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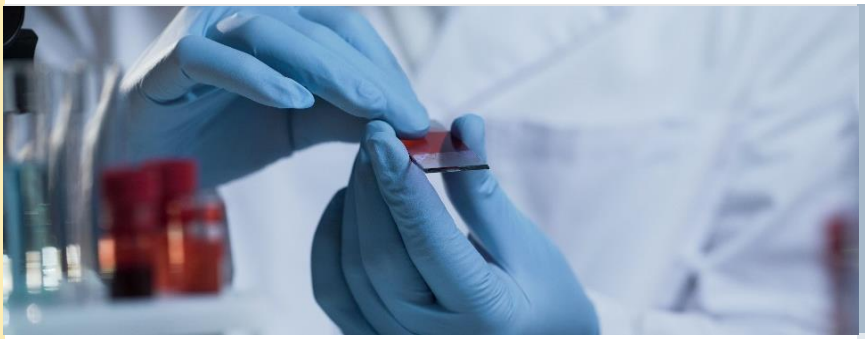
IN-BRIEF SERIES (Part 2 of 3)

Beyond DNA: The Role of Biological Evidence in Sexual Assault Investigations



"Sexual assault evidence should be evaluated holistically. The identification of the type of biological fluid may further substantiate and clarify how events took place."

-Dr. Patricia Melton, RTI International

Preface

Sexual assault remains prevalent in the United States, with an average of 300,000 cases reported to law enforcement each year [1]. However, another 600,000 go unreported [2]. The circumstances of and trauma resulting from a sexual assault can pose a challenge to investigators. For example, witnesses are not always present; the impact of trauma or incapacitating substances, such as alcohol, may affect the victim's ability to recount details of the incident; and frequently, corroborating evidence is limited.

DNA evidence, while valuable, is not always probative or present in every case: many DNA samples do not meet the quality standards required to be uploaded into CODIS (38% of profiles were found to be ineligible as noted from recent NIJ-supported research [3]). Even in cases where a DNA profile is present and is CODIS-eligible, a CODIS hit occurs only about half of the time [3]. Additionally, a DNA profile may provide limited probative value in situations where sexual contact is not disputed. Thus, many types of physical evidence play a critical role in the investigation and prosecution of sexual assault cases.

Physical evidence collection, submission, and analysis can be an effective and necessary means of reconstructing at least some of the events that occurred during a sexual assault. Physical evidence provides value to investigations even if a DNA profile is developed and probative, as it can be used to corroborate and supplement a greater understanding of the circumstance and make a stronger case. This three-part Beyond DNA In-Brief series highlights types of physical evidence that can provide crucial information about a sexual assault, so that key stakeholders in the criminal justice community ultimately obtain just resolutions for these crimes.

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Objectives

- Illustrate the impact of biological evidence in sexual assault investigations beyond DNA analysis.
- Provide an overview of biological evidence processing techniques used in sexual assault investigations.
- Identify limitations in current processing methods.
- Highlight examples of current forensic biology research applicable to sexual assault investigations funded by the National Institute of Justice (NIJ).

These reports are designed to provide **law enforcement, policymakers, legal professionals, and the public** with an introduction to different types of physical evidence and the roles they may play in sexual assault investigations.

This is the second installment of a three-part series, which also includes (1) *Beyond DNA: The Role of Physical Evidence in Sexual Assault Investigations* and (3) *Beyond DNA: The Role of Toxicological Evidence in Sexual Assault Investigations*.

Introduction

The collection and analysis of biological evidence plays an important role in the investigation of sexual assault cases. Biological evidence refers to evidence samples that contain biological material [4] and can be found on a victim, on a suspect, or at the scene of a sexual assault. Types of biological material typically encountered in sexual assault investigations include blood (from injury or trauma), menstrual blood, saliva, semen, urine, and vaginal fluid. Additional materials that contain DNA, such as hair and skin cells, may be collected in sexual assault casework. This in-brief report focuses on body fluids—evidence collected as part of a sexual assault kit (SAK) and at the scene.

