A190 Evaluation of Deparaffinization Techniques and DNA Extraction Methods for Formalin- Fixed Paraffin-Embedded Tissue

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After attending this presentation, attendees will gain an understanding of the best extraction method for DNA recovery from formalin-fixed paraffin embedded tissues. Attendees will also learn which organs or tissues are optimal for DNA recovery from paraffin embedded tissues from decomposed and non-decomposed human remains.

This presentation will impact the forensic science community by better defining which tissue type(s) is (are) the best for extraction of DNA from paraffin blocks and which type of DNA extraction method produces the highest quantity and best quality DNA. With this knowledge DNA analysts will be able to make an informed decision as to which organs or tissues to select for DNA testing in order to yield the best DNA profile possible.

Although infrequent, there has been a growing increase in requests to use formalin-fixed paraffin embedded tissue (FF-PET) as reference samples for DNA testing. Re-examination of old Drug Enforcement Administrationth investigation cases in the form of post-conviction review or law enforcement cold case initiatives and cases of unidentified human remains often have no other samples available for testing from the complainant/decedent. Formalin solution is often used in the fixation of human tissue samples. It is an excellent preservative and fixative that enhances the integrity of the tissue for histological sampling. Once the tissue is fixed in formalin, it is embedded in paraffin for long-term storage. In certain instances, such as cold cases, formalin-fixed and paraffin-embedded tissues (FF-PET) may be the only specimens remaining from a decedent for DNA testing.

Although formalin, an aqueous solution of 10% formaldehyde, is an excellent fixative, it cross-links proteins and nucleic acids within a tissue sample, so DNA extracted from formalin fixed tissue is generally fragmented to less than 300 base pairs in length. This study investigated the effect of the time since death and the time of fixation in formalin on the fragment sizes of the DNA purified from various tissue samples.

Four different tissue specimens (heart, liver, muscle, and spleen) were fixed in formalin at three different intervals. These were day one, day five, and day twelve after the autopsy was performed on three different individuals. Once fixed, the tissue specimens were paraffin- embedded in the Histology Laboratory. Since the tissue type and time to fixation can affect the DNA quality, the muscle from day one of one individual was chosen to begin and sizes of three, ten, and thirty microns were cut using the microtome.

Deparaffinization involves the separation and removal of the paraffin wax from the tissue specimen. Two different techniques were evaluated and compared: solvent wash and melting. The solvent wash method involves washing the specimen with varying amounts of xylene and ethanol. The melting method involves melting away the paraffin by the use of a heating block set at 98ºC.

Two different extraction methods were also evaluated and compared. The first method used was the organic extraction, followed by Microcon purification and concentration. The second method involved the use of a robot that performs automated extractions. Each procedure was performed according to the standard operating procedure of the laboratory.

Once the extractions were complete, the tissue specimens were quantified, amplified using Identifiler, and run on the 3130xl by capillary electrophoresis. The electropherograms were analyzed and the results were tabulated. Of the tissue specimens tested, most gave full profiles. A few gave partial results with just one or two loci showing drop out.

Based on the results, the melting deparaffinization technique was chosen to be superior over the solvent wash. Thus, for future samples, only the melting method will be performed. A size of ten microns will be used for future samples and both extraction methods will continue to be performed on all tissue samples.

Future samples will include tissue specimens from the heart, liver, and spleen of each of the three individuals from the different fixative times.

The methods employed by the laboratory for deparaffinization and extraction of FF-PET samples have proven to work thus far with the type of samples available. However, the laboratory is interested in using these methods on tissues from decomposed individuals and is currently working on compiling tissues from decomposed bodies to further evaluate these methods. The laboratory also has several older “cold” cases in which tissue specimens are the only sample left for identification. It is the hope to have a method for FF-PET samples validated soon to address these cases.

FF-PET, DNA Extraction, STR

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