



A125 Increasing the DNA Yield of Biological Samples Stored on Membranes

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After attending this presentation, attendees will have an overview of the yield performance of different extraction methods applied on biological material stored on paper and FTA cards; attendees will also be able to evaluate side-by-side the performance of different methods of whole genome amplification (WGA) in terms of DNA yield and quality.

This presentation will impact the forensic science community by proposing the widespread use of a sample storage method which consumes less energy, space, and minimizes sample collection discomfort.

Storage of biological samples on membranes has traditionally been used in forensics and neonatal registries for enzymatic testing. It is a very advantageous form of storage, reducing the amount of space needed, easing transportation, and collection of biological material for DNA analysis where regulations and cultural issues prevent autopsies. Furthermore, for FTA cards, it is possible to reduce energy consumption as they do not require refrigeration for storage. If the preferred collection method is a buccal swab, sample collection discomfort is also minimized. Different extraction methods have been tested, both automated and manual in order to obtain the highest DNA yields; WGA has also been performed, as applications such as postmortem genetic testing require large amounts of DNA.

Materials and Methods: Blood and buccal samples were immobilized onto FTA cards (GE Healthcare) and 903-cards (GE Healthcare). For DNA extraction, a piece of the card containing biological material was removed and placed into a fresh sample tube. DNA was extracted using the recommended (GE Healthcare) protocol, the EZ1 DNA Investigator Kit (Qiagen), an EZ1Advanced XL robot (Qiagen) or the Generation Capture kit (Qiagen). Following DNA

extraction, quantification was done using the Quantifiler® Human DNA Quantification Kit (Applied Biosystems - AB) on the 7900 HT Real-Time PCR System (AB). WGA was performed using Repli-G (Qiagen), Genomiphi (GE) and Genomeplex (Sigma-Aldrich). WGA amplification products were quantified using the Quantifiler® Human DNA Quantification kit (AB). The quality of the extracted DNA for forensic genetic applications was evaluated by conventional STR analysis using the AmpF!STR® Identifier® PCR Amplification Kit (AB). Detection of the amplified STR fragments and subsequent genotyping was carried out by capillary electrophoresis on AB 3130xL Genetic Analyzers (AB). Results were analyzed using Genescan Analysis version 3.7 (AB) and allele calls were made using Genotyper version 3.7 (AB) macros.

Results and Discussion: One of the limitations for widespread use of membranes as storage medium for biological material is the low DNA recovery efficiency, particularly in the growing field of postmortem genetic testing. Some of these genetic analysis methods require high quantities of DNA (up to 3 µg genomic DNA). Using the Generation Capture kit, it was possible to obtain comparable yields as when extracting the same amount of biological material directly. Furthermore, Repli-g WGA amplification resulted in high quality DNA in quantities sufficient for genetic analysis with the highest DNA requirements. This indicates that the membranes may be used for storing biological material to be used in genetic testing protocols requiring large amounts of DNA.

Forensic Genetics, DNA Extraction, WGA