A64 Comparative Study on Stability of DNA in Alternative Sample Tissues for Use in the Identification of Decomposed Cadavers

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The goal of this presentation is to identify several alternative sample tissues for the DNA identification of decomposed cadavers and compare their DNA yield and stability over variable postmortem intervals (PMIs). The effectiveness of each tissue at providing consistent short tandem repeat (STR) profiles will be determined and evaluated in comparison to the performance of the other tissues. The tissues at the focus of this presentation are vitreous humor, cartilage, tendons, and nails.

This presentation will impact the forensic science community by informing attendees about the possible usefulness of alternative sample tissues for DNA identification of decomposed cadavers, their resistance to degradation, ease of collection, amount of DNA recoverable from each tissue, and the stability of nuclear DNA contained in these tissues. These data will be considered comparatively in order to determine the most suitable alternative sample tissue for DNA identification of decomposed cadavers.

Identifying highly decayed cadavers is a frequent difficulty in death investigations. Developing a DNA profile of such cadavers using STRs typically requires sampling of hard tissues such as bone, because the DNA yield from soft tissues decreases exponentially after death. Bone has been shown to provide sufficient quantities of DNA for amplification over a wide range of PMIs; however, the extraction process is time- consuming and laborious, requiring bone segmenting with an electric saw, several washing steps including decalcification, and pulverization using specialized implements. If alternative sample tissues that are simpler to collect and extract could be identified, forensic DNA analysts would benefit from having additional sample choices that may be less expensive and more expedient to process.

The alternative sample tissues chosen for study in this presentation were selected for their potential to resist postmortem degradation due to their anatomical location and matrix properties. Vitreous humor, found in the eye, is anatomically isolated and has been used previously in postmortem toxicology analyses of alcohol. Cartilage and tendons were chosen because they have a fibrous, collagen-rich structure lacking in vascularization that inhibits autolysis and bacterial putrefaction. Previous studies have already shown that nails offer substantial quantities of DNA. However, in this presentation nails will be compared with other alternative tissues.

All tissues will be extracted, quantified, and amplified using the same method: Samples will be washed prior to extraction in distilled water and ethanol or detergent. Solid tissues will be powderized to facilitate sample preparation. Extraction will be performed using a silica- based spin column method, following the manufacturer's protocol. Real- time PCR will be used for DNA quantitation and determination of inhibition. Amplification will be performed using an STR miniplex kit specialized for 16 loci with the following cycling parameters: a one min hold at 96°C; followed by 30 cycles of 30 s at 94°C, 30 s at 60°C, and 45 s at 70°C; and a final extension for 30 min at 60°C. Amplified extracts will be subjected to capillary electrophoresis for 5-10 s at 15 kV. Allele designations for each of the 16 loci analyzed will be made using allele- typing software.

The percentage of full profiles obtained from DNA analysis of each tissue relative to the total number of samples analyzed will be the gauge of the experiment's success. Consideration will also be given to the tissue most capable of yielding full STR profiles for the most advanced states of decomposition and longest PMIs. Each of the alternative sample tissues studied will be compared against the others to determine which yields the most DNA of the highest quality for degraded specimens and gives full STR profiles with the most consistency. A recommendation will be made based on the results as to which alternative sample tissue provides the greatest potential for use in DNA identification of decomposed cadavers.

DNA Stability, Decomposed Cadaver, Alternative Tissues

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