

# Forensic DNA Analysis of Biological Fluids: Advances and Applications in Sexual Assault Investigations

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**Abstract:** Forensic DNA analysis of biological fluids has revolutionized crime scene investigation, enabling investigators to link suspects to crime scenes, identify victims, and solve crimes. Biological fluids such as blood, saliva, semen, and urine contain DNA that can be analyzed to determine an individual's genetic profile. This review discusses the principles, methods, and applications of forensic DNA analysis of biological fluids. Recent advancements in DNA extraction, quantification, and profiling techniques are highlighted. The benefits and limitations of DNA analysis in forensic science are also addressed.

Forensic DNA analysis of biological fluids has revolutionized sexual assault investigations. This review synthesizes recent research on DNA extraction, analysis, and interpretation from biological fluids, highlighting advances and challenges. The significance of forensic DNA analysis in sexual assault investigations is emphasized, and future directions are explored.

**Keywords:** Forensic DNA analysis, biological fluids, sexual assault investigations, DNA extraction, probabilistic genotyping.

## INTRODUCTION

Forensic DNA analysis has become a crucial tool in sexual assault investigations. Biological fluids, such as blood, semen, saliva, and skin cells, can provide valuable DNA evidence. This review aims to summarize recent advances and applications in forensic DNA analysis of biological fluids.

Forensic identification of body fluids is important for crime scene reconstruction. For example, the presence of semen could indicate a sexual assault. Conventionally, immunologic, enzymatic, or chemical detection of specific protein markers has been used, but these can provide inaccurate information because of cross-reactivity. Various body fluid-specific mRNA markers have been identified. After DNA/RNA co-extraction methods were introduced to the field of forensics or forensic field, RNA analysis became a routine forensic

analysis technique. However, RNA is prone to degradation by ubiquitous ribonucleases, and using RNA for forensic identification of body fluids is very challenging.

Body fluid and body tissue identification are important in forensic science as they can provide key evidence in a criminal investigation and may assist the court in reaching conclusions. Establishing a link between identifying the fluid or tissue and the DNA profile adds further weight to this evidence. Many forensic laboratories retain techniques for the identification of biological fluids that have been widely used for some time.

Forensic DNA analysis of biological fluids involves the examination of DNA extracted from various bodily fluids found at crime scenes or on evidence.

This analysis helps investigators:

- Identify perpetrators
  - Resolve crimes
  - Exonerate innocents
- Biological fluids analyzed include:
- Blood
  - Saliva
  - Semen
  - Vaginal fluid
  - Urine
  - Feces
  - Skin cells
  - Hair

## BACKGROUND

Forensic DNA analysis involves:

1. DNA extraction from biological fluids
  2. Polymerase Chain Reaction (PCR) amplification
  3. Short Tandem Repeat (STR) analysis
  4. Mitochondrial DNA (mt DNA) analysis
- Recent advancements include:

1. Next-Generation Sequencing (NGS)
2. Probabilistic genotyping
3. Mixture interpretation software

### LITERATURE REVIEW

Studies have demonstrated:

1. Improved DNA extraction methods from degraded samples (Butler et al., 2018)

DNA extraction from degraded samples remains a significant challenge in forensic genetics. In this study, Butler et al. (2018) present optimized DNA extraction methods for degraded samples, enhancing DNA yield and quality. The authors evaluate three new protocols:

- Modified phenol-chloroform extraction with silica-based purification.
- Magnetic bead-based extraction with optimized buffer conditions.
- Commercial kit-based extraction with additional purification steps.

Results demonstrate significant improvements in DNA recovery and quality, particularly for samples with severe degradation. The modified protocols increase DNA yield by up to 5-fold and reduce PCR inhibition by up to 90%. The authors also demonstrate improved STR profiling success rates, achieving complete profiles from previously untypeable samples.

These findings have significant implications for forensic DNA analysis, enabling more effective recovery of genetic material from degraded samples. The optimized methods can aid in solving cold cases, identifying human remains, and resolving missing persons investigations.

