B208 Exploring the Relationship Between Quantitative Polymerase Chain Reaction (qPCR) and Short Tandem Repeat (STR) Data for Compromised, Low-Template DNA Samples

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The goal of this presentation is to provide attendees with an understanding of how information obtained from the qPCR reaction, such as the degradation index and Internal PCR Control (IPC) Cycle threshold (C_t), may relate to the resultant electropherogram. This will be accomplished by examining qPCR and STR data obtained from pristine, enzymatically degraded, sonicated, Ultraviolet (UV) -damaged, and humic acid-inhibited DNA extracts.

This presentation will impact the forensic science community by demonstrating that the correlation between qPCR and STR data varies and depends upon the way in which the DNA was compromised. Although results from the qPCR reaction are often used to inform downstream processing and interpretation, the data presented indicate that under certain circumstances, and particularly for low-template, compromised extracts, information obtained from the qPCR reaction is only an approximation of the amount of amplifiable DNA. The information garnered from qPCR becomes less informative as the DNA becomes more compromised and as the extract contains fewer copy numbers.

With the implementation of increasingly sensitive detection systems, compromised (i.e., damaged, degraded, or inhibited), low-template DNA samples are often encountered in forensic casework. During forensic DNA processing, quantitation via qPCR precedes STR amplification. Data obtained from the quantitation reaction, which now include a measurement of total human DNA content, an assessment of the level of degradation, and information pertaining to the amplification of an Internal PCR Control (IPC), may inform downstream processing steps.[1,2] Ideally, with well-paired qPCR and STR amplification kit chemistry, these metrics may be used to optimize the amount of sample added to the amplification reaction or indicate that additional treatment may be worthwhile.

This study seeks to relate and compare information obtained from the qPCR reaction to the resultant STR Electropherogram (EPG) using both pristine and compromised DNA samples obtained from whole blood. Compromised samples were generated via enzymatic degradation, sonication, UV damage, and inhibition with humic acid. For each type of compromised extract, three levels were produced by varying concentration (DNase and humic acid) or reaction time (Fragmentase, UV, and sonication). Extracts were quantified then amplified using 29 cycles at 0.25ng and 0.03ng. Fragment analysis was completed using common electrophoresis settings and an analytical threshold of one Relative Fluorescence Unit (RFU).

Three phenomena were observed: (1) the correlation between the Degradation Index (DI) obtained from the qPCR assay and the peak height slope observed across increasing molecular weight loci in the EPG varied with different types of compromised extracts. Sonicated samples displayed the highest correlation between DI and slope, and the remaining sample types in order of decreasing correlation for 0.25ng templates were: DNase/Fragmentase, UV, and humic acid. For all compromised extracts, this correlation became worse as the template mass decreased; (2) with the exception of inhibited extracts, a decrease in RFU at the lowest molecular weight loci was observed with all

compromised extracts as the DI increased despite the target mass remaining constant (0.25ng). The concentration of the 80bp target in compromised samples may be an overestimate of the amount of amplifiable product available for STR amplification. Although the STR amplicons tested range in size from ~80bp-450bp, the low molecular weight region is largely composed of rare autosomal alleles and non-autosomal markers with low discriminating power.[3] Preliminary results obtained with sonicated extracts quantified with a 140bp target and amplified with STRs that range in size from ~100bp-400bp suggest that using a longer qPCR target more representative of the size of the shortest high-frequency STR alleles may result in a more informative EPG for certain sample types; and, (3) with regard to extracts inhibited with humic acid, no correlation was observed between the DI and IPC C_t obtained from the qPCR assay and the resultant EPG. In the worst-case scenario, characterized by an out-of-range or undetected IPC C_t and an undefined degradation index, the corresponding EPGs exhibited no discernable peak height slope across loci and a drop-out rate of 0% for 0.25ng template samples. Cumulatively, these data indicate that for low-template, compromised extracts, information obtained from the qPCR reaction provides only some predictive value regarding the final EPG and may not be an appropriate method by which to decide whether a sample is likely to produce an EPG with substantive levels of signal.

Reference(s):

1. Holt A., Wootton S.C., Mulero J.J., Brzoska P.M., Langit E., Green R.L. Developmental validation of the Quantifiler® HP and Trio Kits for human DNA quantification in forensic samples. *Forensic Sci Int Genet.* 2016;21:145-57.
2. Ewing M.M., Thompson J.M., McLaren R.S., Purpero V.M., Thomas K.J., Dobrowski P.A., et al. Human DNA quantification and sample quality assessment: Developmental validation of the PowerQuant® system. *Forensic Sci Int Genet.* 2016;23:166-77.
3. Hill C.R., Duewer D.L., Kline M.C., Coble M.D., Butler J.M. U.S. population data for 29 autosomal STR loci. *Forensic Sci Int Genet.* 2013;7(3):e82-3.

DNA Quantification, DNA Degradation, PCR Inhibition