
FLUID EVIDENCE - THE ROLE OF FORENSIC SEROLOGY IN DECODING CRIME

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ABSTRACT

This study explores the crucial position of forensic serology as "fluid evidence" in the decryption of crimes in India and examines the biological fluids such as blood and semen in the light of the Bharatiya Sakshya Adhiniyam, 2023 (Section 39) and the Bharatiya Nagarik Suraksha Sanhita, 2023 (Sections 51, 52). It discusses the difficulties that arise in the areas of collection, preservation, contamination, and admissibility and reviews techniques from presumptive tests to DNA integration considering the FSL backlogs. The judicial precedents highlight its efficacy but still, the problem of the lack of standardization continues. The objectives are to reform the protocols and improve the training in order to increase the number of convictions. The findings suggest that there is a need for alignment between the legislation and the recommended best practices to ensure the reliability of prosecutions.

Keywords: Forensic serology, fluid evidence, Bharatiya Sakshya Adhiniyam, chain of custody, contamination risks.

INTRODUCTION TO ANALYTICAL METHODS IN FORENSIC SEROLOGY

Forensic serology is a significant forensic tool in criminal investigations as it aids in the identification, classification, and analysis of body fluids like blood, semen, saliva, and other bodily fluids. Forensic serology must use scientific techniques that are accurate, reliable, and admissible in court due to the huge impact that they have on linking the suspect, victim, and crime scene. “Forensic serological evidence admissibility in India is regulated by the Bharatiya Sakshya Adhiniyam, 2023, and more specifically Section 39, under which courts have the power to accept expert evidence based on scientific examination. Procedural protection for ensuring that evidence is obtained, analyzed, and presented so as to maintain the integrity of forensic examinations is provided by the Bharatiya Nagarik Suraksha Sanhita, 2023.¹

Forensic serological examination commences with presumptive examination of biological samples. Presumptive tests are quick screening tests for detecting the potential presence of certain body fluids. They are based on colorimetric or luminescence-based reactions when in contact with biological indicators. For example, the Kastle-Meyer test is a common one for detecting blood since it detects peroxidase-like activity of hemoglobin. The acid phosphatase test is used to detect semen through detection of the enzymatic activity unique to seminal fluid. But the findings of presumptive tests are not conclusive and have to be validated by more specific and sensitive confirmatory tests. Such tests should be interpreted and documented in conformity with the procedural requirements of the Bharatiya Nyaya Sanhita, 2023, especially in the case of violent offenses like murder (Section 101) and rape (Section 64), where biological evidence is essential proof.²

Confirmatory tests are utilized to determine unequivocally the nature of a biological fluid within a sample. Confirmatory tests utilize immunological responses, chemical testing, and microscopic analysis to confirm the outcome of presumptive testing. One of the most popular confirmatory tests used in forensic serology is the ABACard HemaTrace test, which detects

¹ “What is the role of forensic serology in criminal investigations?,” StudySmarter UK *available at*: <https://www.studysmarter.co.uk/explanations/law/forensic-science/forensic-serology/> (last visited March 22, 2026).

² Joanne L Webb, J I Creamer and T. I. Quickenden, “A comparison of the presumptive luminol test for blood with four non-chemiluminescent forensic techniques” Wiley, 2006 *available at*: https://www.researchgate.net/publication/7135226_A_comparison_of_the_presumptive_luminol_test_for_blood_with_four_non-chemiluminescent_forensic_techniques (last visited March 22, 2026).

human hemoglobin with high specificity³⁴

The Supreme Court of India has, in all cases, stressed the need to maintain the chain of custody of forensic evidence to avoid tampering and to maintain the credibility of scientific evidence. Section 61 of the Bharatiya Sakshya Adhiniyam, 2023, that deals with the admissibility of electronic records has been judicially extended to place special significance on authentication in forensic reports.⁵

The success of forensic serology in criminal investigations also relies on the training and experience of forensic staff. The National Accreditation Board for Testing and Calibration Laboratories (NABL) and Directorate of Forensic Science Services (DFSS) of the Ministry of Home Affairs lay down guidelines to ensure uniformity and reliability in forensic laboratory practices. Training of the forensic serologists focuses on the application of sophisticated techniques, observance of legal requirements, and documental recording of the forensic results. In India, forensic labs function under the auspices of the Bharatiya Nyaya Sanhita and the Bharatiya Nagarik Suraksha Sanhita, with specific guidelines laid down by the judiciary to preserve the integrity and impartiality of forensic evidence during court trials.

