A57 Evaluation of Commercial Kits for Co- Extraction of RNA and DNA From Human Body Fluids

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After attending this presentation, attendees will learn that for situations for which both RNA and DNA are desired from the same evidentiary sample, valuable material and time can be saved by simultaneously processing the sample using various commercial kits.

This presentation will impact the forensic science community by providing information that may aid forensic scientists in making a decision as to which commercial kit to use for the dual extraction of RNA and DNA. Attributing a stain recovered from a crime scene to a particular anatomical tissue may aid in predicting the success of DNA profiling efforts, as well as helping to reconstruct the crime. RNA is transcribed in a tissue-specific manner and, thus, the reverse transcription of RNA and subsequent amplification of tissue-specific markers have been developed to supplement more conventional methods of body fluid identification. The simultaneous extraction of both RNA and DNA from the same stain is desirable in order to use the least amount of evidence in the analysis.

The purpose of this study was to evaluate several dual extraction methods for their ability to provide DNA and RNA of sufficient quality and quantity for successful downstream applications. Blood and saliva samples were collected from 30 donors. A preliminary study was performed with blood spotted onto cotton swabs for 10 donors. In order to establish a reference to which the dual extraction kits could be compared, samples were purified with traditional DNA and RNA extraction methods (i.e., organic extraction, Ambion® RNAqueous®, and TRIzol® Plus). The dried spots were co-extracted using the Zymo Research ZR-Duet™ DNA/RNA MiniPrep kit, the Qiagen® AllPrep DNA/RNA Mini kit, the Norgen Biotek RNA/DNA/Protein Purification Plus kit, and the Fisher SurePrep™ RNA/DNA/Protein Purification kit. Purified RNA was reverse transcribed to cDNA. Both DNA and cDNA were quantified by real-time PCR. Amplification of cDNA was carried out using primers for fluid-specific markers and purified DNA was amplified using primers for STR markers. All amplicons were detected on a capillary electrophoresis instrument.

Quantification of the DNA extracts indicated that the highest average concentrations were observed with the Zymo and Qiagen kits (0.828 and 0.318 ng/uL, respectively). The lowest average concentrations were observed with the Norgen and Fisher kits (0.013 and 0.007 ng/uL, respectively). The peak heights and the frequency of artifacts or aberrations such as allele dropout were used to assess the quality of the STR profiles generated from co-extracted DNA.

The ability of each kit to yield RNA extracts free of genomic DNA contamination without relying on optional DNase treatments was examined. Laboratories that analyze nuclear DNA are unlikely to allow the incorporation of DNase in the workflow. The detection of a housekeeping gene (beta-2-microglobulin) and a blood-specific marker (erythroid delta-aminolevulinate synthase) were used to assess the ability of co-extracted RNA to yield successful identification of the body fluid source tissue.

The results of the preliminary study were used to select the best of the four kits for further analysis of both blood and saliva samples from the remaining 20 donors. In addition to assessing extraction efficiencies and the quality of genetic profiles generated, the co-extraction methods were evaluated for cost, user-supplied reagents, sample processing time, hazardous waste generation, and required bench skills. Stand-alone DNA or RNA purification methods generally offer better extraction efficiency, but co-extraction methods may still provide a complete genetic profile while simultaneously identifying the tissue source of the stain from which a profile has originated.

RNA and DNA, Co-Extraction, Body Fluid Profiling

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