Body fluid evidence is one of the most commonly collected types of evidence in a sexual assault investigation [4]. A 2012 study of 603 sexual assault cases identified biological evidence as the main type of evidence collected and submitted to the crime laboratory, with SAKs being one of the primary collection methods [4]. In many cases, the biological evidence collected in these investigations is examined for the likelihood of presence of DNA; up to 88% of cases with biological evidence are sent for DNA testing [4]. However, not all of these evidentiary items will produce DNA profiles with sufficient quality to be uploaded into the Combined DNA Index System (CODIS). From a sample of more than 7,000 SAKs submitted for DNA testing, only 38% yielded a DNA profile of sufficient quality to be uploaded to CODIS [3, 5].

The value of body fluids is not limited to being a source for DNA as it can provide details about events that transpired during a sexual assault. Biological evidence testing, also referred to as serology, may:

- **Indicate that sexual or physical contact may have occurred**: the presence of seminal fluid on the inner thigh of a female victim may suggest that sexual contact happened between the victim and a suspect.
- **Demonstrate that force or restraint may have been used**: a vaginal swab taken from a victim that contains non-menstrual blood may indicate that forceful sexual contact took place.
- **Help corroborate or disprove a scenario**: the type and location of biological materials can support or refute victim testimony, and also help determine how a victim was sexually assaulted (e.g., anal, vaginal, or oral penetration); the physical location in which the assault took place (such as in a car or bedroom); and the perpetrator's characteristics (e.g., if male, azoospermic). For example, the presence of saliva on a victim's breast might corroborate the victim's account of being licked or bitten during the assault.

Identifying the specific type of biological evidence supports a victim's account, especially in cases when a suspect claims that DNA found on the victim is attributed to transfer of skin cell DNA.

It is important to note that while the presence of biological evidence on a victim or suspect can indicate that sexual or physical contact may have occurred, it does not necessarily indicate that a crime has occurred. Biological evidence alone cannot establish whether the contact between the suspect and victim was non-consensual, except in cases in which the victim is a minor because the consent defense does not apply. Moreover, absence of biological evidence does not indicate that sexual assault did not occur.

Many labs are moving to a direct to DNA approach, where samples are sent for DNA testing without biological fluid analysis. However, the information gleaned from biological evidence analysis is still valuable and can be requested after DNA analysis.

Successful Use of Biological Evidence in Casework:

Body fluid identification helped convict a man of charges including rape and indecent assault of two minors in Wyoming County, Pennsylvania. A key piece of evidence in the trial was a stain on a mattress, which had no sperm but was presumed as semen based on the concentration of Prostate Specific Antigen (PSA), or p30. The stain was later found to contain DNA from both the suspect and one of the victims. The identification of seminal fluid supported the claim that the suspect had sexually assaulted the victim. Read more about the case here.

Processing of Biological Evidence

Biological evidence is often collected by crime scene professionals, law enforcement officers, laboratory analysts, and forensic healthcare professionals such as sexual assault nurse examiners or sexual assault forensic examiners (SANE/SAFEs) and medical examiners. In a sexual assault investigation biological evidence may be collected (1)

from the individual(s) reporting a sexual assault, (2) the suspect(s), and/or (3) the crime scene. When a person reports a sexual assault, a medical professional or SANE/SAFE typically examines the individual and collects items for forensic analysis in a SAK. Although SAK contents may vary depending on the jurisdiction, a SAK usually contains swabs, slides, and envelopes for materials such as hairs, fibers, and debris [6]. Undergarments may also be collected with the kit as they may contain biological stains. SAKS, which may contain biological fluids such as semen, saliva, vaginal fluid, and blood, as well as hair and skin cells, are submitted to a crime laboratory for body fluid identification and/or DNA analysis. In cases where a drug facilitated sexual assault (DFSA) is suspected, blood or urine may be collected for toxicological analysis. For more information on toxicology and DFSA, consult the in-brief *Beyond DNA: The Role of Toxicological Evidence in Sexual Assault Investigations*. Nurses, law enforcement officers, and crime scene professionals may also collect items from a suspect or an accused individual in another separate process commonly referred to as a suspect exam.

