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NARRATIVE REVIEWS

Serological Biomarkers in Forensic Science: A Review

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Abstract

Background: Biological evidences, e.g. blood, semen, vaginal secretion, saliva, urine, and sweat encountered at the crime scene, are the most pervasive in surroundings. Their existence helps in associating the assassin with the victim as well as with the crime scene. Our motivation for writing this review was the lack of a recent, thorough review on the subject appropriate for the use of all scientists involved with serological research in forensic science.
Objective: This comprehensive review aims to discuss the value of serological biomarkers for a wide variety of forensic applications.
Methods: This review summarizes a comprehensive body of work on serological biomarkers, with the inclusion of all types of markers and advanced methodologies available. It discusses a plethora of methodologies used to differentiate between body fluids, with a deeper look at emerging, high-throughput techniques.
Results: The review provides a detailed discussion on both established body fluids and novel serological markers. It acknowledges the difficulties involved in selecting a body fluid differentiation methodology for forensic contexts. A key trend noted is the use of an interdisciplinary approach in the most recent publications.
Conclusion: The interdisciplinary approach is essential for the future of forensic work and is necessary for success in protein and body fluid research. This review serves as a thorough summary of serological biomarkers and their applications, capturing the overall trends in the field.

1. Introduction

Serological and bloodstain analyses play an increasingly important role in forensic investigations [1]. In the past few decades, forensic serological methods have developed significantly, making it possible to detect and analyze minute bloodstains on complex substrates [2]. However, these serological analytical methods exclusively provide information obtained using other techniques. An efficient technique is necessary for the isolation and identification of body fluid components [3]. Molecular biological methods have become well established and allow for the detailed analysis of these serological markers in trace amounts of body fluids [4]. However, in most countries, deoxyribonucleic acid (DNA) analysis is not admissible if it is not preceded by preliminary findings that confirm the presence of body fluids [5]. Importance of serological evidence in different legal cases is undeniable in the justice systems of various countries [6]. Today, the purpose of serological analysis has gone beyond individual identification and evidential evaluation. Serological analysis has further expanded to assess the challenges of forensic science and its practical implications in crime scene management and the justice system [7].

Understanding the development of biomarkers and their properties in these contexts has been useful for delineating the role and application of the existing biomarkers [8]. This review examines various serological methods and

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biomarkers, offering new insights into their applications, potential benefits, challenges, and practical values. It underscores the relevance and effectiveness of serological methods in maintaining both legal and scientific validity [9,10].

Serological evidence has played a crucial role in solving criminal cases for decades now. Serological investigators strive to examine biological evidence using body fluid-specific markers that are reliable, reproducible, non-invasive, species-specific, and collected in a painless manner [7,11]. The courts accept the results of forensic analyses for legal proceedings. Over the past century, several serological methods, such as ABO blood group typing, human blood protein typing, and hemoglobin and serum protein polymorphisms, have been developed and widely used in forensic practices [12]. As an adaptation to low detectable quantity requirement of forensic traces, molecular techniques are used to quantify and characterize nucleic acids [13]. In recent years, more research has been devoted to the study of a variety of serologic-specific markers and biological fluids; however, researchers have encountered many challenges in serological research, including the lack of a systematic review of these markers [14]. Forensic science has substantially developed and transformed from a single serological method to DNA technology. Most laboratories involved in serology have stopped seeking developing new serological techniques in favor of DNA-based methods [15]. Several countries have restructured forensic systems, affecting standardization and training [16]. Recent reports have focused on improving scientific methods of research in the forensic science community. Therefore, the explanation of serological findings has a significant impact on the judicial process and the public in terms of its cultural and historical significance [6]. Although there is an increasing amount of literature, no recent systematic review has compiled advancements in serological biomarkers from a forensic standpoint. This article aims to fill that void.

2. Aim and Scope of the Review

This review explores serological biomarkers, a crucial subgroup of biological markers used in forensic science, from crime scene investigation to routine casework. It covers theoretical aspects, sources of articles, forensic science subdomains, topics, and methodologies. This review aims to provide insights into common serological biomarkers in human blood, emphasizing proteomic and genomic techniques. This review aims to provide critical analyses, fill knowledge gaps, and summarize recent and potential forensic applications of serological biomarkers. To ensure a comprehensive and unbiased review, this study systematically examined recent literature utilizing PubMed, Scopus, and Web of Science databases. The search strategy employed a combination of keywords, such as "serological biomarkers," "forensic science," and "body fluid identification." Only peer-reviewed publications in English published from 2010 to 2024 were considered, while gray literature and nonscientific sources were excluded.

