INTERNAL VALIDATION STUDY OF THE DIRECT TO DNA WORKFLOW USING THE ZYGEM FORENSICGEM SEXCRIME KIT WITH THE GENEAMP™ PCR SYSTEM 9700 THERMALCYCLER AND APPLIED BIOSYSTEMS™ QUANTIFILER™ TRIO DNA QUANTIFICATION KIT WITH THE APPLIED BIOSYSTEMS® 7500 REAL-TIME PCR SYSTEM AND THE HID REAL TIME PCR ANALYSIS SOFTWARE V1.2

Sherri Barborich, MS, James Anstead, PhD, Elizabeth O'Bannon, MS, Kelsey Lowther, MS, Maria Engel, BS.
DNA Solutions, Inc.

Direct to DNA processing of sexual assault kits saves the laboratory screening time compared to conventional serological testing methods and conserves potentially limited sample substrate for more sensitive, probative downstream DNA analysis. The ZyGEM forensicGEM SexCrime Kit is a commercially available product that directly lyses of sperm cells using a thermalcycler at three different temperatures. This kit utilizes a mesophilic enzyme at a lower temperature to degrade the cell membranes followed by a thermophilic proteinase at a higher temperature, which lyses the cell membrane and removes nucleosomes and deactivates nucleases. A final heat cycle deactivates the thermophilic proteinase and denatures the DNA into single strands for downstream analysis.

In this internal validation study, a direct to DNA workflow for the laboratory was developed utilizing the ZyGEM forensicGEM SexCrime Kit, quantifying the sample lysates using the Quantifiler Trio kit with the ABI 7500 qPCR instrument, and then characterizing the lysates using the Promega PowerPlex Fusion and Y23 systems on 3130XL and 3500 genetic analyzers. Throughout this study, this kit demonstrated high levels of sensitivity, with male DNA detected from single source samples at concentrations of semen as low as 0.1 nL/µL. No issues with direct quantification of lysates were observed, even in sample lysates containing mixtures of blood, semen, and soil. In addition, full, balanced profiles were obtainable directly from the lysates. In mixture studies, male DNA was detected in dilutions as low as 1:10,000 of semen to blood mixtures; however, male allele peaks were not observed in dilutions lower than 1:100 of semen to blood mixtures. To demonstrate the reproducibility and repeatability of the results obtained using this screening method, samples were isolated from known contributors. All DNA profiles obtained were concordant with the known profiles. Comparable results were obtained when two different analysts conducted independent experiments using this method. Over the course of the study, reagent blanks were run and analyzed following the direct to DNA workflow. No reagent blanks yielded DNA peaks above the analytical threshold. Moreover, the ZyGEM ForensicGEM SexCrime kit demonstrated robustness in lysing sperm cells present on a variety of different mock casework sample substrates. Overall, this screening workflow was found to be a high throughput, effective way to screen samples for male DNA.