Integration of Direct to DNA Approach and Body Fluid Identification

Identification of biological evidence has typically preceded DNA analysis as a method to screen for swabs with potential DNA. NIJ's *National Best Practices for Sexual Assault Kits: A Multidisciplinary Approach* recommends a Direct to DNA approach, where DNA analysis is performed before body fluid identification, for laboratories capable of high-throughput DNA analysis [6]. This approach can more efficiently and accurately identify swabs with potential CODIS-eligible profiles. The biological fluid analysis, therefore, is conducted after the DNA analysis to provide additional and supportive information to the case.

In addition to the SAK, crime scene professionals may also collect evidence at the scene that could have relevant biological materials—such as bedsheets, couch cushions, or clothing. Crime scene investigators (CSI) may also collect other types of evidence—such as trace evidence (e.g., hairs, fibers, and fingernail scrapings), impression evidence (e.g., fingerprints and shoeprints), and other types of evidence. The NIJ provides best practice guidelines for the collection and analysis of biological evidence, including the following:

- National Best Practices for Sexual Assault Kits: A Multidisciplinary Approach
- The Biological Evidence Preservation Handbook: Best Practices for Evidence Handlers (NIST/NIJ)

Methods to detect and identify body fluids can be divided into presumptive and confirmatory techniques, described in Table 1.

Table 1. Presumptive and confirmatory techniques for detecting and identifying body fluids.

	Description	Examples	
		Body Fluid	Techniques
Presumptive	**Presumptive techniques** are used to indicate that a body fluid could be present. These types of tests are sensitive, meaning that they are designed to detect a potential body fluid at low levels, but these tests may not be specific to a certain body fluid, or specific to biological materials of human origin. Therefore, presumptive tests should be interpreted with caution and an understanding of the limitations of such testing (e.g., false positives and false negatives). Presumptive methods are typically less expensive, rapid, and portable. Presumptive testing techniques can be used in either the laboratory or at the crime scene.	Saliva	Alternate Light Sources (ALS), Starch-Iodide Test, Phadebas Test, ELISA [7]
		Semen	ALS, Acid Phosphatase Test, Chemical Tests for Prostate Specific Antigen (PSA), Florence Test [7]
		Blood	ALS, Luminol, Fluorescein, Kastle-Meyer Test [8]
		Vaginal Fluid	ALS, Electrophoretic Tests, Lugol's Iodine Test, Dane's Staining Methods [9]
Confirmatory	**Confirmatory techniques** identify a body fluid. These tests are usually performed after presumptive tests and typically take place in a forensic laboratory setting. Although one or more presumptive tests may be used to detect a potential body fluid during an investigation, investigators must use confirmatory testing techniques to confidently identify the body fluid(s) present. Confirmatory techniques are often less sensitive than presumptive tests but are highly specific.	Saliva	Research: Quantum Dot Beacons to Test for RNA [10], RSID Saliva Test
		Semen	RSID Semen Test, ABAcard p30, Christmas Tree Staining, Hematoxylin-Eosin Staining
		Blood	Takayama test, ABAcard Hematrace, Soret Bands [9]
		Vaginal Fluid	Research: Quantification of protein biomarkers in Q-TOF MS [10]

There are a variety of both presumptive and confirmatory methods available to test for biological fluids. **Table 2** provides a snapshot of chemical, electrophoretic, immunological, microscopic, and spectroscopic techniques available.

Table 2. Summary of available types of presumptive and confirmatory tests for body fluid detection and identification.