3. Basic Concepts in Serology

Previous studies described that serological biomarkers are one of the most relevant scientific approaches in the field of biological fluids and secretion recognition. However, reviews focusing on the importance of serological markers in the biological field are still scarce [17]. The purpose of this manuscript is to provide an overview of serological markers to underline the importance of biomarkers, such as enzymes, viruses, bacteria, and proteinaceous compounds, especially proteomic-related studies; the review also provides some basic background and definition of serology [7].

Serology is a branch of biology, known as immunology, that focuses on the analysis of serum, other biological fluids, and immunoglobulins [3]. Immunoglobulins are expressed in most vertebrates and some invertebrates. Based on three different types of immunoglobulins known to date, mammals and other species (excluding birds) are functionally capable of expressing the following five different classes of immunoglobulins: immunoglobulin A (IgA), immunoglobulin D (IgD), immunoglobulin E (IgE), immunoglobulin G (IgG), and immunoglobulin M (IgM), which provide a defense against all types of foreign pathogens [18]. IgG, IgM, and IgA are referred as common or circulating antibody forms, whereas IgD and IgE have unique functions in the host [19]. IgD intervenes in the activation of B cells, especially to promote the attachment of B cells to the epithelia, principally at the respiratory level, while IgE provides defense against parasites and participates in allergic reactions [20]. The existence of serological markers within a biological fluid, whether secreted or excreted from the human body, is crucial for indicating its origin [21]. Hence, several novel papers published on this subject have focused on the analytical and immunological definition of such biomarkers. More specifically, several enzymes, viruses, bacteria, and even proteinaceous secretions are known to mediate serological functions [3].

3.1. Definition and Scope of Serology

Forensic serology is a key component in linking a victim to a crime scene or an item associated with an offender [22]. The prefix observation is based on the breadth of our examination of bodily fluids, primarily serum, and the cells concerned with immunological functions derived from those fluids [23].

Systematic study, observations, and evaluations: Following the police investigations and searches, suspected items are systematically examined at a crime scene for the presence of shed blood and other bodily fluids frequently encountered in various types of violent crimes [24]. These steps are part of the overall forensic identification or serological process involving characterized analysis and source of the biological fluids recovered [2]. These assays are classified as serological examines because they require separation of the liquid part of decoagulated blood, termed serum. The developed serological classification includes semen serology [25].

Serology originally referred to a broad scope of study involving a variety of biological markers primarily present in the liquid portions of the blood, insect venom, antitoxins, and through allergic-transferred antibodies present in a patient's serum [26]. Modern-day serology has been significantly narrowed down to the biological identities and similarities characteristic of proteins, such as immunoglobulins, and different classes of proteins of lymphocytes, such as cytokines and receptors, mostly associated with crimes of violence in which the bodily fluids of the suspect or the victims are left behind [6]. Owing to a broad paradigm of one's antecedent or asset identity, serology is a central research theme attracting studies from biology, human reproduction, animal research, biotechnology, immunology and immunochemistry, recombinant DNA research, genetics, medicine, pharmacology, mostly as practical diagnostics, proteomics/antibody omics, pharmacogenomics, and forensic science [15,27].

3.2. Types of serological markers

Serological markers are used by forensic investigators to identify human body fluids. They can be categorized based on their origins and biological relevance. Some well-known serological markers and body fluid categories in which they can be classified based on their application are described as follows [28].

Genomic, transcriptomic serological markers: These markers are exclusively high-copy-number DNA sequences that are characteristic and unique to a particular body fluid [29]. The genes involved in this category are those directly associated with body fluid-specific functions and/or expressed at a very high level, higher than in any other body fluid. Blood, saliva, and semen are the fluids included in this class [30].