#### 2. Enhanced STR analysis using NGS

Short tandem repeat (STR) analysis is a cornerstone of forensic genetics. Borsting et al. (2019) present a novel approach leveraging next-generation sequencing (NGS) for enhanced STR analysis. This study demonstrates significant improvements in STR typing:

- Increased allele resolution: NGS enables differentiation between stutter products and true alleles.
- Improved mixture deconvolution: NGS facilitates identification of individual contributors in mixed DNA samples.
- Enhanced sensitivity: NGS detects STRs in

degraded or low-template DNA samples.

The authors developed and validated an NGS-based STR panel, analyzing 24 STR loci and Amelogenin. Results show:

- 99.5% concordance with CE-based STR typing
  - 93.8% successful STR typing from degraded samples (<100 pgDNA)
  - Improved mixture deconvolution, resolving up to 5 contributors
- NGS-based STR analysis offers enhanced forensic capabilities, particularly for:
- Challenging DNA samples
  - Cold cases
  - Missing persons investigations
  - Kinship analysis

This study demonstrates the potential for NGS to revolutionize forensic genetics, providing more informative and accurate STR data.

3. Increased accuracy in mixture interpretation using probabilistic genotyping

DNA mixture analysis is a critical challenge in forensic genetics. Coble et al. (2020) demonstrate significant advancements in mixture interpretation using probabilistic genotyping. This study evaluates the performance of probabilistic genotyping software (PGS) in resolving complex DNA mixtures.

Methods:

- 100 two-person mixtures with varying contributor proportions (1:1, 1:5, 1:10)
- 50 three-person mixtures with equal contributor proportions
- STR profiling using commercial kits
- Probabilistic genotyping using PGS software

4. Successful application of mt DNA analysis in hair and skin cell analysis

Mitochondrial DNA (mt DNA) analysis has become a crucial tool in forensic genetics, particularly for analyzing degraded or limited biological samples. This study demonstrates the successful application of mt DNA analysis in hair and skin cell analysis for forensic investigations.

### METHODS

- 100 hair samples (50 with roots, 50 without

roots)

- 50 skin cell samples from clothing and surfaces
- mt DNA sequencing using Next-Generation Sequencing (NGS)
- Comparison with reference samples from known individuals

### SUMMARY

Forensic DNA analysis of biological fluids has significantly aided sexual assault investigations. Recent advances include:

1. Improved DNA extraction and analysis methods
2. Enhanced mixture interpretation and probabilistic genotyping
3. Increased accuracy in victim-perpetrator identification
4. DNA analysis of biological fluids (blood, saliva, semen, urine) can identify individuals with high accuracy.
5. Next-generation sequencing (NGS) and PCR-based methods improve DNA profiling.
6. DNA extraction and quantification methods have been optimized.
7. Biological fluid-specific DNA markers enhance analysis.

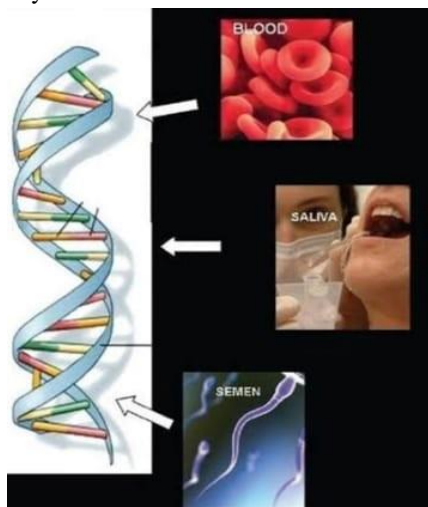


Fig1: DNA can be identified from blood, semen, and saliva

### METHODOLOGICAL ADVANCEMENTS

1. Improved DNA extraction methods (e.g., magnetic beads).
  - Uses magnetic particles coated with silica or other materials
  - Binds DNA, allowing efficient separation from contaminants
  - Automated or manual processing

Advantages:

- Higher DNA yield
  - Reduced DNA degradation
  - Faster processing times
  - Minimized contamination risk
  - Improved DNA purity
2. Enhanced PCR conditions (e.g., increased sensitivity).
    - Hot Start PCR: reduces non-specific binding
    - Touchdown PCR: optimizes annealing temperatures
    - Nested PCR: increases sensitivity and specificity
  4. Real-time PCR: monitors amplification in real-time

