H64 Empirical Validation and Application of the Quality-Control Polymerase Chain Reaction (qcPCR) Inhibitor Detection System

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The primary objective of this study is to optimize the quality control Polymerase Chain Reaction (qcPCR) inhibitor detection system by empirically determining its breadth of effectiveness and its sensitivity range. Effectiveness and sensitivity will be tested by utilizing several commonly encountered PCR inhibitors. The qcPCR detection system will then be compared to several other methods used to detect the presence of inhibitory substances in PCR template solutions. A variety of soft tissue and bone samples with prior negative amplification results are evaluated using qcPCR.

This presentation will impact the forensic community and/or humanity by developing a more accurate understanding of the relative effects of various PCR inhibitors. Investigators will also be able to differentiate between amplification inhibition and DNA degradation beyond the current limits of analysis.

Many current forensic DNA analysis methods are dependent on the successful amplification of a template DNA sample using the polymerase chain reaction (PCR). However, forensic samples often contain co-extracted diffusible compounds that partly or completely inhibit amplification of the template DNA molecules producing false-negative PCR results. Common diffusible inhibitors encountered in forensic contexts include substances produced by the body (e.g., heme-products, bile salts, collagen) and compounds introduced from the environment by diagenetic processes (e.g., humic and tannic acids). Identifying PCR template samples that contain inhibitory substances allows investigators the opportunity to utilize inhibitor neutralization strategies, increasing the chance of a successful re-amplification attempt. This study will provide empirical evidence supporting the development of a PCR-based method to identify false-negative amplification results.

The proposed qcPCR system utilizes a 500 base-pair region of lambda phage DNA as the reporter template that is amplified using lambda phage specific primers. Test template solutions that have failed to amplify during previous attempts are spiked into the reporter amplification reaction. The qcPCR solution is then thermocycled using parameters optimized for reporter (lambda DNA) template amplification. The yield of amplified DNA obtained from the test template-spiked qcPCR is compared to that of a positive-control qcPCR lacking the test template. If both reactions yield the same amount of reporter amplicon (500 bp of lambda DNA), the test template solution does not contain diffusible inhibitory compounds capable of blocking or reducing the amplification efficiency of the reporter reaction. If however the test templatespiked samples yield less reporter amplicon than the control, the test template solution is assumed to contain diffusible inhibitors, suggesting genetic analysis will be compromised.

While a theoretically sound method, little empirical work has demonstrated its applicability to a range of known PCR inhibitors commonly encountered in forensic contexts. A variety of diffusible PCR inhibitors (humic acid, tannic acid, heme-products and collagen) are used to test the effectiveness and sensitivity range of the qcPCR system. Different concentrations of each inhibitor are mixed with varying concentrations of inhibitor-free genomic pig DNA. An amplification success matrix is constructed to determine the minimum inhibitor concentration necessary to reduce amplification efficiency. The inhibitor-genomic template mixes are then spiked into a qcPCR to assess the system's detection capabilities for single inhibitors. The aforementioned inhibitor/DNA templates are also used to evaluate the following inhibitor detection methods: spectrophotometric measurement of template purity, amplification efficiency calculation using real-time PCR, and the presence of oligonucleotide primer dimers in failed amplification attempts. Results are compared to assess the inhibitor detection capabilities of each method. The qcPCR inhibitor detection method is then used to evaluate lung, liver, spleen, psoas muscle, parietal, rib, vertebral body and femoral shaft DNA extractions that have failed to amplify during previous attempts. These samples were obtained over the course of two years from 28 pigs: 14 placed in a pond-shore environment and 14 deposited on a forest floor. Additionally, qcPCR analysis is reported for femoral DNA extractions from historic human burials.

This study will demonstrate that the proposed qcPCR inhibitor detection method allows investigators to reliably identify specimens that require more rigorous DNA extraction and purification procedures, thus facilitating the diagnostic amplification process and minimizing the potential for false-negative results.

Quality Control Polymerase Chain Reaction (qcPCR), PCR Inhibitors, Inhibitor Detection

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