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Identification of forensically relevant body fluids using a panel of differentially expressed microRNAs

Erin K. Hanson [a,b], Helge Lubenow [c], Jack Ballantyne [a,b,d,*]

[a] Graduate Program in Biomolecular Science, University of Central Florida, Orlando, FL 32816, USA
[b] National Center for Forensic Science, Orlando, FL 32816, USA
[c] Qiagen GmbH, 40724 Hilden, Germany
[d] Department of Chemistry, University of Central Florida, Orlando, FL 32816, USA

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ABSTRACT

The serology-based methods routinely used in forensic casework for the identification of biological fluids are costly in terms of time and sample and have varying degrees of sensitivity and specificity. Recently, the use of a molecular genetics-based approach using messenger RNA (mRNA) profiling has been proposed to supplant conventional methods for body fluid identification. However, the size of the amplification products used in these mRNA assays (~ 200–300 nt) might not be ideal for use with degraded or compromised samples frequently encountered in forensic casework. Recently, there has been an explosion of interest in a novel class of small noncoding RNAs, microRNAs (miRNAs, ~20–25 bases in length), with numerous published studies reporting that some miRNAs are expressed in a tissue-specific manner. In this article, we provide the first comprehensive evaluation of miRNA expression in dried, forensically relevant biological fluids—blood, semen, saliva, vaginal secretions, and menstrual blood—in an attempt to identify putative body fluid-specific miRNAs. Most of the 452 human miRNAs tested (~67% of the known miRNA*ome*) were either expressed in multiple body fluids or not expressed at all. Nevertheless, we have identified a panel of nine miRNAs—miR451, miR16, miR135b, miR10b, miR658, miR205, miR124a, miR372, and miR412—that are differentially expressed to such a degree as to permit the identification of the body fluid origin of forensic biological stains using as little as 50 pg of total RNA. The miRNA-based body fluid identification assays were highly specific because the miRNA expression profile for each body fluid was different from that obtained from 21 human tissues. The results of this study provide an initial indication that miRNA profiling may provide a promising alternative approach to body fluid identification for forensic casework.

In the past, standard practice in forensic casework analysis typically included a preliminary screening of evidentiary items recovered during the investigation of criminal offenses to identify the presence, and possible tissue origin, of biological material. The presence of biological material such as blood, semen, and saliva stains can indicate the location of potential sources of DNA that, once recovered, could be used to identify the donor of the biological material. Typically, conventional methods for body fluid stain analysis are carried out in a serial manner, with a portion of the stain being tested for only one body fluid at a time. Frequently, multiple tests are required to first presumptively identify the presence of biological fluids, followed by additional testing to confirm the presence of the fluid or identify the species of origin. Therefore, these methods are costly not only in terms of the time and labor required for their completion but also in terms of the amount of sample consumed during the performance of each assay. Although these conventional methods can confirm the presence of human blood and semen, none of the routinely used serological and immunological tests can definitely identify the presence of human saliva or vaginal secretions. With the large volume of cases that operational crime laboratories are faced with processing every year, a significant amount of the total time spent on an individual case can be devoted solely to the screening of evidentiary items for the presence of biological material. The inability to positively confirm the presence of certain biological fluids, the consumption of valuable samples, and the time and labor required have resulted in a trend to bypass conventional body fluid identification methods and proceed straight to the analysis of DNA present in forensic samples. Proponents of this approach argue that the recovery of human DNA from evidentiary items directly indicates the presence of human biological material, thereby eliminating the need for conventional body fluid testing.

* Corresponding author. Address: Graduate Program in Biomolecular Science, University of Central Florida, Orlando, FL 32816, USA. Fax: +1 407 823 2252.
E-mail address: jballant@mail.ucf.edu (J. Ballantyne).

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There are several disadvantages to bypassing the body fluid identification step during biomolecular forensic analysis. First, the analytical methods used to analyze DNA are considerably more expensive than basic serological testing. Therefore, the use of DNA analysis as a means to identify the presence of human biological material might not be justifiable from a budgetary standpoint. In addition, a smaller number of samplings from an individual piece of evidence containing multiple stains may be collected in an attempt to reduce the associated cost of analysis. Critical evidence may be missed using this type of approach that might have been identified using a larger preliminary screen with basic serological methods. A second disadvantage of the disuse of body fluid identification methods is that the identification of the biological material present might be crucial to the investigation and prosecution of the case. For example, consider a sexual assault of a child with a stepfather suspect where the stepfather's DNA profile was recovered from samples taken from the child's underwear and bedding. The stepfather could argue that the source of the DNA was from his skin cells deposited from casual and frequent contact with the child's clothing and bedding. However, the finding that his DNA originated from a semen stain and not skin cells would be more problematic for him to explain away and would more strongly support the allegation of a sexual assault. Another example of a case demonstrating the importance of identifying the body fluid could be that DNA from a sexual assault victim is found in a suspect's vehicle and the suspect claims it was present due to casual contact given that the victim had ridden in his car numerous times. However, the significance of this evidence would increase if the source of the DNA could be shown instead to originate from the victim's vaginal secretions, a circumstance that would be more difficult to attribute to casual contact as opposed to a sexual assault. The routine use of body fluid identification methods prior to DNA analysis awaits the development of suitable molecular genetics-based methods that are fully compatible with the current DNA analysis pipeline. For any new body fluid assay to be suitable for forensic casework, it must demonstrate a high degree of specificity for each body fluid, permit parallel analysis of the different biological fluids, is completed in a timely and labor-efficient manner, and be sufficiently sensitive.

