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Molecular Signatures of Crime: Biomarkers and Biochemical Evidence in Forensic Science

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Abstract

The molecular biomarkers have revolutionized forensic science by providing a powerful tool for the highly sensitive and reliable analysis of biological evidence in criminal investigations. The present review discusses the use of molecular signatures such as genetic, protein, metabolite, micro-biological and body fluid signatures for better forensic identification and evidence interpretation. It is concerned with the use of sophisticated analytical techniques like genomics, proteomics, metabolomics, spectroscopy, chromatography and mass spectrometry to find and characterize forensic samples with accuracy. The review also emphasizes the significance of molecular signatures in the identification of body fluids, estimation of post mortem interval, forensic toxicology, forensic pathology, human identification and crime scene reconstruction. Various challenges in current research, such as degradation of samples, ethical issues and standardisation of analytical protocols, are also discussed. Some innovations that are currently emerging, such as the use of artificial intelligence, the integration of multiple omics technologies, portable forensic technologies, and advanced bioinformatics are highlighted as potential tools to improve forensic investigations. Overall, molecular biomarkers give a solid scientific underpinning to precise forensic science and further enhance the reliability and credibility of modern criminal justice systems.

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1 Introduction

The field of molecular biology and biochemistry has become part of forensic science, contributing to the identification of individuals, the interpretation of biological evidence and the reconstruction of criminal events, with very sensitive and scientifically sound techniques. In contrast to the traditional forensic methods (which were mainly based on physical features, fingerprint or eyewitness observations), molecular forensic techniques analyse the biological molecules containing information about a person or the physiological state of the biological sample. The molecular signatures, which encompass DNA, RNA, proteins, metabolites, lipids and microbial profiles, are measurable and can provide clues to the origin, identity, condition and history of forensic evidence. They are particularly useful in providing objective and reproducible information, which has greatly improved the accuracy and credibility of forensic investigations.[2]

Biochemical evidence is any biological substance that can be found at a crime scene and includes blood, saliva, semen, urine, sweat, hair, tissue and other body fluids. When the molecular components of these samples are present, they carry valuable biochemical markers that can help investigators identify the origin of body fluids, determine postmortem time, screen for drug or toxic exposure, assess physiological stress, and differentiate individuals. New developments in analytical sciences, such as genomics, transcriptomics, proteomics, metabolomics, and forensic toxicology have broadened the range of biomarker discovery, and allowed forensic scientists to glean meaningful information from degraded, mixed or trace biological samples. The use of high-resolution analytical techniques such as polymerase chain reaction, next generation sequencing, mass spectrometry, chromatography and biosensor based platforms has also enhanced the sensitivity, specificity, and reliability of molecular evidence.[3]

The use of molecular signatures for individual identification is now being used in a variety of other forensic applications, including crime scene reconstruction, victim and suspect differentiation, disaster victim identification, wildlife forensics, anti-doping investigations and

[2] F. Sessa and M. Salerno, "Special Issue 'Molecular Biology in Forensic Science: Past, Present and Future,'" Int. J. Mol. Sci., 2024, doi: https://doi.org/10.3390/ ijms25052883.

[3] K. B. N. De Silva, K. S. Dharmasiri, M. P. A. A. Buddhadasa, and K. G. N. U. Ranaweera, "Criminal Investigation: A Brief Review Of Importance Of Biological Evidence," Eur. Sch. J., vol. 2, no. 8, pp. 8–12, 2021.

forensic pathology. Advances in multi-omics techniques and computing power are also improving the ability to interpret complex biological evidence by combining information from a number of molecular levels. Molecular biomarkers and biochemical evidence are essential tools to support the scientific basis of criminal justice as forensic science progresses to precision-based investigations.[4] This review will discuss recent developments in molecular biomarkers, analytical technology, forensic application, challenges and future prospects that are driving the current generation of forensic investigations.

2 Types of Biomarkers and Biochemical Evidence in Criminal Investigations

Biomarkers are biological markers whose presence that can be measured and give important information for forensic identification, interpretation of evidence and crime scene reconstruction. The evolving science of molecular biology has allowed forensic scientists to examine biological remains (at the genetic, protein, metabolite, microbial, and body fluid level), which has helped enhance the accuracy and reliability of criminal investigations.