IMMUNOLOGICAL AND BIOCHEMICAL METHODS IN SEROLOGY

Immunological and biochemical techniques make up a major component of forensic serology that provides reliable and precise means for the detection and analysis of bodily fluids found in crime scenes. These techniques are not just an instrument used for the identification of particular bodily fluids like blood, semen, and saliva but are also used for identifying the origin of the fluid, linking an individual to the crime. Immunoassays, enzyme-linked immunosorbent assays (ELISA), and other biochemistry testing methods are commonly employed in forensic laboratories to identify the existence of certain markers or molecules which are peculiar to certain body fluids. The Indian legal system acknowledges the significance of such scientific

³ John M. Butler, "Recent advances in forensic biology and forensic DNA typing: INTERPOL review 2019–2022," 6 *Forensic Science International: Synergy* 100311 (2023).

⁴ "Laboratory Orientation and Testing of Body Fluids and Tissues for Forensic Analysts," National Institute of Justice *available at*: <https://nij.ojp.gov/nij-hosted-online-training-courses/laboratory-orientation-and-testing-body-fluids-and-tissues/testing-body-fluids-tissues/overview/presumptive-v-confirmatory-tests> (Last visited March 22, 2026).

⁵ Salem K Alketbi, "DNA Contamination in Crime Scene Investigations: Common Errors, Best Practices, And Insights from a Survey..." Biomedical Research Network, LLC, 2024 *available at*: https://www.researchgate.net/publication/384729096_DNA_Contamination_in_Crime_Scene_Investigations_Common_Errors_Best_Practices_And_Insights_from_a_Survey_Study (Last visited March 22, 2026).

evidence and has guidelines for its use under the Bharatiya Sakshya Adhiniyam, 2023, under Section 39, which permits expert opinion on the basis of scientific principles, including immunology and biochemistry.

Forensic serology immunological techniques rely primarily on antigen-antibody interactions, wherein antibodies are employed to detect and bind to antigens found in body fluids. This specificity gives immunoassays great power in forensic investigation. The specificity principle is very useful when one is trying to identify and determine the presence of biological fluids because various fluids contain specific proteins or enzymes that can be targeted with immunological testing. Agglutination test is one of the most popular immunological tests used in forensic serology. This test is the interaction between certain antibodies and blood antigens, and it is most frequently employed to identify blood type. The Ouchterlony double diffusion test, another immunologic procedure, is employed to identify particular blood antigens and can be helpful in identifying the victim's and suspect's blood type.⁶

Enzyme-linked immunosorbent assay (ELISA) is one of the most useful biochemical procedures in forensic serology. It is also extensively used for detecting the existence of proteins or enzymes of body fluids like semen, saliva, and vaginal fluid. ELISA tests utilize the mechanism of binding antibodies to the target protein or antigen in the sample, where a chemical reaction causes a change in color which can be measured.

Apart from immunological procedures, biochemical methods are also employed in forensic serology for the identification and examination of biological fluids. Biochemical tests tend to target the presence of certain enzymes or chemical markers found in various body fluids. For instance, the acid phosphatase test is widely applied to identify seminal fluid since this enzyme occurs in high levels in semen. Likewise, the existence of amylase in saliva is routinely screened for using biochemical tests. Amylase is a high concentration enzyme in human saliva and is an excellent marker to use for detecting saliva at crime scenes. The biochemical tests are easy, efficient, and economical and are extremely valuable for field forensic serologists.

The legal permissibility of immunological and biochemical techniques for use in forensic examination is founded on the Bharatiya Sakshya Adhiniyam, 2023. According to Section 39 of the Act, expert evidence formed on the basis of scientific investigation is acceptable

⁶ Hanieh Bazyar, "On the Application of Microfluidic-Based Technologies in Forensics: A Review - PMC," 23 Sensors (Basel, Switzerland).

evidence in court. This is highly applicable when proving the outcome of immunological or biochemical tests, which have to satisfy high requirements of reliability and accuracy in order to qualify as valid evidence. In forensic science, there are rigid procedures and guidelines for the experts to adhere to in order to maintain the integrity of evidence and presentation before a court of law. The Bharatiya Nagarik Suraksha Sanhita, 2023, also provides detailed provisions for collection, preservation, and presentation of forensic evidence so that evidence from immunological and biochemical tests is dealt with as per legal requirements.⁷

In addition, admissibility of forensic evidence hinges on its capability to satisfy the standards established in Section 3 of the Bharatiya Sakshya Adhiniyam, which delimits relevant evidence. The evidence derived from immunological and biochemical techniques has to be both scientifically sound and pertinent to the case in question. This condition prevents the introduction as evidence of those findings that will not have any direct role to play in substantiating the facts of the case.

