B120 mRNA Profiling: Identification of Solid Tissues of Forensic Interest by Multiplex Real-Time PCR

Jane S. Juusola, PhD, University of Central Florida, Department of Chemistry, Orlando, FL 32816-2366; Kerri Dugan, PhD, Counterterrorism and Forensic Science Research Unit, Laboratory Division, Quantico, VA 22135; and Jack Ballantyne, PhD, University of Central Florida, Department of Chemistry, National Center for Forensic Science, Orlando, FL 32816-2366*

After attending this presentation, attendees will be briefed on a novel mean of identifying tissues of forensic interest.

This presentation will impact the forensic community and/or humanity by demonstrating a mRNA based approach, such as the multiplex real-time PCR method described here, could allow the definitive identifi- cation of the tissue components present in a forensic casework sample and is one of many assay platforms that conceivably could supplant conven- tional histological methods currently employed in forensic casework analysis.

Fragments of solid tissue from internal organs such as brain or muscle are occasionally encountered at violent crime scenes especially those involving assaults with a firearm and may be associated with bullet frag- ments that have entered and exited an individual. The identification of the tissue source of this solid tissue may be an important investigative aid in itself. However, identification of the tissue is presently accomplished by conventional histological means, which is time consuming, labor-intensive, and requires the services of a highly trained histologist and/or pathologist.

Terminally differentiated cells, whether blood lymphocytes, ejacu- lated spermatozoa, muscle fibers, or adipocytes, 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 bio- chemical or histological 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 has been previously reported that it is possible to isolate total RNA of sufficient quality and quantity from biological stains to enable subse- quent detection of particular mRNA species using the reverse transcription-polymerase chain reaction (RT-PCR) technique and that candidate sets of blood-, saliva-, semen-, vaginal secretions-, and menstrual blood-specific genes have been identified using a combination of literature and database searches. The development of multiplex mRNA-based assays for the iden- tification of body fluids of forensic interest that are compatible with current DNA analysis procedures have been reported.

These same principles have been applied to the development of mRNA-based identification methods for tissues of forensic interest and have identified and tested candidate sets of skin-, muscle-, adipose-, and brain-specific genes in the present work.

A set of multiplex real-time PCR assays for the definitive identifi- cation of skin, muscle, adipose, and brain have been developed. Real-time PCR employs a 5' nuclease assay to detect specific amplimers and elimi- nates 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 mul- tiplex assays for tissue 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 that are composed of two tissue-specific genes and one housekeeping gene have been developed and optimized for the detection of skin, muscle, adipose, and brain RNA. The data analysis 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 tissue-specific genes (TG) (C_t HSK- C_t TG). Depending upon the tissue- specific gene being tested, a positive dC_t value would indicate the presence of a particular tissue, while a negative dC_t value would indicate the absence of that tissue.

mRNA Profiling, Multiplex Real-Time PCR, Tissue Identification

 ** Presenting Author*