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A Comprehensive Review On The Forensic Significance Of Body Fluids In Criminal Investigations

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Abstract

The analysis of body fluids represents one of the most crucial aspects of forensic investigations, providing valuable biological evidence that aids in criminal identification, reconstruction of events, and linking suspects to crime scenes. This review discusses the significance of commonly encountered body fluids such as blood, semen, saliva, urine, sweat, and vaginal secretions, along with emerging sources such as earwax and breast milk. Traditional presumptive and confirmatory methods are compared with recently developed molecular and analytical approaches including mRNA profiling, proteomics, microbiome analysis, and mass spectrometry. The paper also evaluates observations from literature, discusses advantages and challenges of newly developed techniques, and outlines future perspectives. The findings suggest that while traditional methods remain relevant, modern molecular biology and bioinformatics are transforming forensic serology into a multidisciplinary science with greater sensitivity, specificity, and evidentiary value.

KEY WORDS:

Forensic serology; Body fluids; DNA profiling; RNA profiling; Proteomics; Microbiome analysis; Mass spectrometry; Forensic identification; Biological evidence; Molecular forensics

Introduction

Forensic science has become a cornerstone of modern criminal justice systems by providing scientific and objective methods of analyzing evidence collected from crime scenes. Among the diverse types of evidence encountered, biological evidence in the form of body fluids remains one of the most critical. Body fluids such as blood, semen, saliva, urine, sweat, and vaginal secretions not only serve as indicators of physical contact or presence but also contain cellular and molecular information that can be used for individual identification, toxicological investigations, and reconstructing events at a crime scene.

The development of DNA profiling in the 1980s revolutionized the analysis of body fluids, making forensic biology a highly reliable tool for individualization. However, traditional forensic serology methods, while still useful, often faced challenges such as environmental degradation, lack of specificity, and difficulties in distinguishing between different types of body fluids. In recent years, advances in molecular biology, proteomics, microbiome analysis, and analytical chemistry have introduced new approaches that enhance both the sensitivity and specificity of body fluid identification.

This review aims to provide a detailed account of the different body fluids used in forensic investigations, discuss both traditional and recently developed methods of detection, and evaluate their forensic significance. It further highlights observations from literature, discusses limitations and challenges, and outlines future perspectives for the field.

Review of Literature

Blood:

Blood is the most common body fluid encountered in forensic investigations. Presumptive tests such as the Kastle-Meyer and Hemastix rely on peroxidase activity, while confirmatory tests such as the Takayama and Teichmann crystal assays have been used historically (Li & Harris, 2008). However, environmental exposure often affects the reliability of these methods. Recent advances include mRNA profiling to detect blood-specific transcripts such as HBB (hemoglobin beta) and ALAS2, proteomic analysis of hemoglobin isoforms, and DNA methylation patterns to distinguish between menstrual and peripheral blood (Virgili et al., 2020).

Semen:

Semen is vital in sexual assault investigations. Traditional methods involved microscopic detection of spermatozoa and the acid phosphatase test (AP test). PSA (prostate-specific antigen) testing became a widely used biomarker. Modern advancements involve mRNA-based semen markers such as PRM1 and PRM2, as well as proteomic approaches to identify seminal fluid proteins in azoospermic samples (Brennan et al., 2021). The use of Y-STR analysis has also improved detection in mixed DNA samples.

Saliva:

Saliva is often collected from bite marks, drinking containers, and cigarettes. The presence of alpha-amylase has been the traditional marker for saliva detection (Pang & Cheung, 2008). However, alpha-amylase is not entirely specific. Newer methods include mRNA markers such as HTN3 and microbial profiling of the oral microbiome (Sweet & Shutler, 1999; Anslinger et al., 2022). These methods improve specificity and allow better differentiation in complex cases.

Urine and Sweat:

Urine and sweat are less commonly analyzed but can provide toxicological information about drug use or poisoning. Conventional methods include colorimetric assays and immunoassays for drug screening. Advances in LC-MS/MS and metabolomics now allow the detection of trace metabolites, drugs, and steroids in urine and sweat (Kwak et al., 2021). Additionally, epithelial cells from these fluids can yield usable DNA in some cases (Cantu, 2015).

Vaginal Secretions:

In cases of sexual assault, vaginal fluid detection is critical. Historically, methods included the detection of glycogenated epithelial cells using Lugol's iodine staining. Modern approaches employ vaginal fluid mRNA markers such as MUC4, as well as microbial community analysis using Next Generation Sequencing (NGS), which has shown high discriminatory potential (Chen et al., 2020).

Other Emerging Fluids:

Recent studies have also explored the forensic use of earwax (cerumen), nasal mucus, and breast milk. While these are not traditionally analyzed, they can provide supplementary information in certain cases (Rutty, 2002).

Observations

The literature review highlights several key observations:

1. Blood and semen continue to be the most commonly analyzed fluids, but saliva, urine, sweat, and vaginal secretions are increasingly significant due to advances in molecular methods.
2. RNA profiling and proteomics provide highly specific biomarkers that can differentiate between body fluids.
3. Microbiome profiling of saliva and vaginal secretions is an emerging field with strong forensic potential.
4. LC-MS has significantly enhanced the role of urine and sweat in forensic toxicology.
5. Despite technological advancements, environmental degradation, sample contamination, and limited volumes of fluids pose ongoing challenges.
6. Integration of multi-omics approaches is likely to define the future of forensic serology.

Discussion

The application of body fluids in forensic investigations has evolved significantly in the past two decades. Traditional biochemical methods provided quick but sometimes unreliable results. The shift to molecular biology and advanced analytical chemistry has greatly enhanced both specificity and reliability. For instance, mRNA profiling has demonstrated its ability to distinguish between multiple fluid types in a single sample (Juusola & Ballantyne, 2003). Proteomic studies have identified fluid-specific proteins, while microbiome profiling can add another layer of forensic evidence by associating fluids with an individual's microbial signature.

However, these methods also face limitations. RNA is highly susceptible to degradation, proteomic methods require advanced equipment, and microbiome-based identification is still in developmental stages and requires large population-based databases for validation. Furthermore, legal admissibility of these newer techniques remains an issue, as courts generally require robust validation and standardization before accepting forensic evidence.

Future perspectives include combining multiple molecular methods (multi-omics), using AI for interpretation, and developing field-deployable kits for rapid body fluid identification. The integration of bioinformatics and machine learning could further enhance the discriminatory power of these techniques.

Conclusion

Body fluids remain indispensable evidence in forensic casework. With advancements in molecular biology, proteomics, and microbiome research, the scope of forensic serology has expanded beyond traditional limits. While blood and semen remain primary fluids of interest, saliva, urine, sweat, and vaginal secretions are gaining increasing forensic importance. The transition from traditional chemical assays to modern omics-based methods represents a paradigm shift in forensic biology. Ongoing validation, technological refinement, and integration with digital forensic tools will determine how these methods are adopted globally in the coming years.

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