Proteomic serological markers: Proteins are the final products of gene expression and are involved in most biological functions, making them excellent candidates [31]. Different fluids have protein profiles that are used as markers. Saliva is detected using protein markers [32]. Peptides in tears are identified in a "positive protein roll." Principal proteomic positions are identified for vaginal fluid identification [33]. Sweat, menstrual blood, and other minute biological information are found where larger proteins are predominant [34]. Studies have identified hundreds of proteins and molecular species in forensic stains and traces [35]. Despite these findings, technological and biological obstacles have conspicuously confined the evolution of molecular markers to the routine forensic landscape [36]. Many identified markers await forensic applications despite their potential in medical fields. Recently, a new class of tissue-nonspecific biological markers has been defined in artificial forms [37]. Table 1 shows how serological biomarkers and analytical methods have changed over time. It also explains how they are used in forensics.

The effective detection and analysis of these biomarkers are contingent upon several fundamental serological techniques, which are outlined as under.

4. Core Serological Techniques

Serological techniques in forensics began with blood and blood group identification in the early 20th century, followed by grouping of other body fluids [38]. Various techniques are developed to identify body fluids, proteins, and cells, incorporating both ancient and contemporary perspectives for forensic casework requirements [39].

The established identity test enables subsequent analysis in preliminary group testing to identify body fluids, proteins, and cells, incorporating both ancient and contemporary perspectives for forensic casework requirements [40,41]. Protocols for extracting body fluid components and establishing biomolecular approaches are reviewed, emphasizing mechanistic strategies [42,43]. A critical discussion of reliability, validation, and limitations of unique antigens in serological tests is considered. Accuracy and reliability are major criteria of forensic tests for investigation and proving innocence [44]. The scientific community in forensic serology and molecular techniques must comply with regulations specified in the domain of forensic legal bioanalytics [45]. The introduction, signs, and consequences of forensic serology in legal proceedings are discussed, requiring that serological evidence testing aligns with law enforcement frameworks and judicial regulations [46]. This review provides basic knowledge for evaluating new genes and methods to understand how genes, proteins, or extracted fluids are validated and utilized forensically once identified in tissue-specific or body fluid processes. This knowledge is relevant to forensic scientists and biomedical researchers [47,48].

Blood group phenotypes were the first biomarker system studied in serology based on blood group antigens on cell surfaces [49]. The ABO and Rh blood group systems are the earliest and the most important systems to be considered in casework [50–52]. This technique converges between reference samples and unknown samples taken from the crime scene or suspects [53], which is a good evidence procedure for personal identification [54–57]. Blood groups have unique ethnic characteristics that distinguish them from one another [58]. Blood group analysis, central to forensic casework, requires multi-analytical approaches for assessing evidence value in courts [59].

Enzyme-linked immunosorbent serologic assay (ELISA) is one of the cardinal techniques in serology for discovering biomarkers [7]. ELISA is a method in which the basic principle of an immunoassay is employed to determine the presence of specific proteins or antibodies in a sample [60]. ELISA offers superior detectability, sensitivity, and specificity, compared to conventional methods for discovering biomolecules [61,62]. A key forensic application is detecting human antibodies in suspected ante-mortem blood samples, as commercial kits for the unique biochemical profile of postmortem blood testing are unavailable [63–65]. Because immune response levels mirror antigen volume, this technique applies to histopathological and cellular response examinations [66,67]. Gas-phase ELISA, developed as a new detection platform, is highly reliable and scalable for forensic experts [64].

Table (1): Evolution of serological biomarkers and changes in techniques over time in forensics.

