A99 Effectiveness of Three RNA Extraction Methods for Body Fluid Stains

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After attending this presentation, attendees will gain a better understanding of messenger RNA (mRNA) profiling as it pertains to forensic samples, the complexity involved in adapting this technique for forensic samples, and various methods which may be employed to extract RNA from forensic body fluid stains and evidence.

This presentation will impact the forensic community by demonstrating the application of different RNA extraction methods to forensic samples. Additional benefits to the community include decreasing RNA extraction time, decreasing forensic sample consumption, as well as allowing for the possible implementation of robotics for sample processing.

This study was initiated to compare and contrast two common RNA extraction methods (an alternate organic extraction method and a silica filter based method) to the current (phenol: chloroform based) standard operating procedure (SOP), for their ability to extract RNA from body fluid stains. The primary objective was to demonstrate that methods exist that with modification, can be used to extract RNA from forensic samples with more efficiency than the current SOP.

Forensic analysis of body fluid stains traditionally involves serological analysis. In recent years a novel molecular technique, mRNA profiling, has emerged as a possible alternative to serological methods. Several different methods can be employed to extract RNA, although typically the protocols are designed for extraction from tissues or cell lines directly rather than forensic body fluid stains. Ballantyne et al have proposed a SOP for RNA extraction from body fluid stains based on a standard phenol: chloroform extraction and this method was utilized for comparison in these experiments. However, this method is tedious and time consuming. The TRIzol method (Invitrogen) was chosen because it utilizes phenol: chloroform as the primary mode of action for the RNA extraction, while the RNAqueous® -4PCR kit (Ambion) was chosen to represent the popular silica filter based extraction method. For both methods it was necessary to modify and optimize the protocol to accommodate forensic body fluid stains. The modifications primarily involved adding a step to denature and lyse the RNA from the body fluid stains prior to applying the sample to the extraction method. All RNA extraction methods were completed by multiple users to verify the robustness of the method. It was found that the SOP requires a greater degree of training and skill than either the TRIzol method or the RNAqueous® -4PCR kit. Saliva stains allowed to dry overnight on sterile cotton swabs were used for initial comparisons between the methods. When comparing the two phenol: chloroform extraction methods, the results demonstrated that despite the TRIzol method being faster, the SOP method yielded an average of 6.5ng/µL more RNA per sample. The silica filter based RNAqueous® -4PCR extraction method is also much faster than the SOP. A comparison of this method with the SOP shows that a comparable amount of RNA can be extracted. Thus, it can be concluded that with modification, commercially available methods have the potential to be utilized to extract RNA from forensic stains in a more efficient and user friendly manner.

mRNA Profiling, Body Fluid Stains, Extraction

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