**AN ANALYSIS OF BINDING MECHANISMS FOR REAL-TIME POLYMERASE CHAIN REACTION (PCR) INHIBITION USING EFFICIENCY AND MELTING CURVE EFFECTS**

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The purpose of this study is to understand the potential mechanisms underlying the cause of inhibition: interaction of the inhibitor with the DNA, binding of the inhibitor to the DNA polymerase, and interaction with the polymerase during primer extension. This presentation will provide the forensic community with a better understanding of how binding mechanisms affect inhibition of DNA samples and to assist forensic DNA analysts in reducing the effect of PCR inhibitors by selecting the proper methods of relieving inhibition or the type of analysis that can be performed. The authors will present an analysis of eleven inhibitors that are commonly found in forensic DNA samples.

Real-time PCR, also known as quantitative PCR (qPCR), is a relatively new method that allows determination of the amount of amplifiable DNA in a forensic sample. qPCR can also be used to detect inhibition through the monitoring of internal control sequences. The procedure is based upon the incorporation of a fluorescent dye during thermal cycling, therefore monitoring a change in fluorescence that correlates with the accumulation of amplified product. There are different approaches used for fluorescence-based detection assays. Two of these chemistries – Plexor HY and TaqMan Systems – incorporate internal control sequences to detect inhibition. Alternatively, inhibition may be detected through the use of melt curve effects. Such analyses are possible with Plexor and SYBR Green assays.

Previous work using SYBR Green intercalation for qPCR detection has demonstrated that inhibitors can affect melt curves differently depending on their structure and mode of action. Inhibitors that bind DNA can cause melt curve shifts while those affecting Taq polymerase may not. Similar but distinguishable effects are seen when using Plexor based melt curves. Unlike qPCR procedures using SYBR Green, Plexor dyes are fluorescently linked to the modified base – 5'-methylisocytosine (iso-dC) – adjacent to the 5' end of the dsDNA. As a result, the Plexor HY System produces minimal interference in the dsDNA structure and is therefore an ideal procedure for measuring these effects. In this study, inhibition of qPCR was evaluated by observing the effect of various inhibitor concentrations and amplicon lengths on the amplification of forensic biological samples.

Based on the authors preliminary results, humic acid, calcium, collagen and phenol show concentration dependent shifts in melt curves for inhibitors suspected of DNA binding. These data tend to show that there is utility in careful analysis of melt curves and that the data obtained can provide complementary information with that produced by the amplification of internal control sequences. The inhibitory effects of other common PCR inhibitors such as urea, bile salts, guanidine, hematin, tannic acid and melanin are currently being observed. STR results performed in concert with these studies indicate that inhibition can lead to a generic loss of alleles from the larger STR loci, effects are commonly seen in all inhibitors. However, in addition to these generic effects, sequence specific allele dropout may also be observed, producing loss of smaller loci such as Amelogenin and D3S1358.

The goal of this research is to provide the forensic community with information on how inhibitors affect qPCR results and means of mitigating these effects prior to STR amplification. The authors believe that by monitoring qPCR efficiency and melt curve effects, DNA analysts will be able to better interpret results and take corrective action to minimize inhibition based allele dropout in their results.