A13 Human DNA Extraction and Identification From Feces

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After attending this presentation, attendees will understand the criteria for selection of a DNA extraction protocol for human fecal evidence samples that affords the best chance of obtaining a complete genotype profile with the least amount of allelic dropout or degradation.

This presentation will impact the forensic community by comparing the advantages and pitfalls associated with using either the QIAGEN QIAamp® DNA Stool Kit or the BioRobot EZ1 Workstation® for processing human fecal evidence for forensic STR genotype analysis.

Careful consideration and selection of the extraction methods will determine the ability to generate a useful genotype profile, and the choice will depend on awareness of the technical issues and nuances of extracting DNA from fecal samples associated with silica-based manual extraction or automated protocols using magnetic bead technology.

The purpose of this study is to compare the QIAGEN QIAamp® Stool Mini Kit with the BioRobot EZ1 Workstation® for DNA extraction- STR genotyping of human feces. Direct sampling (excision) or swabbing of fecal samples was also compared to determine the most efficient method to generate an optimal amount of DNA. Earlier research by others suggested that epithelial cells, which are able to be isolated for DNA extraction, remain on the outer surfaces of a fecal sample and although DNA can be successfully extracted it was often inhibited during

the amplification process due to bacterial DNA, DNAses, bile salts and/or polysaccharides. Due to this known inhibition the QIAamp® kit utilizes a proprietary InhibitEX® tablet and buffers which allows for the removal of degradative enzymes and inhibitory substances. Because of this dilemma, a modified protocol has been developed for use with the QIAamp® kit to help overcome this PCR inhibition.

It is thought that extraction using the modified protocol will yield more quantifiable and uninhibited, human DNA than the original protocol (QIAGEN QIAamp® Stool Mini Kit) or the use of the BioRobot EZ1 Workstation®. Fecal samples for DNA extraction were obtained using either an excision of ~200mg or a complete swabbing of the outer, fecal "cellular" layer. Samples were then quantified by Applied Biosystems Quantifiler™ method using real time PCR. The original protocol using the QIAamp® kit resulted in the overall highest quantities of amplifiable DNA. Selected samples were concentrated and recovered using a Microcon® 100, then STR-typed by Applied Biosystems Identifiler™ utilizing an Applied Biosystems Prism 3130 Genetic Analyzer. It was learned that those extracted with the stool kit gave the most complete profiles with the least amount of allelic dropout or degradation. Samples which showed inhibition during quantification were successfully amplified after addition of bovine serum albumin.

In conclusion the best possible way to extract quantifiable DNA from feces without concern for downstream PCR inhibition is to use the Qiagen QIAamp Stool Mini Kit with the original protocol. Certainly, it should be realized that each person varies in the amount of exfoliated epithelial cells, the choice of DNA extraction methods may not be that crucial. Crime laboratories also need to consider time and cost factors, so that it my end up being more efficient to use the system already in place, such as the BioRobot Workstation. Ultimately in the forensic community, the efficient extraction of DNA from forensic samples will lead to successful identification which is the primary goal.

Real Time PCR, Inhibition, Feces

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