	Description	Characteristics	Examples	
			Presumptive	Confirmatory
Chemical	Chemical tests suggest the presence of a body fluid through a positive chemical reaction with a particular component of a body fluid. These tests are usually (1) colorimetric, meaning the presence of the body fluid chemically changes the color of the test chemical, or (2) chemiluminescent, meaning light is produced as the result of a chemical reaction.	▶ Inexpensive ▶ High sensitivity ▶ Low specificity ▶ Rapid ▶ Field Portable ▶ Destructive	Starch-Iodine Test **Detects:** Amylase **Suggests:** Saliva [7] Luminol **Detects:** Heme **Suggests:** Blood	None
Electrophoretic	Electrophoretic tests separate the sample into different components using an electric charge.	▶ Distinguishes body fluid types ▶ Requires a laboratory setting ▶ Not rapid	Electrophoretic tests **Detects:** Vaginal Acid Phosphatase (distinguishing from seminal acid phosphatase, SAP) **Suggests**: Vaginal Fluid [12].	None
Immunological	Immunological tests confirm the presence of specific body fluids through an antibody-antigen reaction. These tests provide the antibodies to which antigens in body fluids, such as semen, can conjugate.	▶ Body fluid specific ▶ Species specific	None	ABAcard Hematrace **Detects:** Hemoglobin **Confirms:** Human blood [11]
Microscopic	Microscopy tests suggest or confirm the presence of body fluids through staining or crystallization patterns visible through a microscope.	▶ Rapid ▶ Destructive ▶ Subjective	Florence Test **Detects:** Choline **Suggests:** Semen [6]	Christmas Tree Stain and Hematoxylin-Eosin Stain **Detects:** Nuclei of spermatozoa **Confirms:** Semen Takayama Crystal Test **Detects:** Heme **Confirms:** Blood
Spectroscopic	Spectroscopic methods can identify body fluids through the interaction between electromagnetic radiation and the biological matter.	▶ Non-destructive	ALS* **Detects:** Fluorophores **Suggests:** Various body fluids (e.g., semen, saliva, vaginal fluid, blood)	Soret Bands **Detects:** Hemoglobin **Confirms:** Blood

* For more information about the use of ALS to detect biological evidence in sexual assault investigations, refer to the FTCoE's Landscape Study of Alternate Light Sources.

Technical Limitations of Current Forensic Biology Techniques

Although body fluid identification may provide significant value in sexual assault investigations, current methods of detection have technical limitations that may impact the ability to obtain useful information from this evidence. Technical improvement needs within biological evidence analysis techniques include:

Beyond technical challenges, additional factors—such as cost, training, and other resource demands—may affect access to equipment and techniques.

- **Deconvolution of body fluid mixtures--** in many circumstances, samples collected from areas of the body in an assault are made up of a mixture of body fluids—such as semen, blood, and vaginal fluid [13]. Correctly identifying a mixture of body fluids from a sample can be challenging, especially if more than two donors are involved. Identifying all body fluids present in a mixture provides information that may corroborate events that had taken place during the sexual assault. Current presumptive tests may suggest the presence of multiple body fluids without being specific.
- **Screening techniques at the crime scene**—CSIs would benefit from screening techniques that can quickly and reliably detect biological fluids for further analysis, and potentially be used at crime scene.
- **Nondestructive analysis techniques**— In traditional testing in which biological testing is performed first, presumptive and confirmatory tests may destroy the sample. Because DNA may be present in low concentrations on the victim or at the crime scene, this can be problematic. Conversely, if DNA testing is performed first, which also consumes the biological sample, then additional biological material identification may be insufficient and unable to further corroborate testimony.
- **Methods to determine time of deposition of body fluids**—Although many techniques can effectively detect and identify the type of biological evidence present, there are no widely standardized techniques that can reliably determine when the biological evidence was deposited onto a surface, or what is known as "time since deposition" (TSD). Many proposed techniques have been limited by factors such as low sensitivity.

NIJ Research Addressing Technical Limitations

The NIJ funds a strong portfolio of researchers committed to developing and improving technologies that increase forensic capacity. Multiple grantees are actively addressing technical limitations related to biological evidence; addressing these limitations may directly improve analysis in sexual assault investigations. **Table 3** provides a snapshot of current efforts to enhance biological evidence collection and analysis.

Table 3. Current NIJ efforts to address challenges in biological evidence collection and analysis.