A. Genetic Biomarkers

Genetic markers are the most commonly used forensics markers since they can give very specific information for individual identification. DNA-based markers like Short Tandem Repeats (STRs), Single Nucleotide Polymorphisms (SNPs), mitochondrial DNA (mtDNA) and RNA-based biomarkers like microRNAs are derived from blood, saliva, semen, hair, bone and other biological samples. The markers are useful for human identification, kinship, predicting ancestry, and identifying body fluids, even in degraded and compromised evidence.[5]

B. Protein Biomarkers

Protein biomarkers are molecules that are present in tissues that are more stable in different environments. Blood, semen and saliva are commonly detected through the presence of proteins like hemoglobin, prostate-specific antigen (PSA), semenogelin, and α-amylase. The modern

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[4] V. Andersen et al., "Investigating the Crime Scene—Molecular Signatures in Inflammatory Bowel Disease," Int. J. Mol. Sci., 2023, doi: https:// doi.org/10.3390/ijms241311217.

[5] T. Huebner, M. Steffens, and C. Scholl, "Molecular Genetic Techniques in Biomarker Analysis Relevant for Drugs Centrally Approved in Europe," Mol. Diagn. Ther., vol. 26, no. 1, pp. 89–103, 2022, doi: 10.1007/s40291-021-00567-x.

forensic proteomics also helps to estimate a postmortem interval and to determine tissue origin and to evaluate physiological change caused by damage or illness.[6]

C. Metabolite Biomarkers

Metabolite biomarkers are small molecules generated in the cells as a result of their metabolism. Their levels are related to the organism's physiological condition, to the presence of toxins and to biochemical changes prior to death. Chromatographic and mass spectrometric metabolomics is now used more and more in forensic toxicology, toxicological poisoning and post mortem interval estimation.[7]

D. Microbial Biomarkers

Microbial biomarkers are founded on the exclusive microbial communities harbored by the human body. The microbiomes of skin, mouth and gut can be used to link individuals to crime scenes, and the process of microbial succession following death can be used to help determine the post mortem interval. High throughput sequencing has greatly improved the use of Microbiome Analysis for forensics.[8]

E. Body Fluid Biomarkers

Characteristic DNA, RNA, protein and microbial signatures can be used to accurately identify blood, saliva, semen, sweat and vaginal secretions from body fluid biomarkers. In the field of sexual assault, homicide and violent crime, these biomarkers can be used to accurately be able to determine the source of the biological evidence and enhance the evidentiary value.[9]

3 Analytical Technologies for Biomarker Detection and Molecular Evidence Analysis

Advanced analytical technologies are essential to the successful use of molecular biomarkers in the forensic sciences, enabling the detection and characterization of biological evidence in forensic casework with high sensitivity, specificity and reproducibility. As a tool for the modern

[6] A. Chhikara, V. Gupta, P. Kumari, and J. Dalal, “Protein Biomarkers as Molecular Tools for Forensic Biofluid Identification,” J. Proteome Res., vol. 25, Mar. 2026, doi: 10.1021/acs.jproteome.5c00898.

[7] K. Lech et al., “Investigation of metabolites for estimating blood deposition time,” Int J Leg. Med, pp. 25–32, 2018, doi: DOI 10.1007/s00414-017-1638-y.

[8] D. A. Mohammed, O. A. Mahmoud, S. M. Ibrahim, F. B. Abed, A. Muhammed, and S. H. Muhi, “Serological Biomarkers in Forensic Science: A Review,” Baghdad J. Biochem. Appl. Biol. Sci., vol. 7, no. 2, pp. 110–124, 2026, doi: https://doi.org/10.47419/bjbabs.v7i2.449.

[9] Z. Liu, J. Wang, Z. Li, and G. Zhang, “mRNA for Body Fluid and Individual Identification,” Electrophoresis, vol. 46, no. 1–2, pp. 44–55, Jan. 2025, doi: https://doi.org/10.1002/elps.202400077.

forensic laboratory, molecular biology and analytical chemistry techniques are used to analyze genetic material, proteins, metabolites and other biochemical components that are recovered from a crime scene. These technologies can be used to analyse trace, damaged and mixed biological samples, which can enhance the identification and interpretation of evidence in forensics.[10]

