A130 Implications of a Modified Extraction Method for Degraded Human Skeletal Remains

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After attending the presentation, attendees will learn a new method for extracting DNA from skeletal remains. Attendees will learn how the implementation of this method in a working laboratory has reduced the number of samples unable to be reported.

This presentation will impact the forensic community by providing attendees with the information to improve their own extractions of DNA from skeletal remains. An increase in the number of reportable samples will lead to an increase in the number of individuals identified, as skeletonized remains are often unknown persons.

The Armed Forces DNA Identification Laboratory (AFDIL) routinely processes the osseous remains of United States servicemembers and civilians from past military conflicts for generation of mitochondrial (mtDNA) profiles. These remains are submitted by the anthropologists of the Joint POW/MIA Accounting Command – Central Identification Laboratory (JPAC-CIL) to aid in the identification and/or re-association of skeletal elements by comparison to mtDNA profiles generated from reference materials. Despite recovery from variable environments, examination of the results of more than 4,000 individual fragments has shown that certain, more compact elements have a greater rate of success when processed for mtDNA (Edson, et al., 2004 & 2005). Targeting the better skeletal elements from which to gather mtDNA has allowed scientists from both AFDIL and JPAC-CIL to more efficiently identify the remains of missing personnel.

In 2006 a new protocol for extraction was implemented at AFDIL (Loreille, et al., 2007). Prior to this, a scientist used 2.0-2.5g of pulverized bone incubated overnight at 56ºC in a solution containing an extraction buffer (10mM Tris, pH 8.0, 100mM NaCl, 50mM EDTA, pH8.0, 0.5% SDS) and 100ul of 20mg/ml Proteinase K. The new protocol decreases the input of pulverized bone to 0.20-0.25g. Incubation overnight remains the same, but the solution now contains a demineralization buffer (0.5M EDTA, pH 8.0, 1% N-Lauroylsarcosine) and 200ul of 20mg/ml Proteinase K. Both protocols use an organic extraction for purification following the incubation.

After implementation of this new method, the failure rate of samples decreased from 20% to 5%. This presentation will show the original success rates of 5886 skeletal elements processed using extraction buffer and 736 elements extracted using the demineralization technique. While this method decreases the importance of sample selection, it does not remove it. Initial size and quality of the sample should still be considered. However, a wider range of elements may now be routinely selected for processing rather than attempting to limit selection to the best possible one available; thereby potentially increasing the number of individuals identified.

The views expressed herein are those of the authors and not necessarily those of the Armed Forces Institute of Pathology, the U.S. Army Surgeon General, nor the U.S. Department of Defense.

References:

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Mitochondrial DNA, Skeletonized Remains, Demineralization

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