THE EVALUATION OF MULTIPLE SECONDARY CLEANUP METHODS ON COMMON PCR INHIBITORS ENCOUNTERED IN FORENSIC CASEWORK

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A multitude of forensic unknown samples typically encountered in forensic DNA laboratories are often plagued by the presence of Polymerase Chain Reaction (PCR) inhibitors. Examples of some sources of PCR inhibition can include: soil laden items, blue jeans or dyed clothing, and even items containing large amounts of blood. A few of the compounds that are indeed responsible for this inhibitory effect include hematin, humic acid and Indigo dye. During a typical organic extraction these inhibitors, and others, often coelute with DNA and remain within the final extract volume. It is during subsequent DNA quantitation and typing that the effects of these inhibitors manifest, consequently resulting in analytical problems.

This evaluation serves to demonstrate the efficacy of several, secondary extraction methods for the separation of purified DNA from the abovementioned PCR inhibitors. To do this, known amounts of purified (inhibitor-free) DNA were combined with varying quantities of hematin, humic acid, and indigo dye in order to elicit PCR inhibition. Prior to secondary cleanup, these DNA/Inhibitor samples were subjected to real-time PCR quantitation and the level of inhibition was determined. Subsequently, these samples were subjected to various secondary cleanup methods including use of the Qiagen EZ1 DNA Investigator Kit, Promega DNA IQ, and Thiopropyl Sepharose 6B beads. Through real-time PCR, the quantity of DNA recovered and the quality of the internal PCR control before and after cleanup were compared. Both of these aspects were used to determine the success of each system in overcoming these inhibitors.