Two possible alternatives to the currently used body fluid identification methods have been proposed recently: Raman spectroscopy and messenger RNA (mRNA)[1] profiling [1–7]. The use of Raman spectroscopy, although currently used in forensic laboratories for the identification of trace evidence material (e.g., drugs, fibers, paint, ink) [8–19], has not been investigated extensively for use with biological samples [7]. Although it shows promise for future use because it is a nondestructive method, there needs to be additional studies performed to demonstrate the reproducibility of the method (i.e., intersample and interindividual variation) and the ability to detect and correctly identify multiple biological fluids in an admixed sample. mRNA profiling of tissue-specific gene transcripts with forensic samples for the identification of body fluids has been reported recently and appears to satisfy most of the above-mentioned criteria [3–5]. More studies regarding an mRNA approach have been presented to the forensic community with a greater demonstration of the reliability and suitability of this method for use with forensic biological samples than is available for the Raman spectroscopy approach. Currently, therefore, it would seem likely that an mRNA approach could be integrated into operational forensic casework with greater ease.

The mRNA in aged and compromised dried stains appears to be sufficiently stable for forensic analysis [20]. However, as with DNA, heat and humidity are detrimental to RNA stability and result in a time-dependent fragmentation of the polynucleotide chain [20]. Typically, forensic assays employ some biomarkers whose amplicon sizes are more than 250 bases, resulting in amplification failure when highly degraded samples are encountered [21]. Thus, reduced-size amplicons for short tandem repeat (STR) and mitochondrial DNA profiling methods are being increasingly used for the analysis of degraded samples [22–31]. Similarly, smaller amplicons could be designed for use in mRNA-based forensic assays, although they may present additional technical assay design challenges because of the need to ensure that contaminating genomic DNA does not confound the analyses. In theory, another way to reduce the amplicon size would be to employ short RNA biomarkers instead of mRNA.

Recently, there has been an explosion of interest in a class of small noncoding RNAs, microRNAs (miRNAs), whose regulatory functions in various developmental and biological processes have been identified [32–49]. The roles of miRNAs in various cancers and diseases are also being revealed [50–61]. Several studies have examined the relative abundance of miRNAs in human tissue, with numerous miRNAs reported to be tissue specific [62–72]. However, so far no studies have described miRNA expression in forensically relevant, dried biological fluids—blood, semen, saliva, vaginal secretions, and menstrual blood. We hypothesize that it should be possible to identify miRNA species that, due to differential tissue expression, could be used to identify the body fluid origin of forensic biological stains with a high degree of sensitivity and specificity.

In this article, we provide the first comprehensive evaluation of miRNA expression in dried, forensically relevant biological fluids—blood, semen, saliva, vaginal secretions, and menstrual blood—in an attempt to identify putative body fluid-specific miRNAs. Most of the 452 human miRNAs tested (~67% of the known miRNA*ome* ([73,74]; see also http://microrna.sanger.ac.uk)) were either expressed in multiple body fluids or not expressed at all. Nevertheless, we have identified a panel of nine miRNAs—miR451, miR16, miR135b, miR10b, miR658, miR205, mi124a, miR372, and miR412—that are differentially expressed to such a degree as to permit the identification of the body fluid origin of forensic biological stains using as little as 50 pg of total RNA. The assays were highly specific because the miRNA expression profile for each body fluid was different from that obtained from 21 human tissues. The results of this study indicate that miRNA profiling may provide a promising alternative approach to body fluid identification for forensic casework.

Materials and methods

Preparation of body fluid stains

Body fluids were collected from volunteers using procedures approved by the University of Central Florida's institutional review board. Informed written consent was obtained from each donor. Blood samples were collected by venipuncture into additive-free vacutainers, and 50-μl aliquots were placed onto cotton cloth and dried at room temperature. Freshly ejaculated semen samples were provided in sealed plastic tubes and stored frozen until they were dried onto sterile cotton swabs. Saliva samples were provided in sealed plastic tubes and stored frozen until they were dried onto sterile cotton swabs. Buccal samples were col-

[1] *Abbreviations used:* mRNA, messenger RNA; STR, short tandem repeat; miRNA, microRNA; snRNA, small nuclear RNA; DEPC, diethylpyrocarbonate; RT, reverse transcriptase; PCR, polymerase chain reaction; cDNA, complementary DNA; C_t, cycle threshold; RT–, samples to which no reverse transcriptase was added; 2D, two-dimensional; 3D, three-dimensional; QT-PCR, quantitative real-time PCR; CLL, chronic lymphocytic leukemia.