BLOOD TYPING AND SPECIES IDENTIFICATION TECHNIQUES

Blood typing and species identification techniques are the essential aspects of forensic serology and are extremely important for investigations. Blood typing is a means for classifying blood based on antigens present on red blood cells. Two of the primary means of determining blood type are the ABO system and Rh factor. The ABO system is most common method that categorises blood into four groups—A, B, AB, and O, based on the presence or absence of A and B antigens. The Rh factor divides blood into Rh-positive and Rh-negative according to the presence of the Rh antigen. The groupings are important in forensic investigations as they can be used to narrow down offenders or potential donors of blood found at a crime scene. While blood typing methods cannot identify an individual uniquely like DNA testing, it can be used for preliminary screening in order to reduce the number of individuals possibly linked to biological evidence.⁸

Modern molecular biology techniques, such as the polymerase chain reaction (PCR) have

⁷ Jeevan Shekar, “The Admissibility of Scientific Evidence in Bharatiya Sakshya Adhiniyam, 2023 and the Need for an Indian Daubert” *available at*: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=5052611 (last visited March 22, 2026).

⁸ “ABO Blood Type Identification and Forensic Science (1900-1960),” Embryo Project Encyclopedia *available at*: <https://embryo.asu.edu/pages/abo-blood-type-identification-and-forensic-science-1900-1960> (last visited March 22, 2026).

revolutionised species identification in forensic science. PCR replicates specific regions of DNA sequences in biological evidence and allows forensic scientists to amplify genetic material and ascertain whether or not it is human, or of a different species. This is particularly useful for species identification as mitochondrial DNA has been shown to be more resistant to degradation than nuclear DNA, so it can be used to analyse even very badly degraded material. Analysis of "Short Tandem Repeats" (STRs), certain regions of DNA, is also carried out to confirm the species. All of these have also contributed to the accuracy and reliability of forensic serology methods in criminal investigations.⁹

Apart from scientific and procedural challenges, forensic serology also faces legal and ethical challenges. Legal acceptance of serology is determined by its scientific soundness and accordance to the requirements of evidence. The Bharatiya Sakshya Adhiniyam, 2023, standards of relevance, reliability and authenticity should be met by forensic evidence to be admissible in court. Serological evidence's admissibility is determined by court with respect to investigative technique used by forensic analysis, qualifications of the forensic experts and authenticity of the chain of custody. In addition, well-versed forensic experts and well-accredited laboratories play an indispensable role in the proper conduct of forensic investigations.¹⁰

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) AND ITS FORENSIC APPLICATIONS

Enzyme-Linked Immunosorbent Assay (ELISA) is a widely-used analytical technique in forensic serology to detect and quantify some proteins, antigens or antibodies in body fluids. ELISA plays a critical role in forensic science when body fluids such as blood, saliva, semen and urine are to be identified and tested to establish a connection between a suspect, victim and scene. This immunological test is based on antigenic-antibody interactions where an enzyme-conjugated antibody will bind to the antigen of interest to produce a measurable change in color confirming the presence of the biological material. The sensitivity and selectivity of the ELISA technique is useful in forensic science because it will yield a positive test even with very low amounts of biological material even when the DNA is severely degraded. This test finds very

⁹ "NCBI," WWW Error Blocked Diagnostic *available at*: <https://www.ncbi.nlm.nih.gov/books/NBK589663/> (last visited March 22, 2026).

¹⁰ admin, "The Role and Admissibility of Forensic Evidence in the Indian Criminal Justice System" Century Law Firm Blog, 2023 *available at*: <https://www.centurylawfirm.in/blog/the-role-and-admissibility-of-forensic-evidence-in-the-indian-criminal-justice-system/> (last visited March 22, 2026).

specific applications in forensic toxicology where it is used for screening for drugs, toxins and other biochemical compounds in urine and blood". ELISA is commonly used as a screening test followed by confirmatory techniques such as spectroscopy (mass spectrometry) and chromatography (high-performance liquid chromatography).