A. Genomics

Genomic technologies are the basis of contemporary forensic investigation and investigation of genomic DNA and RNA biomarkers. The most common technique to amplify small amounts of DNA is the Polymerase Chain Reaction (PCR) and the most common method to identify a human being is Short Tandem Repeat (STR) profiling. With the development of Next-Generation Sequencing (NGS) technology, it is now possible to analyze several genetic markers simultaneously including STRs, Single Nucleotide Polymorphisms (SNPs), mitochondrial DNA and RNA biomarkers. These technologies aid in degraded samples, kinship testing, ancestry prediction and complex DNA mixtures, resulting in more reliable forensic evidence.[11]

B. Proteomics

Proteomics involves the identification and quantification of proteins used as tissue- or body fluid-specific biomarkers. An application for high resolution protein analysis is the ability to detect protein markers such as hemoglobin, prostate-specific antigen (PSA), semenogelin, and α-amylase by combining liquid chromatography with tandem mass spectrometry (LC-MS/MS). The use of forensic proteomics is expanding in body fluid identification, tissue typing, wound analysis and estimation of the post mortem interval, especially in the absence of or when DNA evidence is degraded.[12]

C. Metabolomics

Metabolomics is the study of metabolites, compounds with low molecular weight that are related to the physiological and biochemical processes. Metabolic profiles are commonly determined

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[10] S. T. Gopukumar, V. R. Patil, E. Benedicto, S. Gupta, and A. Sankar, "Innovations in Analytical Chemistry for Biomarker Detection in Chronic Disease Management," J. Appl. Bioanal., vol. 11, no. 4, 2025, doi: http://doi.org/10.53555/jab.v11i4.461.

[11] S. Tang, K. Yuan, and L. Chen, "Molecular biomarkers, network biomarkers, and dynamic network biomarkers for diagnosis and prediction of rare diseases," Fundam. Res., vol. 2, pp. 894–902, 2022, doi: https://doi.org/10.1016/j.fmre.2022.07.011.

[12] M. Babaei, S. Kashanian, H. Teck, and L. Frances, "Proteomics techniques in protein biomarker discovery," Quant. Biol., vol. 12, pp. 53–69, 2024, doi: 10.1002/qub2.35.

for biological samples using analytical platforms such as Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS) and Nuclear Magnetic Resonance (NMR) spectroscopy. Metabolomic analysis can be used to assist in toxicological investigations, detection of drugs and poisons, cause of death determination, and estimation of postmortem interval, tracking biochemical changes after death, in forensic science.[13]

D. Spectroscopy

Spectroscopic techniques measure the interaction of light and biological materials, and are used in forensic analysis to rapidly and non-destructively determine the composition of forensic evidence. Fourier Transform Infrared (FTIR) spectroscopy, Raman spectroscopy, and ultraviolet-visible (UV-Vis) spectroscopy are popular methods for identifying body fluids, biological tissues, drugs, explosives, fibers and trace chemical residues. The techniques involved in the screening require little sample preparation and provide a rapid investigation with the preservation of useful forensic evidence for subsequent analysis.[14]

E. Chromatography

Biochemical analysis of mixtures is impossible without separating them first by chromatographic means. Volatile compounds are usually analysed using gas chromatography (GC) and non-volatile and thermally unstable molecules are analysed using liquid chromatography (LC). When used in conjunction with the use of sensitive detectors, chromatography can be used to identify drugs, toxins, metabolites, and environmental contaminants in biological samples. Due to its high separation efficiency and high accuracy, it is still an important instrument for the forensic toxicological and biochemical evidence analysis.

F. Mass Spectrometry

For the purposes of forensic science, mass spectrometry (MS), which is an analytical technique with outstanding sensitivity, specificity and molecular identification power, has proved to be one of the most powerful tools. MS is used to accurately identify proteins, peptides, metabolites, drugs, toxins and other biomolecules found in forensic samples, by measuring the mass-to-charge ratio of the ions. Forensic science has greatly benefitted from techniques like LC-MS/MS,

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[13] D. Vo and K. T. L. Trinh, "Emerging Biomarkers in Metabolomics: Advancements in Precision Health and Disease Diagnosis," Int. J. Mol. Sci., 2024, doi: https://doi.org/10.3390/ijms252313190.

[14] S. Roy, J. Hauer, and K. Sankar, "Development of non-invasive diagnosis based on FTIR spectroscopy," Vib. Spectrosc., vol. 134, p. 103724, 2024, doi: https://doi.org/10.1016/j.vibspec.2024.103724.

MALDI-TOF, and Orbitrap MS, which have facilitated detailed molecular profiling, quantitative analysis, and biomarker identification in forensic investigations. The combination of mass spectrometry with genomics, proteomics and metabolomics remains a powerful tool for molecular evidence analysis and aids in very confident forensic decision making.