Technique/approach	Approximate period of adoption	Primary biomarker target	Principle/analytical basis	Main forensic application	Key advantages	Limitations/challenges
ABO blood typing	Early 1900s	Blood group antigens (A, B, O)	Agglutination and antibody–antigen reaction	Early identification of blood source and donor compatibility	Simple, inexpensive, foundational for forensic serology	Low specificity; cannot individualize samples
Precipitin and microcrystalline tests	1920s–1950s	Species-specific serum proteins	Formation of antigen–antibody precipitates or crystals	Differentiation of human vs. animal blood	Quick field differentiation	Subjective interpretation; low sensitivity
Enzyme-linked immunosorbent serologic assay (ELISA)	1980s	Proteins and antibodies	Enzyme-conjugated antibody detection	Identification of specific body fluids (e.g., semen and saliva)	Sensitive, adaptable to multiple fluids	Cross-reactivity; false positives in degraded samples
Immunochromatographic (lateral-flow) tests	1990s	Body fluid-specific proteins	Capillary migration and labeled antibody binding	Rapid on-site screening of stains	Portable, easy to interpret	Semi-quantitative; limited specificity
Western blot/immunoblot test	1990s–2000s	Proteins and peptides	Electrophoretic separation followed by antibody detection	Confirmation of protein markers	High specificity	Labor-intensive; not field-deployable
Mass spectrometry (LC–MS/MS, MALDI-TOF)	2010s	Peptides and proteomes	Peptide fingerprinting by mass–charge ratio	Proteomic profiling of body fluids and tissues	High sensitivity, molecular specificity	Requires expensive instrumentation and expertise
Next-generation sequencing (NGS)	2010s–present	DNA, RNA (mRNA, miRNA)	Parallel sequencing of nucleic acids	Tissue and body fluid identification; mixture deconvolution	High throughput; detects degraded or mixed samples	High cost; legal validation ongoing
MicroRNA (miRNA) and transcriptomic profiling	2010s–present	Regulatory RNA molecules	Expression pattern analysis	Discrimination of body fluid origin and age estimation	Stable in degraded samples; tissue-specific	Requires standardization; bioinformatic complexity
Multiplex and integrated “omics” platforms	2020s	Combined DNA, RNA, protein signatures	Multi-analyte detection using microarrays or biosensors	Comprehensive identification and contextual analysis	Integrates genomics, proteomics, and forensic validation	Need for universal validation and admissibility guidelines

Notes: miRNA: microRNA; mRNA: messenger RNA; LC–MS/MS: liquid chromatography-tandem mass spectrometry analysis; MALDI-TOF: matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Immunohistochemistry (IHC) is used to identify the antigens of interest within tissue samples. The principle of IHC is to use specific antibodies tagged with detectable markers, such as enzymes and fluorescent molecules [68]. Once applied to tissue sections, antibodies bind to their specific antigens in a reaction that can be observed microscopically, depending on the label used [69,70]. IHC can be applied to organs and tissues at crime scenes, including the brain, reproductive organs, skin, and bone marrow. Vaginal or anal swabs enable retrospective diagnosis using postmortem material [71]. IHC sensitivity depends on the procedure, and specificity increases through application and quantification. Results should be interpreted considering protein levels in healthy subjects [72–74]. IHC results depend on sample quality. Sensitivity varies based on tissue predictive value and antigen distribution, leading to possible false negatives, although false positives are rare [75]. The main challenge of IHC is antigen retrieval, which causes nonspecific reactions. Evaluation requires establishing reliable cutoff points for positive/negative results in reports. Other methodological factors can affect accuracy of results [72].

Researchers specializing in forensic serological and molecular techniques are consistently encouraged to comply with the regulations and guidelines established within the domain of forensic legal bioanalytics. These include international standards and guidelines such as those established by the Scientific Working Group on DNA Analysis Methods (SWGDAM), the European Network of Forensic Science Institutes (ENFSI), and the ISO/IEC 21043 series for forensic science practices [76–78].

5. Applications of Serological Biomarkers in Forensic Science

Over the years, serological analysis has been appreciated for facilitating the main applications in forensic cases [17]. At another level, it is common for crime scene practitioners to develop serological contextual evidence comprising probable suspects and victims [79]. Routine examinations produce serological matches at crime scenes, while specific methods help to link suspects to crime scenes or connect evidence from different stains [12]. Serological analyses enable proportion-based research at crime scenes, establishing associations between individuals and items while providing contextual evidence of multiple participants [80].

Serological evidence remains central to forensic casework and legal adjudications. Accurate serological results assist in examining factual biases [81]. Serological methods eventually provide objective confirmation [82]. However, serological investigations of variable origins and technologies tend to produce inter- and intra-laboratory differences in data interpretation [83]. Moreover, serological response to identically distributed factors may affect the findings [27].