AGGLUTINATION TESTS AND THEIR ROLE IN FORENSIC SCIENCE

Agglutination tests are an important part of forensic serology, used for the typing of blood and the identification of certain antigens in body fluids. Agglutination tests work on the principle of antigen-antibody reactions, where the reaction between antibodies and target antigens results in visible clumping or agglutination of cells or particles. Agglutination reactions are most often applied in forensic blood typing, which helps narrow down the list of possible suspects or victims on the basis of "ABO and Rh blood group systems. In addition to blood typing, these tests are employed in the detection of microbial infections, the confirmation of human proteins in stains, and the identification of the species origin of biological fluids.¹¹

ADVANCES IN DNA EXTRACTION AND AMPLIFICATION FROM BIOLOGICAL FLUIDS

The technological developments in DNA extraction and amplification have revolutionized forensic serology by enabling individual identification from the trace deposits of biological fluids including blood, saliva, semen and sweat. Innovative techniques for extracting DNA, including organic extraction, Chelex extraction and silica-based extraction, have made it possible to obtain good quality DNA from complex forensic evidentiary samples. Rapid DNA extraction kits have also streamlined forensic practices, allowing for rapid processing of forensic evidence. Sufficient quantities of DNA are prepared for analysis by amplification of DNA via the PCR technique. PCR-based technologies such as Short Tandem Repeat (STR) profiling and mitochondrial DNA sequencing have revolutionized forensic science, enabling precise individual identification from degraded and/or old samples.¹²

The legal framework for conducting forensic DNA analyses in India includes: the DNA

¹¹ "Latex agglutination test: MedlinePlus Medical Encyclopedia," *available at*: <https://medlineplus.gov/ency/article/003334.htm> (last visited March 22, 2026).

¹² Pankaj Shrivastava et al., "Biological Sources of DNA: The Target Materials for Forensic DNA Typing" Springer Singapore, 2022 *available at*: https://link.springer.com/10.1007/978-981-16-4318-7_2 (last visited March 22, 2026).

Technology (Use and Application) Regulation Bill to regulate the collection, storage and use of DNA evidence into criminal proceedings. The collection of all DNA samples of suspects is done under the BNSS section 52, especially in sexual offences and other serious crimes. The Bharatiya Sakshya Adhinyam, 2023 provides for the admissibility of DNA evidence to the extent that forensic DNA analysis techniques would be in accordance with scientific principles. The updating of the Identification of Prisoners Act, 1920 also allows the taking of samples from suspects to aid in their identification. DNA labs used for forensic analysis need to follow National Accreditation Board for Laboratories (NABL) accreditation standards and Bureau of Indian Standards (BIS) guidelines to ensure that forensic DNA evidence is reliable and admissible in court proceedings.

MULTIPLEX ASSAYS AND THEIR SIGNIFICANCE IN MODERN FORENSICS

Multiplex assays are a huge improvement to forensic serology, this is where multiple biological targets are detected in a single forensic sample. Techniques such as multiplex PCR, multiplex ELISA, and microarray technology are used to measure multiple proteins, genetic probes and antigens in biological body fluids. The primary advantage of multiplex assays is that they can provide comprehensive forensic profiles from minimal sample volumes, which eliminates the risk of sample depletion and maximises the efficiency of forensic analyses. Multiplex PCR, for instance, enables the simultaneous amplification of multiple STR loci, enhancing the discriminating potential of DNA profiling. Similarly, multiplex immunoassays simplify the detection of multiple serological markers in biological specimens that might help detect specific biological products of criminal acts.¹³

Multiplex assays are being recognised as being of legal and forensic value, as reflected in the regulatory evolution of forensic science in India. The DNA Technology (Use and Application) Regulation Bill (DTUAR) paves the way for formalising the implementation and use of new DNA technologies such as multiplex assays, in criminal cases on their appropriate use. The Bharatiya Sakshya Adhinyam, 2023, has the provisions for the admissibility of the multiplex forensic analysis provided they conform to certain scientific standards. Also, the Bharatiya Nagarik Suraksha Sanhita (BNSS) in Section 329 permits the admission of the evidence of forensic experts such as multiplex assays, through the reports of the laboratory. It is vital that

¹³ “Multiplex Polymerase Chain Reaction - an overview,” ScienceDirect Topics *available at*: <https://www.sciencedirect.com/topics/neuroscience/multiplex-polymerase-chain-reaction> (last visited March 22, 2026).

forensic laboratories performing multiplex analysis follow the NABL and BIS standards to ensure precision, accuracy and compliance with judicial standards.

SPECTROSCOPIC AND CHROMATOGRAPHIC TECHNIQUES IN SEROLOGY

Spectroscopy and chromatography play a crucial role in forensic serology because they can be used to detect and analyse biological fluids, proteins and other biological constituents at crime scenes. They are highly specific and sensitive, allowing forensic scientists to distinguish human from non-human biological samples, to detect low concentrations of items and to analyse complex mixtures. Spectroscopic methods such as ultraviolet-visible (UV-Vis) spectroscopy, infrared (IR) spectroscopy and Raman spectroscopy are often used for analysis of bloodstains and other biological evidence. UV-Vis spectroscopy helps to identify hemoglobin byproducts to confirm a bloodstain. IR and Raman spectroscopy helps in the non-destructive analysis of biological samples so that forensic scientists can discriminate between body fluids on the basis of their chemical composition. Fluorescence spectroscopy aids in the identification of specific proteins and other biomolecules, thereby increasing the specificity of tests.¹⁴

