B26 A Safe Methodology to Identify Biologically or Chemically Contaminated Evidence

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After attending this presentation, attendees will understand the efficacy of a protocol designed to safely identify, through PCR DNA testing, the source of blood, semen, and saliva stains, and epithelial cells recovered from biologically or chemically contaminated evidence.

This presentation will demonstrate how irradiation provides a safe method of decontamination while preserving the integrity of the evidence for PCR DNA testing. Specifically, the methodology limits the exposure of the evidence collection team to the contaminant and obliterates the risk to laboratory personnel.

In an event that involves weapons of mass destruction, biologically or chemically contaminated evidence may require PCR DNA analysis for criminal investigations or body identification. Since most forensic science laboratories operate at biosafety level two, protocols must be designed that ensure the safety of forensic examiners, while preserving the integrity of the evidence. Irradiation at doses adjusted to destroy the structure and functionality of a pathogen, likely leave identifying DNA sequences intact. Therefore, evidence samples were irradiated and analyzed by PCR DNA testing, and compared to identical non -irradiated samples. Most samples, with the exception of low copy number DNA samples, generated full DNA profiles, although the amount of DNA produced was reduced.

Two identical sample sets were assembled. Samples such as blood, semen and saliva stains at various concentrations and combinations, cigarette butts, clothing, hair, lip prints on tape, and toothbrush bristles were equally divided. Alternatively, duplicate samples were obtained from the same sources for cookie crumbs, cosmetics, ear and eye swabs, razors, swabs of fingerprints on glass, a metal doorknob, a metal file rack, a stapler, and a tackle box, and swabs of touched objects such as bottles, cell phones, credit cards, cups, jewelry, keys, and wallets. Evidence was packaged within two sealed envelopes or containers and stored within two boxes that were irradiated intact with 32kGy of energy from an LX 2 High Power X-ray, courtesy of Titan Inc. Control non-irradiated samples were similarly stored at room temperature in the laboratory.

Samples from both sets were processed simultaneously. Semen and mixed epithelial cell samples were extracted by differential lysis using Chelex beads. According to a modified DNA IQ™ (Promega) protocol for degraded samples, blood and saliva samples were extracted on the Biomek 2000. Low level DNA samples, for example swabs of fingerprints and touched objects, were digested with 0.01% SDS and Proteinase K at 56º C for two hours, incubated at 100º C for 8 minutes, and following the addition of 1mg of Poly A RNA, were purified and concentrated with a Microcon 100. Samples were quantitated with either the Quantiblot® kit (Applied Biosystems) or with an ALU based PCR method with an endpoint determination using a fluorescent plate reader. 1ng or 19.2 µL of each sample were amplified with Promega's PowerPlex® 16 kit for 32 or 35 cycles, as needed, and separated on an ABI 3100 Prism® Genetic Analyzer.

Irradiation reduced the yield of DNA for the majority of the samples. For example, irradiated blood, semen and saliva stains and swabs of fingerprints and touched objects contained 68.8%, 64.4%, 97.5%, 54.1%, and 33.4% less detectable DNA than non-irradiated samples, respectively. Despite this decrease, blood, semen, and saliva stains were still robust and produced full DNA profiles. However, DNA profiles for the minor components of blood stain mixtures were compromised. Although 93.8% of the alleles could be deduced correctly for the minor components in the control samples, only 21.1% of the alleles were apparent in the irradiated samples. Similarly, 55% of the alleles were called correctly for control swabs of touched objects whereas only 7.2% of the alleles were designated for the same irradiated swabs. Samples that have very low copies of DNA, fingerprint swabs, generated at least 400% less accurate DNA profiles following radiation.

The decrease in DNA yield suggests that irradiation degrades DNA. Samples with low amounts of DNA are significantly compromised by radiation, and thus produced partial or no PCR Nuclear DNA profiles. These low copy number DNA samples would be better candidates for SNP analysis. However, irradiation does not affect allelic calling when the DNA recovered is sufficient. Therefore, our study demonstrates that irradiation may be employed to decontaminant most forensic samples

for PCR Nuclear DNA testing. Implementing this described irradiation process to decontaminate evidence would limit the exposure of the evidence collection team and obliterate any risk to laboratory personnel.

Decontamination, Degradation, Low Copy Number DNA

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