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Temporal Estimation of Semen Deposition on Garments in Sexual Assault Cases: A review on Advances in Biochemical and Microbiological Forensic Techniques

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ABSTRACT

As a forensic DNA expert in a forensic laboratory, the author frequently encountered the intricate challenge of determining and reporting the time of semen deposition on garments or in other exhibits in rape cases. This remains one of the most demanding aspects of forensic investigations in sexual assault cases. The complexity of this task stems from the dynamic biochemical and microbiological changes that occur in seminal stains over time. The ability to estimate the time of deposition holds significant forensic and legal implications, as it can play a crucial role in establishing case timelines, corroborating or contradicting witness or victim statements, and providing critical evidence to strengthen the prosecution or defence.

The present review highlights the need for continued research in this area, considering the intricacies of the problem and the diverse range of factors that influence the degradation and persistence of seminal stains. Achieving reliable and precise temporal analyses is far from straightforward, as it requires sophisticated methodologies and a deep understanding of the interplay between biological, chemical, and environmental variables. Moreover, the review emphasizes the potential for novel interdisciplinary collaborations to advance forensic methods. While recent advancements in chemical profiling, microbiota analysis, and protein degradation studies offer promising avenues, the reliability of these methods depends on careful consideration of environmental, biological, and situational factors. Continued research and technological innovation are essential to enhance the accuracy and applicability of temporal analyses in forensic investigations.

As the forensic community continues to integrate technological innovations, such as artificial intelligence and machine learning, with traditional forensic analysis, the potential for more precise and automated TSD estimation tools increases. These tools could ultimately lead to faster, more accurate determinations of Time since deposition (TSD), enhancing the overall effectiveness of forensic investigations.

Key words: Forensic, Time since deposition (TSD), semen, chemical profiling, microbiota analysis, mRNA degradation

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Introduction

in forensic investigations the assessment of the time since deposition (TSD) of body fluid is critical requirement. Accurate determination of TSD not only aids in pinpointing when a crime may have occurred but also helps to establish whether the deposition is after or before the crime, thereby enabling the exclusion of irrelevant samples.

Determining of the time since deposition (TSD) of body fluids, particularly seminal fluid, is a highly complex and unresolved challenge. Forensic DNA experts are frequently tasked with this intricate responsibility, as the temporal analysis of seminal stains plays a pivotal role in reconstructing case timelines, corroborating or disputing statements made by witnesses or suspects, and providing robust evidence that supports prosecution or defence arguments. Despite its critical importance in forensic investigations, reliable methods for estimating the TSD of seminal stains remain elusive and are a subject of ongoing research and debate.

Addressing these challenges is essential for improving forensic practices and ensuring that forensic evidence plays a critical role in the pursuit of justice. By continuing to refine the methodologies for estimating TSD, the forensic community can move closer to developing precise and reliable tools for temporal analysis. These tools are indispensable for resolving the intricate questions surrounding sexual assault cases, where every piece of evidence, including of deposition of semen, can be pivotal in the pursuit of truth and justice. In the past certain criteria for TSD estimation like Chemical profiling, Microbial Analysis, m-RNA studies etc. are supposed to be important but more studies are needed in forensic exhibit which are being examined in Forensic Laboratory

Methodology

To address the current challenges, it is essential to develop standardized methodologies that account for the wide range of influencing factors. Collaborative research efforts should focus on developing robust databases that document the degradation patterns of semen under varying conditions. Standardizing sample collection, storage, and handling procedures to minimize variability. Validating emerging techniques, such as chemical profiling and microbiota analysis, across diverse environmental and situational contexts. Integrating advanced computational tools to analyse complex datasets with accurate temporal predictions. It has been observed that the scientist is aware of the importance of TSD in criminal cases, still the available techniques for TSD are few and it is also true that till now no accurate technique is available for determination of the time of deposition of biological fluid. There are different approaches for evaluating the time since deposition (TSD) of semen or other body fluid in criminal cases. These approaches are mainly related to biochemical profiling, microbiological analysis and RNA studies. A comprehensive Literature review of on TSD determination was conducted which mainly concentrating on chemical profiling in which molecular markers such as proteins, lipids, and small metabolites were studied and Microbial analysis is another area in which microbial communities associated with seminal stains over time is explored. In addition, some authors used chemical profiling to estimate TSD. Incorporating spectroscopy, chromatography, and direct mass spectrometry to assess temporal markers are considered as advanced Analytical techniques (Doty et al., 2017). Some authors described method of isolation of DNA and RNA molecules simultaneous from