4 Applications of Molecular Signatures in Crime Scene Investigation and Human Identification

The use of molecular signatures has proven to be an essential tool in forensic science, for the accurate identification, interpretation and reconstruction of crime scenes. DNA, RNA, proteins, microbial communities, and metabolites are sources of biomarkers that can be obtained from biological samples, even in the absence of significant quantity, quality, and/or mixing, and can assist in forensic investigations. Their use with the use of advanced analytical technology has helped to make forensic analyses more reliable and more admissible in court.[15]

A. Body Fluid Identification

Identifying body fluids is an important part of a forensic investigation, especially in homicide, sexual assault and violent crime investigations. Molecular biomarkers like tissue-specific RNA transcripts, proteins like hemoglobin, prostate-specific antigen (PSA), semenogelin and α-amylase are used to accurately differentiate blood, semen, saliva, urine and other biological fluids. These biomarkers are more sensitive and specific than traditional chemical tests, and can help investigators determine the source of biological evidence without compromising the samples available for DNA profiling.[16]

B. Postmortem Interval Estimation

Estimating the post mortem interval (PMI) is crucial for estimating the time since death. Biomarkers at the molecular level such as RNA degradation patterns, protein decomposition profiles, metabolite changes and microbial succession offer valuable insights into the post mortem biochemical processes. These biomarkers, when analyzed together, are more accurate

[15] R. Sinha, B. Sharma, A. Sharma, and H. Pradesh, "Emerging Applications Of Microbial Forensics In Criminal Investigations: A Review," Int. J. Eng. Appl. Sci. Technol., vol. 10, no. 02, pp. 86–93, 2025.

[16] H. Dash and A. Das, "Detection and identification of forensically relevant body fluids," 2026, pp. 35–48. doi: 10.1016/B978-0-443-30128-5.00010-2.

in estimating PMI than the traditional ones, which are dependent on body temperature, rigor mortis, and livor mortis, especially in the case of variable environmental conditions.[17]

C. Forensic Toxicology and Pathology

In forensic toxicology, molecular biomarkers are highly important in the detection of drugs, alcohol, poisons and their metabolites from biological specimens. The metabolome and the proteome are used to help determine the cause of death, toxic exposures and physiological responses to injury or disease. In a forensic context, biomarkers can aid in the characterization of tissues, in the estimation of wound vitality and in the distinction between ante mortem and post mortem injuries, thus complementing medical and legal conclusions.

D. Human Identification and Crime Reconstruction

Short Tandem Repeats (STRs), mitochondrial DNA and Single Nucleotide Polymorphisms (SNPs) continue to be the preferred markers for human identification. These molecular signatures help identify victims, to analyze kinship, disaster victim identification (DVI), and suspect verification (SV). In addition, the use of genetic, proteomic, metabolomic, and microbial evidence can be combined to reconstruct the events of a crime and establish links between individuals, biological materials, objects, and crime scenes. This all-encompassing molecular information helps to ensure that forensic information is scientifically accurate and aids towards just and reliable verdicts.[18]

5 Challenges, Emerging Innovations, and Future Perspectives in Molecular Forensics

Although molecular forensics has advanced rapidly, a number of scientific, technical, ethical and legal issues remain that impact the real-world use of the science in criminal investigations. At the same time, new technologies are enhancing the forensic analysis capabilities, offering more efficient, accurate and intelligent ways to interpret evidence.

A. Challenges

Although great advances have been made, molecular forensic analysis is hampered by the presence of degraded, contaminated and small template biological samples that can affect the ability to detect or interpret biomarkers. There can be more reasons for loss of analytical

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[17] J. L. R. Lopez and M. P. Navadijo, "Estimation of the post-mortem interval: a review," Forensic Sci. Int., vol. 369, 2025, doi: https://doi.org/10.1016/j.forsciint.2025.112412.

