B119 Age Determination by RNA Profiling: Validation of a Newborn Child-Specific Real-Time PCR Assay

Michelle S. Alvarez, BS, University of Central Florida, PO Box 162366, Orlando, FL 32816-2366; and Jack Ballantyne, PhD, National Center for Forensic Science, PO Box 162367, Orlando, FL 32816-2366*

After attending this presentation, attendees will be informed of the spe- cific requirements, applications and limitations of two real-time PCR duplex systems which determine if a bloodstain originated from a newborn baby.

This presentation will impact the forensic community and/or humanity by setting forth the requirements for accurate and reliable real-time PCR results in relation to the reproducibility, sensitivity and specificity of the assays to newborn individuals, and the extent of their cross-reactivity with other nonhuman species and non-blood body fluids. Also the applicability of this method to aged bloodstains will be described.

It is now a matter of routine for the forensic scientist to obtain the genetic profile of an individual from DNA recovered from a biological stain deposited at a crime scene. Potential contributors of the stain must either be known to investigators (*i.e.* a developed suspect) or the questioned profile must be searched against a database of DNA profiles such as those main- tained in the CODIS National DNA database. However, in those instances where there is no developed suspect as yet or there is no match with any database sample, the DNA profile *per se* presently provides no meaningful information to investigators, with the notable exception of gender determi- nation.

To aid in these investigations another useful biometric that could provide important probative information is the age of an individual. For example, the ability to provide investigators with information as to whether a DNA donor is a newborn baby, an adolescent teenager or an elderly indi- vidual could be useful in certain cases, particularly those involving young children such as kidnapping or in providing additional intelligence during terrorist investigations. Currently no reliable validated molecular tests are available for age determination. The lifecycle of humans comprises a number of developmentally recognized stages. As the human proceeds through these developmental stages, sub-sets of the 30-50 thousand human genes will be differentially expressed. Theoretically, and given sufficient knowledge of developmental genetics, a determination of the global gene expression profile could reveal constellations of genes whose expression is correlated with a specific age.

It has been previously reported that it is possible to determine if a blood- stain originated from a newborn baby. Two novel hemoglobin isoforms were discovered and shown to have an expression pattern which was restricted to newborn individuals. Based on this information two duplex real-time PCR (qPCR) assays were developed to determine if a bloodstain originated from a newborn baby. The two duplex qPCR assays have undergone a develop- mental validation study that evaluated their reproducibility and specificity to newborn individuals, species specificity, body-fluid specificity, sensitivity and ability to analyze environmentally-aged bloodstains.

The reproducibility of the two duplexes to positively identify newborn individuals was determined by testing newborn and infant individuals, aged 1 hour to 9 months (n=20). The specificity of the assays for the accurate iden- tification of newborns was determined by testing male and female indi- viduals, aged 1 hour to 102 years (n=76). Human specificity was tested against blood obtained from various animal species. The ability of these assays to positively identify newborns in other commonly encountered body fluids was also examined to determine if the assays are blood specific. Saliva, semen, vaginal secretion and menstrual blood samples were obtained from multiple individuals of varying ages and examined. The sensitivity of the method was determined by varying the concentrations of genomic DNA and cDNA, input into the qPCR reaction. Finally, stability studies were conducted by subjecting bloodstains to a variety of environmental conditions for 1, 3, 6, 9, 12 and 15 months, followed by qPCR analysis.

Age Determination, Identification of Newborns, Validation

 ** Presenting Author*