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blood and semen stains (Auer and Patzelt, 2003). Several analytical techniques were used to determine TSD. For chemical profiling, Chromatographic Analysis was also applied to isolate and quantify chemical markers (Alshehhi and Penelope, 2019). Achetib et al. (2019) conducted a study exploring the potential of a non-contact spectroscopic approach for estimating the age of semen stains. This method relied on intrinsic fluorescence measurements combined with a chemical rate equation.

Discussion

Precise estimation of TSD of body fluid is critical in corroborating victim testimonies, confirming the timing of forensic evidence with the sequence of events as described by witnesses or suspects, and supporting or refuting alibis. It can also be a decisive factor in establishing the timeline of the crime, which is often central to the investigation and legal outcomes.

The effect of environmental variables on degradation rates were also investigated (Sirker et al 2016). Ishikawa et al. (2023) demonstrated the successful amplification of most allele peaks up to 9 hours after saliva deposition recovered from bite marks. Some researchers have explored methods for determining the age of body fluids. Doty et al. (2017) used Raman spectroscopy as a non-destructive tool for analyzing bloodstains and genes specific to semen and vaginal secretions were completely degraded. This highlights the challenges and temporal limitations of genetic analysis in forensic investigations.

Direct mass spectrometry offers benefits like analysis in less time, high sensitivity, and with small sample size. Their method holds promise for providing forensic investigators with precise temporal information, even in cases where the deposition occurred weeks prior. However, the reliability of this method may vary depending on external conditions, such as exposure to air, light, or moisture. Further research is necessary to refine this approach and adapt it for diverse environmental and situational contexts. Understanding the age of a biological stain, could offer law enforcement crucial insights to aid in the prosecution of criminal cases. However, a consistently reliable method for accurately determining TSD remains unavailable.

In addition to biochemical changes, the microbial flora present on seminal stains undergoes significant alterations that can also be used as a marker for determining the TSD. Microbial growth, bacterial succession, and the formation of biofilms all contribute to the temporal dynamics of the stain. Another innovative methodology involves analysing changes in the microbiota structures present in seminal stains. Microbial communities, including bacteria and fungi, naturally colonize semen and exhibit predictable growth patterns over time. They also assessed microbial community dynamics under different environmental conditions. Researchers have observed that the composition and activity of microbiota in seminal stains evolve in response to environmental factors and the duration since deposition (Lira Neto, 2024). These changes can serve as temporal indicators, enabling forensic scientists to approximate the age of a semen stain. Microbiota analysis offers a unique perspective by leveraging the biological interactions between microbes and their environment. However, this approach requires careful consideration of external variables, such as temperature, humidity, and fabric type, which can significantly influence microbial growth rates. Additionally, the specificity of microbial markers to semen stains must be ensured to avoid cross-contamination or false positives in forensic settings. Zhang et al. (2024) presented emerging evidence that microbial characteristics hold great potential as biomarkers for both the identification and estimation of time since deposition (TSD)

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of body fluids. Their findings suggest that microbial signatures can effectively address the challenges associated with TSD determination.