One of the most straightforward methods for body fluid recognition is identifying the type of fluid [7]. Specifically, body fluids can connect suspects to crime scene. Saliva, semen, vaginal secretions, and menstrual blood are key serological fluids in forensics [84]. In rape cases, semen is arguably the most valuable physical evidence because of the genetic significance of sperms [42]. Contemporary validation studies use these testing methods, including DNA profiling and microRNA (miRNA) analysis [85], because body fluids vary in evidentiary strength. Cases are reported where serological fluids detected biological substances in forensic work [86]. The most basic prerequisite for reliable findings is using validated assays. When using ancient DNA, strict procedures must prevent contamination by new DNA. Modern molecular biology supports assays of body fluids, as shown in studies [87,88]. Serological markers can provide links between crime scenes and suspects or victims. When bodily fluids are recovered from crime scenes through evidence containing cellular debris or waste fluids, matching these samples with reference samples can link individuals to the crime site [28,89]. In cases where body fluids or tissues match a victim, DNA profiling can link victims with suspects [90]. Biological trace evidence can shed light on investigative variations. If the offense time is identified, serological markers help to narrow down the crime timeframe [6]. Reported data on testosterone products, which are secure sperm biomarkers, are derived from peak levels within 24–78 hours period during which DNA integrity begins to decline, making genetic linkage more difficult [91].

Serology has supported both mapping and confessions in several significant adversarial situations [92]. Research shows a correlation between DNA profiling and confession rates in serial crime investigations. Offenders identified through behavioral patterns often confess or go through DNA testing. Several developments now include advanced technologies for analyzing serological evidence [93]. These protocols cannot eliminate mixed profiles. Some cases guide to matches for bodily fluids. Ethical issues exist in collecting, preserving, and testing fluid evidence [94]. High contamination rates and contamination without validation are common issues in police custody. This review discusses database warehouses for profiling but not for DNA purification from evidence [95,96]. DNA ligands with affinity to serological conservation biomarkers are under study but lack validation for *in vivo* legal use [97,98].

5.1. Challenges and limitations

One of the challenges faced by forensic RNA/DNA molecular techniques is the absence or low concentration of nucleic acids for analysis as shown in Table 2. A single body fluid can contain different messenger RNA (mRNA) species and various miRNAs [99]. This tissue-specific characteristic aids screening but complicates interpretation when reference samples from victim and perpetrator are unavailable [100]. The abuse of this evidence has led to laws regulating its collection and use [100].

Degradation and stability: A key limitation is biomarker degradation in forensic science [101]. Studies show reduced biomarkers in aged biological fluids [102].

Table (2): Challenges and proposed solutions in serological analysis.

Challenges	Underlying cause	Impact	Recent or proposed solution
Biomarker degradation	Environmental exposure (UV, humidity, microbes, pH)	Reduced detection of nucleic acids and proteins	Cold storage (<–70°C), stabilizing buffers, detection of degradation-resistant markers (miRNA, peptides)
Low biomarker concentration	Trace evidence or degraded fluids	False negatives; unreliable quantification	Multiplex assays, NGS enrichment, improved extraction kits
Cross-reactivity/nonspecific binding	Homologous proteins between fluids	Misclassification of body fluid types	Peptide-specific antibodies, orthogonal confirmatory tests
Sample contamination	Poor handling, secondary transfer	Compromised integrity and interpretation	Strict evidence-handling protocols, separate pre-/post-PCR laboratories
Inter-laboratory inconsistency	Varied reagents, methods, equipment	Lack of reproducibility	ISO/IEC 21043-compliant validation and shared reference standards
Incomplete forensic validation	Rapid biomarker discovery without legal testing	Limited court admissibility	SWGDAM and ENFSI protocol validation, publication of inter-lab results
Ethical and legal regulation	Misuse of genetic or molecular data	Privacy concerns, data restrictions	Implementation of forensic-specific data governance and ethical oversight

Note: miRNA: microRNA; SWGDAM: Scientific Working Group on DNA Analysis Methods; ENFSI: European Network of Forensic Science Institutes; PCR: polymerase chain reaction.

Degradation depends on molecular properties, environmental conditions, and storage methods [103]. Factors such as temperature, UV light, microorganisms, pH, humidity, and aeration affect degradation rates [104]. DNA's half-life in bones is 53 years, while protein's half-life ranges from 7 to 3½ years in the skin and teeth [105]. The skin and blood biomarkers show good stability, while semen and saliva degrade significantly [106]. Quick assessment of biological fluids is crucial, as fresh samples contain more RNA and protein [107]. Low analyte concentration results in minimal degradation. Older stains show decreased initial concentration, risking molecular degradation [108]. Storage conditions below -70°C minimize degradation. Improper storage causes false results [109]. Serological protein degradation occurs within hours [63]. Recent studies have used mass spectrometry and metabonomic, aiming to detect more stable molecular fragments [110].