Separation and analysis of constituents in biofluids are achieved using chromatographic methods like gas chromatography (GC), liquid chromatography (LC), and high-performance liquid chromatography (HPLC). These techniques are commonly employed in forensic toxicology to identify drugs, toxins, and metabolites from blood and urine specimens. HPLC with UV or fluorescence detection is commonly utilized in forensic serology for the identification of blood group substances. Moreover, thin-layer chromatography (TLC) is a preliminary screen for the detection of several biochemical constituents in forensic specimens. The reliability of such methods in forensic serology requires strict compliance with legal and procedural guidelines to establish their admissibility in courts.

The Bharatiya Sakshya Adhinyam, 2023, in Section 39, makes forensic expert opinion admissible as evidence, including findings obtained from spectroscopic and chromatographic tests. Section 329 of the Bharatiya Nagarik Suraksha Sanhita (BNSS), 2023, permits reports of forensic examinations by accredited labs to be filed as evidence so that scientific reports from

¹⁴ Romy Elise Keane et al., "Mass spectrometry based proteomics: Changing the impact of protein analysis in forensic science," 6 *Wiley Interdisciplinary Reviews: Forensic Science*.

chromatography and spectroscopy are taken into account in judicial proceedings¹⁵

THE ROLE OF MASS SPECTROMETRY IN SEROLOGICAL ANALYSIS

Mass spectrometry (MS) has had a profound impact on forensic serology by ensuring precise detection of biological molecules, proteins and metabolites. It provides forensic scientists with the means for sensitive and specific analysis of complex biological samples, which is a successful approach in blood, semen, saliva and other bodily fluid investigations. High specificity and sensitivity in mass spectrometry is achieved by the ionization of the sample, the separation of the ions according to their mass-to-charge ratio, and their precise detection. MS is often combined with other chromatographic methods, such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) in forensic serology to provide exact identification of biomarkers, drugs and toxins found in biofluids.

Perhaps the most important aspect of MS in forensic science is the identification of protein markers in blood, which help in the discrimination of human from non-human blood. This feature is helpful in forensic investigations requiring animal/human identification. Another application of MS-based proteomics is to investigate protein degradation profiles in bloodstains to aid in determining the age of the bloodstain and its exposure to various environmental conditions. In cases of sexual abuse, MS can be applied for profiling semen proteins to connect the victim and perpetrator. MS techniques are also used to determine illicit drugs in body fluids in forensic toxicology, to strengthen prosecution for drug crimes.¹⁶

CHALLENGES IN SEROLOGICAL TESTING AND INTERPRETATION

Despite the advances in forensic serology, there are a range of challenges in testing and interpretation of serological data in forensic science. The most prominent ones are the degradation of biological evidence in the environment, which affects forensic testing. High temperature, moisture and bacterial or fungal contaminants might contribute to degeneration of proteins and DNA present in the bloodstains and other body fluids, which might preclude the production of consistent results. To address such issues, the forensic community is in search

¹⁵ Jeevan Shekar, "The Admissibility of Scientific Evidence in Bharatiya Sakshya Adhinyam, 2023 and the Need for an Indian Daubert" *available at*: <https://papers.ssrn.com/sol3/Delivery.cfm/5052611.pdf?abstractid=5052611&mirid=1> (last visited March 22, 2026).

¹⁶ Akshat Jain, "What new criminal law says about forensic evidence & how this could put 'immense stress' on labs" *theprint*, 8 July 2024.

of new preservation technologies to stabilise the sample.

The interpretation of serological analytical results is also a problem, particularly in mixtures of biological samples. When the body fluids involved belong to more than one person, it falls on the forensic scientists to use advanced techniques such as differential DNA recovery and probabilistic genotyping to discriminate between the contributors. The mixed nature of samples may lead to dispute in court, thus requiring expert witness and science-admissible methods. The strength of serological evidence is also impacted by the chances of false positive and false negative results in forensic investigations. Factors such as the specificity of antibodies in immunological tests, the presence of inhibitors in biological samples and technical issues with instruments can cause misleading results. Research and validation studies are required to enhance the methods of forensic serology and to minimise false results.¹⁷

¹⁷ Bruce Mccord et al., "Forensic DNA Analysis" American Chemical Society, 2018 *available at*: https://www.researchgate.net/publication/329259545_Forensic_DNA_Analysis (last visited March 22, 2026).