collection to remove food debris and contaminants. Saliva was obtained by swabbing buccal mucosa using sterile cotton swab. Indoor samples were collected and then stored at room temperature in laboratory until DNA extraction. Outdoor samples were left exposed to environment elements (e.g. direct sunlight, ambient temperature, humidity) for duration of 15 days in sterile vials placed on cotton substrates, simulating real world evidence recovery conditions.

Then DNA extraction was performed in the laboratory as followed

- Place the buccal swab in 1.5ml microcentrifuge tube
- Add 200 ul of proteinase K.
- Incubate at 56^0C For 30 minutes.
- Add 600 ul of buffer AL and vortex for 15 seconds.
- Incubate at 56^0C for 10 minutes.
- Add 600 ul ethanol to the sample to the sample and vortex it.
- Centrifuge the sample for 10 minutes and then remove the supernatant.
- Transfer the sample to mini spin column and then centrifuge it for 1 minute.
- Add 500 ul of buffer AW1 and centrifuge again for 1 minute.
- Discard the flow through.
- Add 500 ul of buffer AW2 to the collection tube and centrifuge for 3 minutes.
- Transfer the column to a new centrifuge tube.
- Centrifuge again for 1 minute to remove residual wash buffer.
- Place the spin column in a clean 1.5ml centrifuge tube and add 150 ul of buffer AE.
- Incubate at room temperature for 1 minute, then centrifuge for 1 minute to elute the DNA
- Then DNA is stored at -20^0C for the further use
- Then after the completion of DNA extraction, Nanodrop spectrophotometry was performed for the quantification of samples.
- The concentration and purity of the extracted DNA were evaluated using a nanodrop spectrophotometer.
- The device was first blanked using water to set baseline readings.1 ul of each DNA sample was loaded on the nanodrop pedestal for analysis.
- Readings of A260/280 close to 1.8 indicate a good DNA purity. The A260/280 ratio helped identify the presence of residual organic compounds or salts.
- All results were tabulated for statistical comparison between indoor and outdoor group for sample quality assessment.



RESULTS

The study analysed a clear distinction between the DNA yield and purity of saliva sample collected under controlled indoor conditions and those exposed to uncontrolled outdoor environments. DNA concentration

and purity were assessed using the NanoDrop spectrophotometric analysis, which produced quantifiable variations that emphasize the significance of environmental influences in forensic investigations.

Results of sample kept in indoor Environment for 15 days

Under stable laboratory conditions, the DNA concentration was consistently high in all samples, suggesting little degradation. The ideal range of \~1.8 for pure DNA was closely matched by the A260/280 purity ratios, which averaged between 1.7 and 1.9. Microbial activity was inhibited by regulated temperature and humidity, which stopped DNA molecules from breaking down. High reproducibility of results was ensured by minimal environmental contamination. These results support the idea that salivary DNA is best preserved indoors.

Result of sample kept in outdoor environment for 15 days.

In outdoor samples The DNA concentration ranged from 3.1 ng/µl (Outdoor 13) to 41.7 ng/µl (Outdoor 10). The A260/280 ratios showed very inconsistent purity values, ranging from 0.98 to 9.07, while the ideal range for pure DNA is \~1.8. The purity ratios of many samples were either abnormally high or very low, indicating contamination with RNA, proteins, or environmental contaminants like dust and microbiological growth. The majority of samples had low absorbance values at A260 and A280, which further supports the DNA's compromised integrity. Most samples were unsuitable for forensic analysis because of inconsistent purity and degradation, even though some samples, such as Outdoor 10, showed relatively higher concentrations Samples exposed to sunlight, temperature changes, and varying humidity showed a marked decrease in DNA concentration.

Limitations and Challenges

1. **Low DNA yield in some samples:** Saliva typically contains fewer nucleated cells compared to blood, which can result in limited quantities of DNA. This becomes more critical when the sample is small or partially degraded, impacting the reliability of downstream analyses such as STR profiling.
2. **Microbial Contamination**: Saliva naturally contains a large population of oral bacteria. When stored improperly or exposed to outdoor environments, additional environmental microbes can colonize the sample, further degrading DNA and interfering with quantification and amplification processes.
3. **Difficulty in Sample Collection and Preservation**: Saliva traces may be partial, invisible, or mixed with other substances. Proper collection, transportation, and storage are crucial, but may be compromised due to: Delayed collection at the crime scene, Lack of proper refrigeration, and Absence of standardized protocols.
4. **Legal and Evidentiary Concerns**: In cases where DNA profiles are partial or potentially contaminated, the evidentiary value may be questioned in court. Therefore, chain-of-custody documentation and expert interpretation become vital to ensure admissibility.

Legal and Ethical Considerations

Saliva collection, storage, and analysis must comply with strict chain-of-custody requirements to ensure evidentiary integrity. Given that saliva contains an individual's complete genetic profile, ethical considerations regarding consent, privacy, and data security are paramount. The potential misuse of genetic data particularly in vulnerable populations has led to calls for stronger regulatory oversight.

CONCLUSION

The review paper, titled "Forensic Significance of Saliva: A Comparative Study of Indoor and Outdoor Cases", demonstrates the importance of saliva as a reliable biological fluid in forensic investigations. The analysis of salivary samples collected under both controlled indoor and uncontrolled outdoor conditions revealed that saliva is a rich and non-invasive source of DNA, enzymes, and biomarkers. Its composition makes it highly useful in criminal investigations for personal identification, toxicological assessment, and reconstruction of crime scenes. The comparative results clearly highlighted that environmental factors such as temperature, humidity, sunlight exposure, and storage duration significantly influence the quality and quantity of DNA retrieved from saliva samples. Indoor samples maintained higher stability, purity, and concentration of DNA compared to outdoor samples, where degradation was more prominent due to exposure to variable conditions. This confirms that while saliva is a powerful tool in forensic science, its evidentiary value is dependent on collection, handling, and preservation conditions. Overall, the findings strongly support the forensic applicability of saliva as a biological sample. Its easy accessibility, non-invasive collection, and rich DNA content make it indispensable in modern forensic science. However, proper handling, timely collection, and improved storage protocols are essential for maximizing its evidentiary value, especially in outdoor crime scenes.

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