Protein Degradation Studies is another area which is concerned with time-dependent changes in semen-specific proteins like PSA and acid phosphatase. The Protein Degradation Studies are related to quantification of levels of PSA and acid phosphatase activity estimated by using enzyme-linked immunosorbent assays (ELISA) (Bauer and Patzelt, 2003). While protein degradation studies offer valuable insights, the rate of degradation is highly influenced by external factors such as temperature, light exposure, and the nature of the fabric on which the stain is deposited. Moreover, the initial concentration of proteins in the semen can vary between individuals, adding another layer of complexity to the analysis. Despite these challenges, advancements in proteomics and bioanalytical tools continue to enhance the reliability of protein-based temporal assessments. Jimenez et al.1994 conducted a study to examine how four enzymatic biomarkers present in semen degrades over time. The research analyzed the impact of different storage temperatures and durations on the degradation rates, aiming to determine whether these rates could be correlated with the age of the stains. One of the core difficulties is the highly variable nature of seminal fluid, which is a complex mixture of proteins, lipids, sugars, and other biomolecules. These compounds break down over time, and their degradation can provide crucial clues about the age of the stain. However, the rate at which these compounds degrade is not constant and can be influenced by external factors such as the surface the stain is deposited on (e.g., fabric, carpet, or skin) and whether the stain has been exposed to environmental stresses such as heat, moisture, or microbial contamination.

Determination of degradation of biomolecules like DNA or RNA over time is an alternative method for estimating time since deposition (TSD). Specific mRNA is being used for identify vaginal secretions in rape cases (Hanson and Ballantyn2013). Messenger RNA, from blood and semen also recovered reliably recovered across extended time periods without adversely affecting the success rate of DNA profiling (Berge and Sijen, 2017). These findings highlight the robustness of molecular recovery techniques in forensic analysis, ensuring the viability of both DNA and RNA as critical tools in solving criminal cases. Fordyce et al (2013) reviewed the literature available on the variability and deterioration mechanisms of RNA and found that limited experimental studies are available on this subject. RNA Degradation Analysis is considered as advanced techniques for estimating TSD. In a notable study,

Alshehhi and Penelope (2019), Anderson et al, (2005) utilized reverse transcription quantitative PCR (RT-qPCR technique and estimated age of body fluids by degradation of RNA. Notably, Salzmann et al (2021), Haas, et al, 2013 identified body fluid-specific substances and pattern changes in rnRNA through comprehensive expression and degradation analyses, providing promising advancements in this area of forensic science. The time of deposition of semen stains is not easily determined due to the variability of factors that influence degradation and persistence. The methods available particularly valuable in sexual assault cases, where the determination of timelines can have profound legal and investigative implications. For example, temporal analysis of semen stains can help establish or refute alibi claims, provide clarity on the sequence of events, and strengthen the evidentiary value of biological samples. However, the practical implementation of these techniques requires careful consideration of multiple factors that can influence their accuracy and reliability.

It is expected that the advent of artificial intelligence and machine learning will improve accuracy of TSD estimation. This holds potential for creating robust, user-friendly forensic tools capable of precise

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and efficient TSD determination, thereby increasing the evidentiary weight of biological samples. To ensure reliable and practical results, future research should focus on creating comprehensive databases detailing decay patterns under diverse conditions and establishing standardized protocols for sample acquisition, handling, and analysis. In addition to this rigorous validation of novel techniques to guarantee forensic reliability is needed. Addressing these shortcomings is critical for improving the accuracy and dependability of TSD estimation in forensic practice, ultimately contributing to a more just outcome in sexual assault cases.

Conclusion

The precise determination of the time elapsed since deposition (TSD) of semen stains in forensic science remains a significant hurdle in sexual assault investigations. While advancements in biochemical profiling, microbial analysis, protein decay studies, and RNA analysis have been substantial, a universally dependable method for TSD assessment remains elusive. Each technique presents unique strengths, yet is hampered by factors including environmental influences, sample heterogeneity, and technological limitations. Recent studies show promise in multidisciplinary approaches, combining advanced analytical techniques like spectroscopy, mass spectrometry, and molecular biology. Microbial and proteomic investigations have illuminated the decay and succession patterns of biomolecules and the microbiome, offering potential temporal markers. However, these methods need standardization, validation across diverse contexts, and careful consideration of extraneous variables to ensure forensic utility.

Conflict of Interest

The authors declare no conflicts of interest.

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