MESSENGER RNA PROFILING: BODY IDENTIFICATION USING MULTIPLEX REAL-TIME PCR

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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, we 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 though a common assay format, improved timeliness, decreased sample consumption and compatibility with DNA extraction methodologies.

Previously we have 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 we have 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, we report the development of a set of multiplex real-time PCR assays for the definitive identification of blood, saliva, semen, and menstrual blood. Real-time PCR employs a 5' nuclease assay to detect specific amplimers 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.

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