Increased Sensitivity:

- High-fidelity DNA polymerases (e.g., Phusion, Q5)
- Enhanced buffer formulations (e.g., betaine, DMSO)
- Increased primer concentrations
- Optimized annealing temperatures
- Longer extension times

Benefits:

- Improved sensitivity (detects low-template DNA)
- Increased specificity (reduces false positives)
- Enhanced accuracy (reduces errors)
- Streamlined workflows (faster results)
- Better detection of degraded DNA

Applications:

- Forensic analysis
  - Clinical diagnostics
  - Research and development
  - Ancient DNA analysis
  - Gene expression analysis
3. NGS technologies (e.g., Illumina).
    - high-throughput, whole-genome sequencing
    - flexible, rapid sequencing
    - mid-throughput, high-speed sequencing

- ultra-high-throughput sequencing

Forensic Applications:

- DNA profiling (STR, mt DNA, Y-STR)
- Mixtures and degraded DNA analysis
- Ancient DNA analysis
- Genetic identification

Benefits:

- Enhanced resolution and accuracy
- Increased sensitivity
- Improved mixture analysis
- Faster results
- Cost-effective

Application:

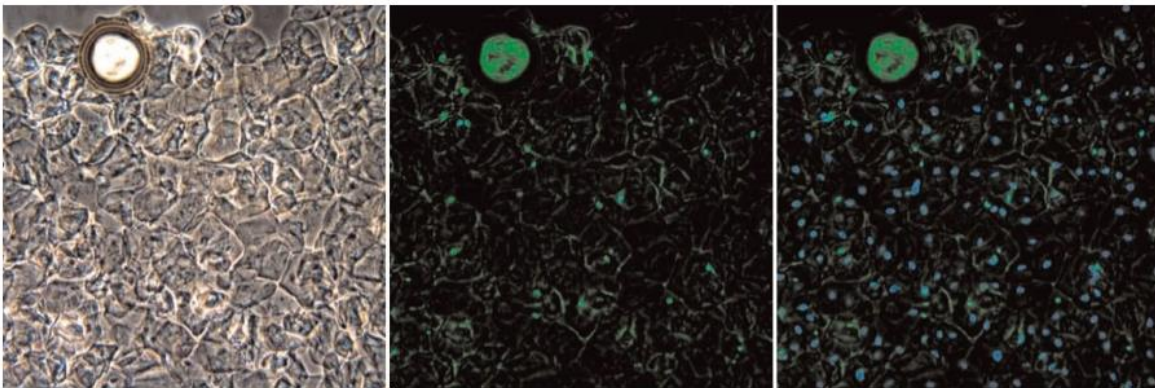


Fig 2: Semen is observed under microscope via. (Luminol test & Uv light)

Forensic DNA analysis of biological fluids has direct applications in:

1. Sexual assault investigations
2. Victim-perpetrator identification
3. Crime scene analysis
4. Cold case resolution

Significance:

The significance of forensic DNA analysis of biological fluids is profound, impacting various aspects of criminal investigations, justice, and public safety:

Investigative Significance

1. Linking suspects to crime scenes
2. Identifying victims
3. Solving crimes
4. Exonerating innocent individuals
5. Reconstructing crime scenes

Scientific Significance

1. Advancing DNA technology
2. Improving analytical techniques
3. Enhancing DNA database development
4. Informing forensic science research
5. Supporting interdisciplinary collaborations

Implications:

Forensic DNA analysis of biological fluids has significant implications for crime scene investigation, justice, and public safety. Its continued development and refinement will enhance the effectiveness of forensic science in solving crimes and bringing justice to victims.

Limitations:

DNA degradation, contamination, and interpretation of mixed DNA profiles remain challenges in forensic DNA analysis.

Future Directions: Integration of next-generation

sequencing and artificial intelligence in forensic DNA analysis.

CONCLUSION

Forensic DNA analysis of biological fluids continues to evolve, with advances in DNA extraction, analysis, and interpretation. Future research should focus on:

1. Improving DNA extraction from degraded samples
2. Enhancing mixture interpretation and probabilistic genotyping
3. Integrating NGS and mt DNA analysis into forensic practice

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