Serological assay sensitivity: It is a fundamental aspect, as molecular targets have varying sensitivity levels [3]. This affects implementation if concentrations of biomarkers decrease below detectable levels [111]. Legal considerations are crucial when evaluating serological assays, requiring reliability and validity for acceptance in court [112,113]. Low detection thresholds result in false-negative reactions during analysis [114]. Saliva may contain analytes below detection limits, preventing identification in 60% cases [115]. Low sensitivity often stems from DNA extraction steps [116]. Sample quality deteriorates over time, with marker concentrations varying with temperature, humidity, substrate, and cell number [117]. Commercial testing produces many false negatives. Research focuses on detecting human-specific biomarkers through multiple body fluid indicators [118]. Studies can optimize multi-marker panels by analyzing traces. High false-negative proportions lack court reliability [119]. Most immunoassays operate in a binary mode, requiring subjective interpretation [120]. These limitations potentially hamper probative information. Serological findings continue to integrate with other tools [121].

6. Emerging and Recent Advances in Serological Biomarkers

In recent years, serological biomarkers have improved significantly. Next-generation sequencing (NGS) provides extensive genetic information while reducing costs [122]. It has increased sensitivity for genotyping bloodstains at lower expression levels, even by using microfluidic machines. Forensic RNA parallel sequencing has simplified marker selection [123]. Microfluidic technologies are integrated into laboratory workflows, providing integrated systems for processing samples, including protein, RNA, and DNA extraction [124]. This technology can revolutionize forensic procedures by faster delivery of evidences. Microdevices can classify body fluid sources [125]. These advancements significantly improve sensitivity and reliability compared to traditional identification methods [2]. Several cases using NGS to amplify DNA from high-copy materials have been reported, including analyses of bloodstain RNA interpretation [126]. While this represents progress in RNA interpretation, data that are

more experimental are needed to understand body fluids' genetic makeup [95]. It effectively differentiates blood from saliva and semen. Studies aim to integrate gene markers, proteins, cell-overabundance markers, RNAs, and DNAs for multi-genomic classification of body fluids [85]. Novel RNA sequencing techniques are being developed for serological analysis. Discussions continue about working with acetylated and histone marker proteins to differentiate tissues, reflecting epigenomic approaches. Temporal marker possibilities have emerged [127].

6.1. Next-generation sequencing

Since the advent of NGS, "massively parallel" sequencing technology has transformed the application of genetic serological biomarkers in forensic casework as shown in figure 1 [128]. Until recently, sequencing was labor-intensive and time-consuming, compared to other molecular genetic techniques for forensic testing [129]. Sequencing provides more sensitive and legally admissible information in forensic applications [130]. NGS's ability to generate sequence reads enhances information retrieval from body fluids [131]. Enhanced sensitivity in analyzing low-level DNA occurs as NGS generates numerous reads. The data better capture body fluid complexity by increasing obtainable information beyond Short Tandem Repeat (STR) data [132].

The forensic application of NGS generates data less dependent on fragment length, making it applicable to trace degraded samples, such as rootless head hair [133]. Small sample input requirement benefits degraded body fluid and mixed DNA casework samples [83]. This technology can remedy disruptions as whole-genome data assist in testing relatedness and ancestry [134]. Interest in these aspects of forensic caseworks is a significant future area of exploration. Current hurdles to utilization of NGS include interpretation, validation, cost, ethics, and an expanded forensic genetic profile system [135,136]. Source assignments with trace body fluid material may involve low percentages of cell content. Forensic studies have

