B67 Optimization of DNA Extraction Procedures for Low Copy Number Degraded Samples

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Attendees will learn modified versions of common DNA extraction procedures that can be used for low copy number degraded samples

This presentation will impact the forensic community and/or humanity by providing optimized procedures to ensure that a high yield of DNA is obtained when a LCN degraded sample is encountered. Ultimately, a higher yield of DNA from the extraction will provide more DNA for amplification.

As LCN degraded samples are encountered more frequently in forensic casework, it would be useful to optimize current DNA extraction procedures in order to obtain a higher yield of DNA. The following DNA extraction procedures were investigated: DNA IQ□ (Promega), QIAamp□ (Qiagen), 5% Chelex® (BioRad), and a LCN extraction procedure developed at the Office of Chief Medical Examiner with the use of 0.01% SDS. Initial experiments were performed with purified DNA in amounts of 100 pg or less. The DNA was degraded with DNase I and subjected to each of the four extraction procedures, before and after optimization. Human embryonic kidney cells were also degraded with DNase I; and known amounts (~20 cells) were used in each of the extraction procedures. Bloodstains that were degraded with irradiation were also used in this study. As a control, LCN undegraded samples of the same nature were subjected to each extraction procedure, before and after optimization. All of the samples were quantitated with real-time PCR, amplified with the Power Plex 16® (Promega) multiplex system, and separated with the 3100 Genetic Prism® Analyzer (ABI).

For the DNA IQ□ (Promega) extraction procedure, the following parameters were changed to optimize the extraction of the LCN degraded samples: smaller volumes of resin and lysis buffer, shorter incubation time, and the addition of Poly A RNA. For the QIAamp□ (Qiagen) extraction procedure, the addition of Poly A RNA and an increased temperature during the elution step optimized the extraction. The extraction procedures with 5% Chelex® and 0.01% SDS both gave optimal results with shorter incubation times. DNA yield, or percentage recovery, was calculated for each extraction. The results from the optimized procedures were compared to those before optimization. An increase in DNA yield was seen with all of the optimized procedures. Usable DNA profiles were obtained for all of the samples subjected to the optimized procedures.

DNA Extraction, Low Copy Number, Degraded DNA

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