A121 An Analysis of Binding Mechanisms for Real-Time Polymerase Chain Reaction (PCR) Inhibition Using Efficiency and Melting Curve Effects

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The goal of this presentation is to inform the forensic community on how inhibitors affect qPCR results and ways in reducing the effects of PCR inhibitors.

This presentation will impact the forensic science community by assisting DNA analysts with means of better interpreting results of inhibited 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 binding 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 therefore is 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 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 utility in careful analysis of melt curves and the data obtained can provide complementary information with that produced by the amplification of internal control sequences. The inhibitory affects of other common PCR inhibitors (e.g., urea, bile salts, guanidine, hematin, tannic acid and melanin) are currently being evaluated. 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 (e.g., 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. DNA analysts will be able to better interpret results and take corrective action to minimize inhibition based allele dropout in their results by monitoring qPCR efficiency and melt curve effects.

DNA Analysis, Real-Time PCR, Inhibitor Binding

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