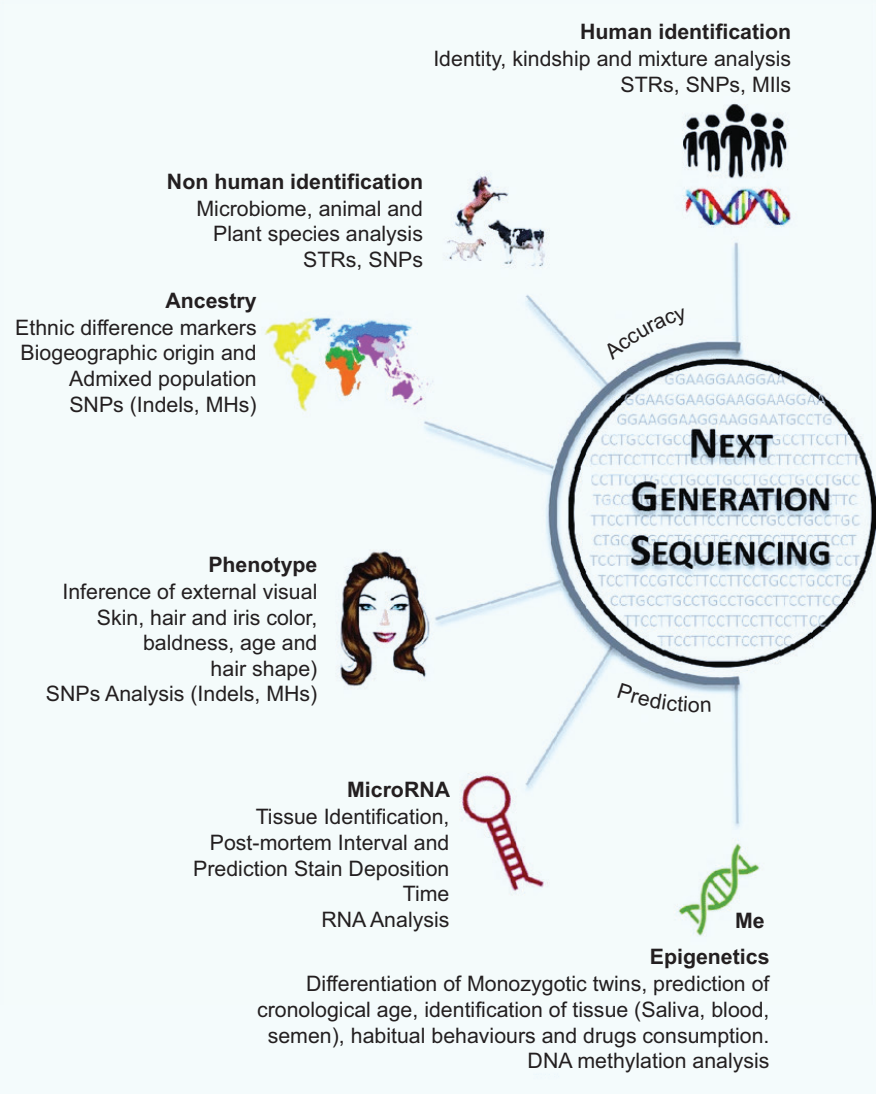

Figure (1): Diverse applications of next-generation sequencing (NGS) in forensic science, highlighting its capacity to enhance accuracy, prediction, and depth of analysis. NGS is a cutting-edge molecular tool used to analyze DNA and RNA, enabling various types of biological and forensic investigations [137].

demonstrated application of NGS in degenerated trace body fluid samples [95].

Nevertheless, challenges persist regarding validation, cost, and ethical implications, emphasizing the need for standardization under forensic guidelines (SWGDAM; ENFSI, and ISO/IEC 21043).

6.2. Microfluidic technologies

Technological advancements in microfluidics and nanofluidics have led to innovative techniques for serological analysis [138]. Miniaturized biological fluid-handling systems utilize volume system range from microliter (μL or 10^{-6}) to attoliter (aL or 10^{-18}) quantity [139]. These systems have proven important for high-quality serological examinations in forensic contexts [143]. Microfluidic devices contain interconnected channels for fluid handling, enabling pumping, mixing, and sorting assays [140]. Microfluidics integrates fluid handling with micro-total analysis systems for extraction and purification after DNA and RNA analyses. These systems enable high-throughput screening using small sample volumes, crucial for forensic examinations [13].

Microfluidics offers advantages include low consumption of reagents, ultra-low power usage, faster reactions, and shorter assay period [141]. These systems allow examinations to be completed in time comparable to expert serological examination of evidence [142]. Integration of flow processing into forensic laboratory workflow could reduce result reporting time [143]. These techniques have successfully developed sensitive screening systems and antibody-based identification of body fluids [106]. However, adoption of flow processing may be limited by operational complexities, low reliability, limited practical applications, commercial unavailability, and need of experienced personnel [144]. While flow processing could revolutionize forensic serology, it requires broader development and determination of DNA specificity and legal validation [145].

