B148 mRNA Profiling: Body Fluid Identification Using Multiplex RT-PCR

Jane S. Juusola, BS, University of Central Florida, Department of Chemistry, PO Box 162366, Orlando, FL 32826-2366; Kevin W.P. Miller, PhD, FBI Academy, Building 12, CTFSRU, Quantico, VA 22135; Jack Ballantyne, PhD, National Center for Forensic Science, University of Central Florida, PO BOX 162367, Orlando, FL 32816-2367*

After attending this presentation, attendees will have been presented with a novel means of identifying body fluids of forensic interest.

The forensic community will be shown how the highlighted system could supplant the battery of serological and biochemical tests currently employed in the forensic serology laboratory.

Since it can be important to identify the nature of the body fluids present in a stain recovered at a crime scene, the development of a body fluid identification system that is compatible with current DNA typing procedures is desirable. 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. Moreover, 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 considered assays based upon protein and messenger RNA (mRNA) since both are expressed in a tissue specific manner. However, multiplex analysis of complex, partially degraded protein mixtures such as those present in body fluid stains awaits further developments in proteomics. Messenger RNA is considered a better option because the technologies for massively parallel analysis continue to be developed due to the rapidly evolving field of functional genomics.

Terminally differentiated cells, whether they comprise of blood monocytes or lymphocytes, ejaculated spermatozoa, or epithelial cells lining the oral cavity become such during a developmentally regulated program in which certain genes are turned off whereas others are turned on. Thus, a pattern of gene expression is produced that is unique to each cell type, which is evinced by the presence and relative abundance of specific mRNA species. If the type and abundance of mRNAs could 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-automatic 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 RT-PCR technique and that we have identified candidate sets of saliva-, and semen-specific genes. Since that time, we have also identified and tested candidate sets of bloodand vaginal secretions-specific genes using a combination of literature and public database searches.

In the extraction method that we employ, total RNA is isolated from biological stains by extraction with guanidine isothiocyanatephenol:chloroform and precipitated with isopropanol. The extracted total RNA is treated with DNase I, and then reverse-transcribed using random decamers as the first strand primer. Finally, the cDNA is amplified using gene-specific primers. The RT-PCR amplimer sizes are carefully chosen to span the range of 100bp–350bp to allow facile separation on agarose gels followed by visualization with a nucleic acid stain or by other platforms, such as capillary electrophoresis.

In the present work, we report the development of a multiplex RT-PCR assay for the definitive identification of all of the body fluids commonly encountered in forensic casework analysis, namely blood, saliva, semen, and vaginal secretions. The tetraplex is composed of four body fluid specific genes and has been optimized for the detection of blood, saliva, semen, and vaginal secretions as single or mixed stains. The methodology is based upon gene expression profiling analysis in which the tissue specific genes are identified by detecting the presence of appropriate mRNA species.

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

mRNA Profiling, Multiplex RT-PCR, Body Fluid Identification

Copyright 2004 by the AAFS. Unless stated otherwise, noncommercial *photocopying* of editorial published in this periodical is permitted by AAFS. Permission to reprint, publish, or otherwise reproduce such material in any form other than photocopying must be obtained by AAFS. ** Presenting Author*