B8 Comparative Analysis of the DNA IQ™ and QIAamp DNA® Extraction Kits for the Processing of Forensic Evidentiary Samples

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Participants will be able to evaluate the differences in DNA yield and the subsequent ability to amplify the DNA that has been extracted using alternative isolation methods in conjunction with more traditional extraction methods. The nature of these differences, specifically between the DNA IQ, QIAamp, organic extraction protocols, will be discussed.

Experiments which compared the DNA IQ™ and QIAamp® extraction systems to the more traditional phenol/chloroform and Chelex® methods were performed. The DNA extraction kits chosen were based on DNA yield, ability of resulting DNA to amplify, ease of use, efficiency, and the potential for robotic automation. The data were compiled to evaluate total DNA yield, DNA concentration and amplification performance of the resulting extracts. Because the final volume may affect the amount of DNA needed for amplification, additional steps may be required which concentrate the DNA sample prior to amplification.

Data were collected to evaluate two main issues. The first issue is the difference in DNA yield obtained when different isolation procedures were used. The second addresses an evaluation of the ability of the DNA obtained from each of the extraction procedure to amplify. To evaluate DNA yield, cells from blood, semen and mixed stains were lysed (SDS/Proteinase K). The substrate was then removed and the resulting lysate was then serially diluted (1, 2, 4, 8, 16-fold dilutions) to evaluate DNA recovery, particularly when a minimal amount of DNA is available. Each sample in the series was then split into 5 separate sets: 1) QIAamp® extraction with a 25ul elution volume, 2) QIAamp® extraction with a 200ul elution volume, 3) DNA IQ™ extraction with a 25ul elution volume, 4) DNA IQ™ extraction with a 200ul elution volume, and 5) an organic extraction suspended in 25ul. DNA yield from each extraction method was determined using the QuantiBlot® Human DNA Quantitation kit (ABI) and the results were calculated using the BioImage. To evaluate amplification performance, each sample was diluted to a concentration of 0.75ng/10ul and was amplified using the AmpFlSTR Profiler Plus™ PCR Amplification kit. It was observed that the DNA concentration of some samples eluted in 200ul was too diluted to amplify the target DNA amount without further concentrating the samples.

Because sample recovery during concentration is a concern, experiments were performed that compared sample recovery during an alcohol precipitation versus the amount of sample recovered during a Microcon® filtration procedure. The samples that were eluted in 200ul of elution buffer were concentrated using both of these methods and subsequently quantified using the QuantiBlot® kit.

The results of these experiments show a relative difference in the ability of each system to capture/elute DNA, with the QIAamp® (200ul elution) resulting in the highest DNA yield for all samples in the dilution series. It was also observed that although the elution volume affected the QIAmp® yield, it did not affect the DNA IQ™ results. The DNA yield obtained after concentration indicated DNA recovery was slightly higher during the Microcon® than the alcohol precipitation. However, for all samples that contained less than 60ng of DNA, the total amount of DNA decreased after each concentration procedure and the percent of DNA recovered was directly proportional to the original concentration. As DNA concentration decreased, the percent of DNA recovered decreased. Does the 200ul QIAamp® elution accompanied by a Microcon® step result in a lower DNA yield than the currently used organic extraction method? Results showed that the Microcon® concentration in association with the QIAamp® 200ul elution extraction procedure, resulted in a higher concentration of DNA than the traditional phenol/chloroform procedure.

As an alternative to concentrating the samples, a 25ul elution volume was considered for the QIAamp® extraction kit. For all samples, more DNA was lost using a 25ul elution volume than was lost using a 200ul elution followed by concentration.

Although commercial DNA extraction kits offer improved efficiency and enable the rapid extraction of forensic evidentiary samples, attention must be given to DNA yield and amplification performance while maintaining the integrity of these samples in processing. This is of particular importance given that many forensic evidence samples contain very little or degraded DNA where retesting may not be possible. Therefore, a poor isolation procedure could lead to a potential loss of interpretable data. Other considerations include ease of use, elution volumes, final DNA concentration, efficiency and the potential for robotic processing.

QIAamp, DNA IQ™, DNA Extraction

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