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COMPARATIVE EVALUATION OF METHODS FOR DIFFERENTIATING BIOLOGICAL FLUIDS BASED ON MRNA PROFILES

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Abstract

(1) Background: Accurate identification of biological fluids at crime scenes is a cornerstone of forensic molecular biology. Traditional presumptive and confirmatory tests are often limited in specificity and sensitivity, and mRNA-based profiling has emerged as a powerful tissue-specific identification approach. **(2) Methods:** This review systematically compares four primary mRNA detection platforms — reverse transcription quantitative PCR (RT-qPCR), DNA microarray analysis, NanoString nCounter technology, and RNA sequencing (RNA-Seq) — applied to the differentiation of blood, saliva, semen, vaginal secretions, menstrual blood, and skin cells. Published validation studies and forensic case reports were analyzed. **(3) Results:** RT-qPCR demonstrated the best balance of sensitivity, specificity, and cost-effectiveness for routine forensic application. NanoString nCounter showed superior multiplexing capacity and reproducibility. RNA-Seq offered the highest resolution but remains impractical for most forensic laboratories due to cost. LAMP-based methods represent a promising rapid alternative. **(4) Conclusions:** mRNA profiling provides reliable, tissue-specific identification of biological fluids superior to conventional methods. RT-qPCR and NanoString platforms are currently best suited for forensic implementation, while RNA-Seq may become standard practice as costs decrease.

Key words

mRNA profiling, biological fluid identification, forensic genetics, RT-qPCR, NanoString, RNA-Seq, tissue-specific markers, crime scene investigation.

Introduction

The identification of biological fluids recovered from crime scenes is of critical importance in forensic investigations. Traditionally, this has relied on presumptive chemical tests (e.g., luminol for blood, prostate-specific antigen for semen) and microscopic examination. While widely employed, these methods suffer from

significant limitations in specificity, sensitivity, and the inability to simultaneously identify multiple fluid types from a mixed sample [1, 3].

The analysis of messenger RNA (mRNA) has emerged as a highly promising approach for body fluid identification. Unlike DNA, which is identical in virtually all cell types, mRNA expression patterns are tissue-specific — a property that allows the cellular origin of a biological stain to be determined with high precision [2, 5]. Seminal studies by Juusola and Ballantyne established that specific transcript markers could reliably distinguish between blood, saliva, semen, vaginal secretions, menstrual blood, and skin cells [4, 7].

Since these pioneering reports, multiple detection platforms have been evaluated for forensic mRNA analysis. Each offers distinct advantages and limitations with respect to sensitivity, multiplexing capacity, resistance to RNA degradation, and operational requirements. A systematic comparative evaluation of these methods is essential to guide practitioners in selecting the most appropriate platform for their specific forensic context [6, 9].

The aim of this study is to systematically compare the principal mRNA-based methods — RT-qPCR, microarray, NanoString nCounter, RNA-Seq, and LAMP-based approaches — for the differentiation of biological fluids of forensic relevance.

Materials and Methods

This study was conducted as a systematic comparative review. A literature search was performed using PubMed, Scopus, and Web of Science databases covering publications from 2000 to 2024, using the search terms 'mRNA profiling forensic,' 'body fluid identification mRNA,' 'tissue-specific RNA markers,' and 'forensic transcriptomics.' Studies reporting original validation data for mRNA-based biological fluid identification methods were included.

Six categories of biological fluid were considered: peripheral blood, saliva, semen, vaginal secretion, menstrual blood, and skin/epithelial cells. For each fluid type, tissue-specific mRNA markers with documented forensic validation were identified (Table 1). The detection platforms evaluated were: (1) RT-qPCR; (2) DNA microarray; (3) NanoString nCounter; (4) RNA-Seq; and (5) LAMP-based methods. Evaluation criteria included analytical sensitivity, specificity, multiplexing capacity, RNA integrity requirements, processing time, and cost per analysis.

Where available, data from degraded or aged sample studies were included to reflect realistic forensic conditions. Statistical results from original validation studies were extracted and summarized descriptively.

Table 1

Tissue-Specific mRNA Markers Used for Forensic Biological Fluid Identification

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Biological Fluid	Marker Gene	Specificity	Detection Method
Blood	HBB, SPTB	High	RT-qPCR
Saliva	HTN3, STATH	High	RT-qPCR / NanoString
Semen	PRM1, TGM4	Very High	RT-qPCR
Vaginal secretion	CYP2B7P1, MUC4	Moderate–High	RT-qPCR
Menstrual blood	MMP10, MMP11	High	RT-qPCR / Microarray
Skin cells	LCE1C, CDSN	Moderate	RNA-Seq

Note: RT-qPCR – reverse transcription quantitative PCR; RNA-Seq – RNA sequencing.

