B127 Effects of Swab Collection and Storage Methods on DNA and Messenger RNA (mRNA) Profiling of Forensic Stains

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The goal of this presentation is to evaluate the effects of different swabbing methods and the subsequent storage conditions of collection swabs on combined DNA and mRNA profiling of trace evidence.

This presentation will impact the forensic science community by providing crime scene investigators with directions on optimal procedures for the collection and storage of biological stains that are expected to undergo DNA/RNA co-analysis.

The increasing sensitivity of multiplex Polymerase Chain Reaction (PCR) assays for the simultaneous amplification of Short Tandem Repeat (STR) loci now "trace DNA"). Determination of tissue origin of trace DNA is often crucial for the reconstruction of crime dynamics. Recently, several robust protocols for DNA/RNA co-extraction from stains have been described and validated for forensic purposes, thus enabling the single pipeline analysis of both STRs and mRNA profiles to identify body fluids.[1] So far, limited research has been dedicated toward identifying an optimal solution for the retrieval and preservation of RNA from forensic stains. Trace samples are usually collected by means of wet swabs moistened with water; however, it has been suggested that this may not be the most suitable method for RNA because of its high susceptibility to hydrolytic breakdown.[2] Storage conditions of swabs after collection may also affect the quantity and quality of retrievable nucleic acids. It has been shown, for example, that freezing rather than drying swabs prior to extraction results in an improvement of DNA recovery.[3]

The goal of this study was to evaluate the impact on DNA/RNA retrieval rates and integrity for downstream applications of: (1) different moistening agents applied to collection swabs; and, (2) different storage conditions prior to DNA/RNA extraction of the swabs used for stain collection.

Mock stains were created by applying the following to glass slides: whole blood; 1:10 diluted blood treated with luminol; saliva; semen; and skin (by rubbing one thumb/index fingertip over the designated area for approximately 10s). Moistening agents applied to collection swabs were: water; ethanol; and RNAlater™. Swabs were either air-dried or frozen for one to seven days prior to DNA/RNA extraction. DNA and RNA were co-isolated and quantified with the Plexor® HY System and 2100 Bioanalyzer, respectively.[2] DNA was genotyped using the AmpF*l*STR® Identifiler® Plus PCR amplification kit and 3500 Genetic analyzer and mRNA profiling was performed.[4]

Concentration of total RNA isolated from mock stains was significantly higher for swabs treated with RNAlater™ compared with water and ethanol, with consequent effects on mRNA profiling. Interestingly, the impact of moistening media on DNA recovery rates varied among tissues. In skin samples, both ethanol and RNAlater™ significantly outperformed water, also generating more complete STR profiles. On the contrary, DNA retrieval from whole blood and semen stains was significantly lower with ethanol; however, because of the higher DNA yields obtained, on average, from whole blood and semen when compared to skin, moistening agents had no effective impact on STR

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typing in this case. Finally, no significant differences in DNA/RNA recovery were observed depending on storage conditions and storage time of collection swabs.

Reference(s):

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Trace Collection, Swab Storage, MRNA Profiling

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