B108 An Evaluation of QIAGEN® Investigator® 24plex GO! Using Crime Scene Substrates and Direct Amplification

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The goal of this presentation is to educate attendees in direct amplification of body fluids without the time-consuming and labor-intensive extraction and quantification steps. After attending this presentation, DNA analysts will be able to generate DNA profiles from body fluids when commonly found crime scene substrates containing inhibitors remain in the amplification reaction during the thermal cycling procedure.

This presentation will impact the forensic science community by generating DNA profiles quickly. The results can be obtained within a very short time period. This in turn can help to quickly locate a perpetrator or exonerate the innocent. No new instrument is needed and the method can be easily implemented in the forensic laboratory.

Short Tandem Repeat (STR) analysis is a technique performed routinely in forensic laboratories. Markers in these regions are amplified by Polymerase Chain Reaction (PCR) technology using specific primers, which have made them valuable in the forensic community. In violent personal crime cases, blood, saliva, semen, and nasal secretions are routinely encountered on various substrates. Direct amplification of these body fluid stains without time-consuming, labor-intensive extraction and quantitation steps has proven useful in forensic science laboratories.

Direct amplification entails amplifying the DNA present in a body fluid stain without extraction or quantitation steps and injecting the amplified product in a capillary electrophoresis system. The QIAGEN® 6-dye amplification system, Investigator® 24plex GO! allows the identification of 22 polymorphic STR loci and includes two innovative internal PCR controls (quality sensors). The quality sensors, QS1 and QS2, provide information regarding whether there is degradation and/or inhibition. These also indicate the absence of DNA or a failed PCR reaction. The current research utilizes this amplification kit to directly amplify minute amounts of blood, saliva, semen, and nasal secretions placed on various simulated crime scene substrates while the substrates remained in the reaction during amplification. Substrates chosen for deposition of these body fluids included various types of fabric ranging from white cotton to blue denim jeans and leather as well as cigarette butts, chewing gum, woodchips, straw, grass, and other objects.

Blood from four deceased donors was obtained from a forensic pathologist. Four semen samples were purchased from commercial vendors. Saliva and nasal secretions were collected from four individuals following the guidelines of the Office of Research Protection (ORP). Each body fluid was diluted in a 1:1 ratio. For blood, semen, and nasal secretions, 0.2µL of each diluted sample was deposited on a 1.2mm punch or cutting of each substrate. For saliva samples, 0.5µL of the body fluid was deposited on each substrate. After drying overnight, the stains were transferred to individual tubes. Next, 5µL of Investigator® GO! Lysis Buffer was added to each punched substrate containing blood, saliva, and nasal secretions and left at room temperature for 20 minutes with occasional mixing.

For semen samples, after drying overnight, 0.2µL of 1M DTT solution was added to each substrate containing semen samples. Each semen-stained substrate was then incubated at 56°C for 30 minutes. In the next step, each sample was shaken at 600rpm for another 30 minutes using a thermal mixer.

Each substrate containing only one type of body fluid was then subjected to amplification. For this step, 20µL of the reaction mixture was added to all tubes and amplification was performed following recommended protocol. During the amplification step, each substrate containing one of the four body fluids remained in the reaction mixture. Amplified products were injected into the 3130xl Capillary Electrophoresis (CE) system. GeneMarker® HID analysis software v 2.9 from SoftGenetics® was used for fragment analysis.

All stains created from the four body fluids using simulated crime scene substrates were amplified successfully, even when the substrates remained in the reaction mixture during amplification steps. All stains were created as single-source samples to generate DNA profile from one single donor. Thus, no mixture analysis was necessary in this project.

Consistent and concordant profiles were obtained from all body fluid-stained substrates. The S peak on QS2 locus occasionally dropped out, indicating inhibition in the sample, even when a complete profile was obtained. Known inhibitors such as soil or dye were present in some of the substrates. Therefore, this observation was not unexpected. Despite the substrates being present during the thermal cycling steps, the reagents were able to overcome inhibition and amplify DNA from challenging samples.

This study suggests that the Investigator® 24plex GO! is a valuable tool that can be easily incorporated in the analysis of body fluids such as blood, saliva, semen, and nasal secretions in forensic laboratories. Since there is no extraction and quantitation involved in the described procedure, the results can be obtained within a very short period of time. This can quickly help find a perpetrator or exonerate the innocent.

Direct Amplification, Investigator® 24plex GO!, Inhibitors