A155 mtDNA and STR Analysis of Stored Maggot Crop Content Extractions Following Real- Time PCR Quantitation

Changpin Zhang, MD, University of Alabama at Birmingham, CHB 116, 1600 6th Avenue South, Birmingham, AL 35233; and Jason G. Linville, PhD, University of Alabama at Birmingham, UBOB 317, 1201 University Boulevard, Birmingham, AL 35294*

After attending this presentation, attendees will understand a comparison of real-time PCR and Quantiblot method used for quantitating human DNA recovered from maggot crop contents, and learn the relationship between the success of mtDNA and STR analyses and the quantity of DNA in the extraction.

This presentation will impact the forensic science community by providing mtDNA and STR data from maggot crop content extractions that have been quantitated using real-time PCR.

DNA analysis of maggot crop contents can be used to identify a missing victim if maggots are discovered at a suspected crime scene in the absence of a corpse or can be used to associate a maggot with a specific corpse in a chain of custody dispute. Maggot crop DNA analysis is a new area of study; many of the limitations of this method have been explored in recent years. In a previous study by Linville and Wells, maggots fed on human tissue were preserved under eight different conditions, where the presence and type of preservation fluid, storage temperature, and length of storage time were varied. In a second study, the ability to recover human DNA from the maggot throughout its development and the persistence of DNA in the crop after the maggot's removal from human tissue were observed. In both studies, maggot crops were removed, extracted, and human DNA was quantitated using Applied Biosystems' Quantiblot Human DNA Quantitation Kit. Amplification and analysis of mitochondrial DNA (mtDNA) and short tandem repeat (STR) loci were attempted on all samples.

While the Quantiblot method provided an adequate method for quantitating samples, the test relied on subjective interpretation of band intensities, was unable to detect low levels of DNA in samples, and did not provide any information on the presence or absence of inhibitors in a given extraction. For example, in the preservation study, 45.8% (33/72) of the quantitation results fell below the level of the last visible standard, which was 0.06 ng/ul. In the development study, 88.9% (64/72) of the quantitation results fell below this standard. Several samples that fell below 0.06 ng/ul were successfully analyzed, producing mtDNA sequences and/or STR profiles. Successful analysis of the samples did not directly relate to the quantity of DNA in the sample, suggesting other factors, such as inhibition, may affect the ability to amplify.

The objective of this study was to lower the detection limit, increase the precision of results, and evaluate the presence of inhibitors by retesting the same maggot crop extractions from the previous studies using real-time PCR and the Quantifiler™ Human DNA Quantification kit (Applied Biosystems). Also, based on the real-time PCR quantitation results, mtDNA and STR analyses were reattempted on 8 maggot crop content extractions selected from each study in order to examine the consistence between the current test and the original test.

Compared with the Quantiblot method, real-time PCR lowered the detection limit, increased the precision of the quantitation results, and provided some evidence that inhibition was not an issue in these samples. The Quantifiler kit detected DNA in more samples than the Quantiblot kit used in the previous studies. Quantifiler failed to detect DNA in 22.2% (16/72) of the maggot crop content extractions from the preservation study and 66.7% (48/72) from the development study. The lowest amount of DNA detected by real-time PCR was 0.001 ng/ul. The internal positive control (IPC) included in each Quantifiler reaction did not indicate any PCR inhibition in most of the samples. The success of previous and repeated mtDNA and STR analyses did not always directly relate to the quantity of DNA in the extraction.

Forensic Entomology, Maggot Crop, DNA Analysis

 ** Presenting Author*