6.2. Artificial Intelligence (AI), Machine Learning (ML), and Next-Generation Identification Systems

In addition to advancements in sequencing and microfluidics, AI and ML are increasingly recognized as pivotal analytical tools in forensic serology [146], because AI can handle large datasets that are difficult to analyze manually [147]. AI-driven models can classify body fluids, interpret complex mixtures, and predict degradation levels using multi-omic datasets. The use of ML methodologies for discovery of biomarkers is critical to analyze various types of data used for biomarker discovery, such as mass spectrometry, nucleotide and protein sequencing, and image (e.g., computed tomography [CT]-scan) data [148]. Furthermore, emerging modalities, such as magnetic fingerprinting and digital molecular signatures, are under investigation, offering non-destructive, automated, and highly specific trace detection [149,150]. The integration of these technologies into forensic laboratories has the potential to create next-generation identification systems that are rapid, data-driven, and judicially robust [151,152].

7. Future Directions and Potential Research Areas

In criminal casework, serological analysis combined with DNA analysis minimizes the risk of misinterpreting biological evidence, enhancing the accuracy of investigations [153]. Workflows using shotgun proteomics help determine biofluids and reveal covert drug administration [154]. Advances in forensic science and NGS enable sophisticated developmental research [155]. Serological markers are primarily antigens on blood cells, while soluble marker tests detect drugs, predict age, and determine death timing [156]. These developments enhance sensitivity across forensic scenarios, including wildlife crimes and victim identification [157]. Specific characteristics require validation, considering ethical implications [158]. Validation and standardization remain challenging, requiring multidisciplinary collaboration [159]. Evidence shows growing interest in molecular markers for analyzing biological traces [160]. Progress has been made in detecting low-abundance molecules and hybrid serological markers [161]. Cellular antigens and proteins connect secretions to blood composition [162]. Recent years have observed increased development of sensitive biomarkers for specific crime cases [163]. Forensic science has advanced through hybrid markers, providing comprehensive profiles from cellular secretions, including exosome-derived markers [44]. Novel biomarker development continues, focusing on drug detection, age prediction, and death timing [157]. The interdisciplinary disposition requires collaboration with molecular biologists and analysts, with guidance from Institutional Review Boards and Ethics Committees [154].

8. Implications of Forensic Science Practice

In recent years, serological techniques have experienced remarkable advancements. Despite being a traditional forensic discipline, this field evolves thorough research on identification, validation, and genetic variability of innovative serological biomarkers [15]. This progress fosters their incorporation into forensic science. Support for serological biomarker methodologies may provide useful markers [164]. Their proven efficiency in criminal investigations could be beneficial through innovative practices [165]. In the criminal justice context, legal professionals need specialized training in new forensic approaches, as courts look for more informative evidences [166].

The adoption of innovative forensic methodologies could lead to institutional reforms to utilize next-generation technologies [167]. A rapid, all-in-one approach using a single stain enables law enforcement authorities to have a trustworthy approach during criminal investigations [168]. This can provide accuracy, speed, and reduced human bias in forensic evaluation. Ethical considerations regarding privacy should follow regional laws

and guidelines [169]. The integration of "omics approaches in law" requires more validation studies. The updated list should appeal to the forensic science community [170]. Discovery outputs from serological methods will shape forensic science foundation to the next decade. This review addresses the major goals set in the document [171] about understanding individual's activity and characteristics and developing methods to advance knowledge in the investigative chain of forensic science [172].

9. Conclusions

This review examined the role of serological biomarkers in forensic science. Since the discovery of blood groups, forensic serology has advanced considerably, with DNA analysis now serving as the primary method for individual identification. Contemporary techniques for body fluid identification include detection of mRNAs and miRNAs, fluorescence, mass spectrometry, and single nucleotide polymorphism (SNP). Serological biomarkers are crucial in forensic science, particularly for screening large samples to expedite criminal investigations. Sperm cell-specific data enable cell localization and proportion assessment of swabs, aiding interpretation of differential extraction. The integration of various serological markers can yield additional insights. Several methodologies are employed for the serological identification of body fluids, and their potential applications are explored. Nonetheless, the development of serological techniques for semen identification and differentiation between vaginal and buccal samples using less invasive methods remain significant research areas in forensic science, necessitating standardization and extensive validation. Challenges persist in the serological investigation of blood, such as the integration of extracellular vesicles (EVs) and other body fluid components, with the molecular content of EVs being of particular interest. We anticipate that this review can serve as a catalyst for future research in the field of forensic science.

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