[18] M. S. Balbudhe, B. S. K. Shetty, N. A. Kumar, D. Singh, and A. I. Sherekar, "Crime scene reconstruction: a scoping review," Egypt. J. Forensic Sci., vol. 15, no. 79, 2025, doi: https://doi.org/10.1186/s41935-025-00495-5.

reliability, such as mixed DNA profiles, environmental degradation, or improper sample handling. The standardization of laboratory procedures, the validation of new biomarkers and the quality control are still essential for guaranteeing reproducible and legally valid results. Ethical and legal issues are also emerging in connection with the growing application of molecular evidence, including issues of genetic privacy, informed consent, data security, forensic DNA databases and the misuse of sensitive genomic information. It is thus very important that there is transparency, regulatory compliance and scientifically proven methodologies in order to ensure public trust and to ensure judicial integrity.[19]

B. Emerging Innovations

In recent years, technological advances have been making molecular forensic investigations more dynamic, including the incorporation of artificial intelligence (AI), machine learning, and multi-omics approaches. These algorithmic tools which can be assisted by AI will help to interpret complex DNA mixtures, automate pattern recognition, and help to improve the predictive analysis of large forensic datasets. However, the new fields of genomics, transcriptomics, proteomics, metabolomics and microbiomics allow for a more comprehensive characterization of the biological evidence, offering higher accuracy than single-biomarker analyses. Portable forensic technologies are also helping to lower laboratory turnaround times and enable real-time analysis at the crime scene, such as rapid DNA systems, hand-held spectroscopic instruments, biosensors and microfluidic lab-on-a-chip platforms. These innovations enhance the efficiency, sensitivity and availability of molecular forensic investigations.[20]

C. Future Perspectives

Future molecular forensic studies are likely to center on the identification of very specific biomarkers, standardized multi-omics workflows, and more powerful computational tools to allow for integrated evidence interpretation. The future will see further advances in next-generation sequencing, high-resolution mass spectrometry and artificial intelligence-powered bioinformatics, which will allow for improved analysis of trace, degraded and mixed biological samples. International harmonization of analytical protocols, ethical guidelines and legislation

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[19] Y. Sai, “Advancing forensic science: Addressing challenges and embracing emerging technologies,” Forensic Sci. Today, vol. 8, pp. 1–5, 2022, doi: https://orcid.org/0000-0002-6151-5687.

[20] S. S. Daga, U. Ahmed, and R. K. Kumawat, “Forensic Tools and Techniques of Absolute Human Identification: Physical and Molecular Approach,” J. Forensic Sci. Crim. Investig., vol. 15, no. 3, 2021, doi: 10.19080/JFSCI.2021.15.555913.

will be vital to maintaining uniformity of forensic practice across jurisdictions. The future of precision forensic science as it advances will enable molecular biology, analytical chemistry and intelligent data analytics to further enhance the reliability of forensic evidence, improve crime scene reconstruction, and further create more objective and scientifically sound criminal justice systems.[21]

6 Conclusion

The advent of molecular biology and biochemical analysis has greatly helped the science of forensics, offering legally sound, highly sensitive and precise methods for criminal investigations. The use of molecular signatures, based on DNA, RNA, protein, metabolites, microbial community and body fluids, has broadened the range of forensic evidence that can be used to identify a human subject, determine nature of a body fluid, estimate the postmortem interval, establish a toxicological profile, and reconstruct a crime scene. The development and use of new analytical technologies such as genomics, proteomics, metabolomics, spectroscopy, chromatography and mass spectrometry have improved the identification and analysis of trace and degraded biological samples. However, sample quality, analytical standardization, ethical issues and legal admissibility are key issues that should be under constant debate. The advent of AI, multi-omics analyses, mobile forensic platforms, and computational bioinformatics will enhance the pace, precision, and breadth of forensic science. Ongoing research, collaboration across borders and uniform forensic protocols will further support the use of molecular biomarkers for a better decision-making process, and make the criminal justice system more reliable, transparent, and scientifically sound.

7 Reference

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[4] T. Huebner, M. Steffens, and C. Scholl, “Molecular Genetic Techniques in Biomarker Analysis Relevant for Drugs Centrally Approved in Europe,” Mol. Diagn. Ther., vol. 26, no. 1, pp. 89–103, 2022, doi: 10.1007/s40291-021-00567-x.

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[8] Z. Liu, J. Wang, Z. Li, and G. Zhang, “mRNA for Body Fluid and Individual Identification,” Electrophoresis, vol. 46, no. 1–2, pp. 44–55, Jan. 2025, doi: https://doi.org/10.1002/elps.202400077.

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[13] S. Roy, J. Hauer, and K. Sankar, “Development of non-invasive diagnosis based on FTIR spectroscopy,” Vib. Spectrosc., vol. 134, p. 103724, 2024, doi: https://doi.org/10.1016/j.vibspec.2024.103724.

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