Result and Discussion

RT-qPCR remains the most widely adopted platform in forensic mRNA analysis. It offers high sensitivity (capable of detecting mRNA from samples as small as 0.5 µL), excellent specificity when validated marker panels are used, and relatively low cost. Studies have demonstrated that RT-qPCR-based panels targeting two to four markers per fluid type achieve identification accuracies exceeding 95% for fresh samples [2, 4, 8]. However, performance decreases with degraded RNA, a common challenge in casework where samples may be exposed to heat, humidity, UV radiation, and microbial activity.

Microarray technology allows simultaneous interrogation of thousands of transcripts, enabling genome-wide expression profiling. While microarray studies have contributed significantly to the discovery of novel tissue-specific markers, the platform's requirement for relatively large amounts of high-quality RNA and its high cost limit its routine forensic application. Its primary value lies in the research and discovery phase rather than operational casework [6, 10].

NanoString nCounter technology has gained considerable attention as a forensic tool due to its ability to directly quantify up to 800 mRNA targets simultaneously without requiring reverse transcription or amplification. This digital counting approach results in superior reproducibility and reduced susceptibility to RNA degradation compared to RT-qPCR. Validation studies have demonstrated high specificity and sensitivity even from samples stored for up to 12 months under adverse conditions [9, 11]. The main limitation is the moderate cost of the instrumentation and consumables.

RNA-Seq provides the most comprehensive transcriptomic data, enabling identification of all expressed transcripts and detection of novel markers. It

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demonstrates superior sensitivity and allows discrimination of mixed biological fluid samples through computational deconvolution. However, RNA-Seq requires substantial bioinformatics infrastructure, high-quality RNA, significant processing time, and considerable cost per sample. At present, it is best suited to research settings, though ongoing reductions in sequencing costs may render it practical for forensic laboratories in the medium term [7, 12].

LAMP-based approaches perform isothermal nucleic acid amplification, representing a promising alternative for rapid field-deployable testing. They require minimal instrumentation, are highly cost-effective, and can be performed without sophisticated laboratory infrastructure. Current limitations include lower multiplexing capacity and moderate specificity compared to RT-qPCR [3, 13].

Table 2

Comparative Performance of mRNA Detection Methods for Forensic Biological Fluid Identification

Method	Sensitivity	Specificity	Cost	Forensic Suitability
RT-qPCR	High	High	Low	Excellent
Microarray	Moderate	Moderate	High	Good
NanoString nCounter	High	Very High	Moderate	Excellent
RNA-Seq	Very High	Very High	Very High	Research stage
LAMP-based	Moderate	Moderate	Very Low	Promising

Note: Ratings are based on synthesis of published validation studies under forensic-relevant conditions.

A key challenge across all platforms is RNA instability. mRNA degrades rapidly under environmental conditions typical of crime scenes. Studies consistently show that RNA integrity number (RIN) values below 3 significantly reduce detection efficiency for all methods. However, short amplicon RT-qPCR designs and NanoString's hybridization-based counting are more tolerant of degraded RNA than full-length transcript approaches. The selection of robust, validated marker panels — including internal RNA quality controls — is therefore essential for reliable forensic application [5, 8, 11].

Mixed biological fluid samples present additional complexity, particularly relevant in sexual assault casework. RT-qPCR multiplex panels can resolve mixtures when individual fluid contributions exceed approximately 10% of the total. NanoString and RNA-Seq offer improved resolution for complex mixtures but require more sophisticated data interpretation. Standardized interpretation

guidelines and proficiency testing programs remain areas requiring further development within the forensic community [4, 9].

Conclusions

mRNA profiling represents a scientifically robust and increasingly validated approach for tissue-specific identification of biological fluids in forensic casework. Among the available platforms, RT-qPCR offers the best combination of sensitivity, specificity, cost, and operational practicality for routine forensic laboratory use. NanoString nCounter technology provides superior multiplexing and degradation tolerance, making it a strong alternative where resources permit. Microarray and RNA-Seq platforms currently serve primarily as research and discovery tools, though RNA-Seq may transition to routine practice as costs decline. LAMP-based methods offer potential for rapid field screening.

Future priorities include the development of internationally standardized marker panels, validation guidelines for degraded samples, and consensus interpretation frameworks for mixed fluid specimens. As mRNA profiling continues to mature, its integration into standard forensic workflows will significantly enhance the evidentiary value of biological trace evidence.

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