G62 Comparative Study of DNA Yield and STR Profile Quality Obtained From Various Tissue Types of a Decomposed Body

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The goal of this presentation is to present the forensic community with comparative DNA quantification and STR profiling results obtained following routine extraction of DNA from a variety of tissue types obtained during the autopsy of a decomposed body.

When DNA testing is necessary on bodies that have undergone decomposition, the pathologist may wish to focus collections on bone and nails prior to collection of hair or muscle for downstream typing purposes. Collection of the most appropriate samples for DNA typing will eliminate the need to repeat the extraction and profiling process and will ultimate expedite the identification process.

STR DNA profiling of decedents, either for identification purposes or for casework applications, is commonly used in the forensic setting. Obtaining DNA profiles from decedents where substantial decomposition has occurred presents a challenge to the DNA analyst due to the compromised nature of the cellular material housing nuclear DNA. The forensic pathologist is tasked with collecting tissue from the decomposed body that will most likely yield DNA of high quality and quantity such that nuclear DNA profiling will be possible. This presentation will describe the results of DNA quantification assays and STR DNA profiling results obtained following the DNA extraction of a variety of tissue types collected from a decomposed body at autopsy. The results presented here are useful for predicting the most appropriate tissue types to collect during autopsy when subsequent DNA analysis will be required.

The study involved collecting a variety of tissue types from various areas of the body. Scalp hair, pubic hair, deep muscle tissue (psoas), nail material (toenails), and cross-sectioned vertebral bone were harvested from a body exhibiting moderate decomposition. According to investigative reports, the body was initially placed in a grassy field for two days. Suspects then returned to the scene and buried the body in a shallow grave, where it remained until exhumation eight days later. Temperatures during this period ranged from 48-88^{O} F, with a mean temperature of 67.5^{O} F and a total rainfall of 1.84 inches. The body exhibited mild to moderate postmortem insect larvae activity.

A general description of each tissue type was made prior to further processing for DNA extraction. The scalp hair was matted and evaluation of individual hairs and/or roots was not possible; approximately 36.7 mg of this material was collected for DNA extraction. Pubic hairs were separable, therefore root ends were identified macroscopically; approximately 7 mm of the root ends of ten hairs were collected for DNA extraction. The muscle obtained at autopsy was further sectioned prior to DNA extraction, yielding two visually different tissue samples. One was central to the mass submitted for testing and was pink in color (referred to as “deep” hereafter), while the second sampling was more superficial and gray in appearance (referred to as “superficial” hereafter). The muscle tissues collected for DNA extraction from these two visually distinct samples had a mass of 159.6 mg, and 147.9 mg, respectively. Toenails were selected as nail material that would least likely bear DNA from a foreign source (as compared to fingernails); 190.4 mg of nail material was cut for DNA extraction. Finally, 190.5 mg of bone matrix was shaved from the wedged aspect of the cross-sectioned vertebra for subsequent DNA extraction.

Resulting tissue cuttings were each extracted using organic extraction methods and by Microconâ (Millipore, Corp., Bedford, MA) concentration and purification of the DNA. Extracted DNA from all samples was quantified using 1% agarose yield gel analysis in conjunction with the QuantiBlot® (Applied Biosystems, Foster City, CA) human DNA quantification kit. The quantity of DNA detected for each tissue type using the agarose product gel and human specific quantification systems, as well as estimated overall human DNA yield, was calculated in terms of nanograms of DNA per milligram of tissue extracted.

Quantification results indicated that high molecular weight DNA was detected all tissue types, with the exception of the hairs. Also with the exception of the hair samples, human DNA was detected from all DNA samples using the QuantiBlot® system; the bone sample yielded the highest overall quantity (ng DNA/mg tissue). Although the superficial muscle appears to exhibit a slightly higher overall yield than the deep muscle sample, the slight difference may be due to the subjective nature of visual comparative determination employed when determining quantifications from slot blot. Nevertheless, it can be concluded that resulting muscle tissue yields were similar regardless of sample stratification.

Samples were then amplified using the PowerPlex™16 BIO System (Promega Corp., Madison WI) and amplified products were detected using the Hitachi FMBIO® II Fluorescent Imaging Device (MiraiBio Inc., Alameda, CA). A target template amount of 0.5 ng was incorporated into each amplification reaction based on the QuantiBlot® quantifications; since no DNA was detected for the hair samples, the sample retentates were consumed during amplification.

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The DNA profiles obtained from the pubic hair and both muscle tissue samples were partial in nature, while the nail material and bone yielded complete DNA profiles. No profile was obtained from the scalp hair sample. Although high molecular weight DNA of human origin could be harvested from deep muscle tissue of various strata, nail material, and bone, the extracted DNA did not type with equal efficiency using common nuclear DNA typing techniques. While moderate results were obtained from pubic hairs that were separable upon extraction preparation, hairs submitted in bulky masses were not useful for typing using conventional methods. Deeper muscle tissue yielded a more complete profile than did the more superficial sample collected from the same muscle group. Although all were partial in nature, a more complete profile was generated from the pubic hair sampling than from either of the muscle cuttings. Nail material and bone both generated full DNA profiles and are were therefore determined most useful for nuclear DNA typing following decedent body decomposition.

It can be concluded that although the agarose product gel indicates the presence of quality DNA from these samples, the DNA may have undergone damage not explicitly detectable by this method. Furthermore, the presence of microbial DNA complicates the analyst's ability to accurately determine the quantity of amplifiable human DNA present. As expected, due to the relatively short nature of the probe primarily responsible for recognition of human DNA in the blotting quantification system, the presence of adequate quantities of human DNA using these conventional blotting techniques is not necessarily indicative of the quantity of amplifiable DNA. However, data from both the agarose gel and blotting system can be used to predict the amount of human DNA within the total observed high molecular weight DNA, which can be useful for predicting which sample will most likely generate a well-balanced DNA profile across all 16 loci. In summary, this study indicates that nail material and bone tissue collected from decomposed bodies are more likely to yield full DNA profiles than are hairs or muscle tissue when straightforward processing and DNA extraction techniques are employed. When DNA testing is necessary on bodies that have undergone decomposition, the pathologist may wish to focus collections on bone and nails prior to collection of hair or muscle for downstream typing purposes.

Decomposition, PCR DNA Profiling, DNA Extraction

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