	Snapshot of Current NIJ-Funded Research Efforts	Potential Impact
Screening Techniques at the Crime Scene	**De-convolution of Body Fluid Mixtures: Cell Type Identification and Genetic Profiling of Micro-Dissected Cells** **Awardee:** National Center for Forensic Science | **Award Number:** 2008-DN-BX-K007 **Research Goal:** Isolate single epithelial cells using laser capture microdissection and identify the origin of cells from body fluid mixtures using DNA/RNA profiling. [14] **Bioinformatic Analysis of Big Proteomic Data: A New Forensic Tool to Identify Menstrual Blood and Body Fluid Mixtures** **Awardee:** City of New York, Office of Chief Medical Examiner | **Award Number:** 2017-NE-BX-0003 **Research Goal:** Use Q-TOF mass spectrometry to develop a proteomic database to distinguish menstrual blood from venous blood [15].	► Provide valuable evidence that can support or disprove a scenario.

	Snapshot of Current NIJ-Funded Research Efforts	Potential Impact
Screening Techniques at the Crime Scene	**Hand-Held Multispectral Camera for Crime Scene Investigation** **Awardee:** New Jersey Institute of Technology | **Award Number:** 2014-DN-BX-K003 **Research Goal:** Develop a compact, multispectral forensic survey camera to detect and document evidence at the crime scene, including body fluids, and improve the resolution of evidence signal from background noise. [16] **Rapid Visualization of Biological Fluids at Crime Scenes using Optical Spectroscopy** **Awardee:** University of South Carolina | **Award Number:** 2007-DN-BX-K199 **Research Goal:** Develop a mid-infrared (IR) camera with thermal imaging capable of visualizing blood at a crime scene. [17] **A Confirmatory Test for Sperm in Sexual Assault Samples using a Microfluidic-Integrated Cell Phone Imaging System** **Awardee:** Stanford University | **Award Number:** 2017-NE-BX-004 **Research Goal:** Develop a cell phone imaging platform using microchip technology to confirm the presence of sperm cells in sexual assault samples. [18]	► Identify quickly any potential sources of biological materials at a crime scene. ► Improve the ability to detect biological evidence and drive submission of evidence for cases.
Nondestructive Testing	**Application of Raman Spectroscopy for an Easy-to-Use, on-Field, Rapid, Nondestructive, Confirmatory Identification of Body Fluids** **Awardee:** University of Albany, SUNY | **Award Number:** 2009-DN-BX-K186 **Research Goal:** Develop software algorithms that identify body fluids based on Raman spectra. [7] **Raman Spectroscopy for Analyzing Body Fluid Traces: Universal Method Development** **Awardee:** University of Albany, SUNY | **Award Number:** 2017-DN-BX-0135 **Research Goal:** Develop a universal method for identifying body fluid traces recovered at crime scenes using Raman spectroscopy. [19] **Development of SERS-Active Forensic Evidence Swabs for Rapid, Non-Destructive Confirmatory Serological Screening and STR Typing of Human Bodily Fluids** **Awardee:** Western Carolina University | **Award Number:** 2015-NE-BX-K003 **Research Goal:** Develop forensic swabs for human body fluid analysis by non-destructive surface-enhanced Raman spectroscopy (SERS). [20]	► Detect and identify traces of biological materials without destroying evidence. ► Increase the probability of DNA recovery.
Time Since Deposition	**Determination of Age (Time Since Deposition) of a Biological Stain** **Awardee:** University of Florida | **Award Number:** 2005-MU-BX-K071 **Research Goal:** Monitor the age of biological stains using RNA degradation. [21]	► Corroborate or refute testimony based on TSD.

Conclusion

Although collection and analysis of biological evidence plays a vital role in obtaining DNA evidence in a sexual assault investigation, this evidence provides valuable information beyond a possible CODIS hit. The identity of biological evidence found on the victim or suspect, or at the scene of the crime—as well as the location of the evidence—could suggest that certain events took place during the assault and serve to corroborate or refute a scenario. A variety of biological techniques are available to identify evidence and addressing technical challenges of collecting and analyzing this evidence is an area of active research. Biological evidence is just one of the many evidence types that can help a jury understand the events transpiring during the incident and ultimately bring just resolutions to these crimes.

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