



B172 mRNA Profiling: Body Fluid Identification Using Multiplex Real-Time PCR

Jane S. Juusola, BS*, University of Central Florida, Department of Chemistry, PO Box 162366, Orlando, FL 32816-2366; Kevin W.P. Miller, PhD, FBI Academy, Building 12, CTFSRU, Quantico, VA 22135; and Jack Ballantyne, PhD, University of Central Florida, Department of Chemistry, PO Box 162366, Orlando, FL 32816-2366

Attendees will learn a novel means of identifying body fluids of forensic interest.

This presentation will impact the forensic community and/or humanity by demonstrating the mRNA based approach, such as the multiplex real-time PCR method described here, possibly allowing for the facile identification of body fluid stains and is one of many assay platforms that could conceivably supplant the battery of serological and biochemical tests currently employed in the forensic serology laboratory.

Conventional methods of body fluid identification use labor-intensive, technologically diverse techniques that are performed in a series, not parallel manner, and are costly in terms of time and sample. Furthermore, for some frequently encountered body fluids no confirmatory technique exists. There is no definitive test, for example, for the presence of saliva or vaginal secretions. In seeking to develop novel multiplex (i.e., parallel) analysis procedures for body fluid identification that are compatible with current DNA analysis procedures, The authors have chosen assays based upon messenger RNA (mRNA) since it is expressed in a tissue type specific manner. Terminally differentiated cells, such as blood lymphocytes, ejaculated spermatozoa, or epithelial cells lining the oral cavity, have a unique pattern of gene expression, which is evinced by the presence and relative abundance of specific mRNA species. If the type and abundance of mRNAs can be determined in a stain or tissue sample recovered at the crime scene, it would be possible to definitively identify the tissue or body fluid in question. Advantages of an mRNA-based approach, compared to conventional biochemical analysis, include greater specificity, simultaneous, and semi-automated analysis through a common assay format, improved timeliness, decreased sample consumption, and compatibility with DNA extraction methodologies.

It was previously reported that it is possible to isolate total RNA of sufficient quality and quantity from biological stains to enable subsequent detection of particular mRNA species using the reverse transcription-polymerase chain reaction (RT-PCR) technique and that has identified candidate sets of blood-, saliva-, semen-, vaginal secretions-, and menstrual blood-specific genes using a combination of literature and public database searches.

In the present work, the authors report the development of a set of multiplex real-time PCR assays for the definitive identification of blood, saliva, semen, vaginal secretions, and menstrual blood. Real-time PCR employs a 5' nuclease assay to detect specific amplicons and eliminates the need for post-PCR processing and gel electrophoresis. The real-time instrument is capable of multi-color detection, and so by using probes labeled with different reporter fluorophores, it is possible to develop multiplex assays for body fluid identification. Real-time PCR also has the ability to quantitate target sequences, which is important in establishing the tissue-specificity of a gene product, particularly when the relative abundance of a number of different mRNAs can demonstrate a unique or restricted pattern of expression.

Real-time PCR triplexes were developed that are composed of two body fluid-specific genes and one housekeeping gene and have been optimized for the detection of blood, saliva, semen, vaginal secretions, and menstrual blood as single or mixed stains. The methodology is based upon determining the delta C_t (ΔC_t) values generated using the C_t for the housekeeping gene (HSK) and the C_t for each of the body fluid-specific genes (BFG) (C_t HSK t BFG). Depending upon the body fluid-specific gene being tested, a positive ΔC_t value would indicate the presence of a particular body fluid, while a negative ΔC_t value would indicate the absence of that body fluid.

An mRNA based approach, such as the multiplex real-time PCR method described above, could allow the facile identification of the tissue components present in a body fluid stain and is one of many assay platforms that conceivably could supplant the battery of serological and biochemical tests currently employed in the forensic serology laboratory.

mRNA Profiling, Multiplex Real-Time PCR, Body Fluid Identification