B27 Post Blast DNA Persistence: A Comparative Analysis of Three Extraction and Amplification Methods

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After attending this presentation, attendees will understand the capabilities and limitations of three singular methods for the extraction and amplification of nuclear DNA from an array of evidentiary materials recovered during controlled detonations of parcel bombs. Attendees will further appreciate the persistence of viable DNA on typical evidentiary items that have been subjected to the harsh environment of an explosion.

This presentation will impact the forensic community by introducing successful methods for obtaining full or partial DNA profiles from post-blast evidentiary materials.

A practical and reliable method for extracting and amplifying small quantities of DNA from evidence in criminal investigations is sought. Extracting and amplifying trace amounts of DNA for the purpose of obtaining a DNA profile may be essential to the association of suspects to evidentiary items. Recovery of very small quantities of DNA may be improved through the preferential use of optimized methods that can render suitable full or partial DNA profiles.

The sensitivity and reproducibility of varied methods for extraction of DNA was tested and validated through the use of known serial dilutions of whole blood using seven protocol variations for extraction. From these initial experiments three optimized extraction methods and two amplification methods were selected. The extraction methods included Orchid Cellmark's Optimized Organic Extraction procedure, Orchid Cellmark's EZ1 Biorobot extraction with a 100µl elution volume and Orchid Cellmark's EZ1 Biorobot extraction with a 50µl elution volume. Amplification was accomplished using either a PCR based method (Ampf*l*STR™ Identifiler Kit or Minifiler STR™ Kit by Applied Biosystems) or a whole genome amplification method (REPLI-g UltraFast™ Mini Kit by Qiagen). The extraction and amplification methods were applied to a set of evidentiary materials including blood, saliva, tape, hair and fingerprints (applied to a solvent-based adhesive) that had been prepared in triplicate. Each set of evidentiary materials was packaged within a parcel containing a pipe bomb and subjected to the controlled detonation of the improvised explosive device (IED) with the package.

All samples collected were extracted following an overnight lysis. After extraction, each sample was concentrated using a Microcon column and eluted using 20 µl of water. Each sample was quantitated using a Quantifiler™ Kit by Applied Biosystems. Samples were either diluted or concentrated to obtain a target amount of 250-500 pg of DNA in a 5 µl volume for the PCR amplifications or a target amount of 1 ng for the whole genome amplifications (WGA). The samples were amplified in a 12 µl amplification reaction volume. For the samples not yielding complete profiles, a re-amplification was performed if sufficient DNA was available, otherwise, the samples were exposed to a variety of post-PCR clean-up methods to improve the genetic profiles generated.

Successful typing of the evidentiary samples was demonstrated with all three of the extraction methods tested; however, the amplification method selected post-extraction resulted in significantly different success. The Optimized Organic Extraction coupled with amplification via the Ampf*l*STR™ Identifiler kit resulted in full DNA profiles obtained from the blood, saliva, full fingerprint and the partial fingerprint samples. When the samples were amplified using Ampf*l*STR™ Minifiler™, full profiles were obtained from the blood, duct tape, packaging tape, full fingerprint and partial fingerprint samples. The EZ-1 100 µl elution coupled with amplification via the Ampf*l*STR™ Identifiler kit resulted in full profiles from the blood, hair and partial fingerprint and full profiles with the Ampf*l*STR™ Minifiler™ kit amplification of the blood, saliva, and the partial fingerprint. Finally, the EZ-1 50 µl elution coupled with WGA did not result in any full profiles and demonstrated failures at most alleles for the samples that did produce partial results. Additionally, the electropherograms produced from the WGA samples exhibit a significant difference in inter-locus balance and a large number of shoulder peaks and extraneous peaks. Of interest, when some of these same samples were amplified using the AmpfISTR™ Identifiler kit prior to WGA, they produced full profiles.

DNA, Post-Blast, Persistence

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