H84 MtDNA From Degraded Human Skeletal Remains: Is Quality Affected by Storage Conditions?

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After attending this presentation, attendees will learn the effects of storage practices on degraded skeletal remains as demonstrated by a case study involving remains of missing U.S. service members from the Korean War.

This presentation will impact the forensic community and/or humanity by providing the forensic community with a case study describing the impacts of storage conditions upon the quality and quantity of mtDNA from degraded skeletal remains. Forensic laboratories facing similar situations can take some guidance from the results of this study; potentially implementing the suggestions for their own labs. Osseous samples need to be stored in a manner in which DNA will be preserved. Failure to do so could lead to an inability to identify a missing individual in the future.

In the missing persons program for the United States military, skeletal remains are recovered from a variety of environments. Anthropologists from the Central Identification Laboratory of the Joint POW/MIA Accounting Command (JPAC-CIL) go into the field to recover the remains and any other archaeological information that may lead to the identification of the individuals. Frequently, samples taken from the osseous remains are sent to the Armed Forces DNA Identification Laboratory (AFDIL) for generation of a mitochondrial DNA (mtDNA) profile for comparison to profiles garnered from maternal reference materials.

Examination of remains submitted to AFDIL for testing over the past ten years has shown that there is a marked difference between osseous elements and the mtDNA sequence information that they yield, a result possibly dependent not only on the structure of the bones themselves, but on environmental conditions (Edson, et al., 2005; Leney, 2006). Hence, there is also a difference in ability to generate full mtDNA profiles between the different conflicts. The environments in which remains are located vary in the extreme, depending on the conflict in which the individuals were lost. Incidents from World War II tend to have the most varied recovery sites, from salt marshes in Tunisia to mountainous regions in China, while remains from the conflict in Southeast Asia are found across sites that are more environmentally homogeneous.

Remains from the Korean War have been recovered in two specific manners. First, remains from this conflict are not always recovered *in situ* from the incident site. Between 1990 and 1994, the Democratic People's Republic of Korea (DPRK) repatriated 208 caskets of osseous samples to the U.S. government. These sets of remains were supposedly comprised of single individuals. However, upon anthropological examination by JPACCIL and mtDNA testing by AFDIL, multiple individuals were found to be present in each group of samples. These remains were stored for an indeterminate period of time since death, presumably at room temperature. Secondly, anthropologists from JPAC-CIL have been allowed into the DPRK under extremely regulated conditions to recover remains from burial sites in what are called Joint Recovery Operations (JRO).

The quality of the mtDNA obtained from skeletal elements submitted from both JRO and the unilateral turnovers will be presented. In this instance, quality is determined by the number of bases of mtDNA sequenced from each skeletal element in each group of remains. Success rate is presented as having a reportable sequence of 100 or more base pairs. In order to report sequence information for a sample, AFDIL requires two separate amplifications from a single extract or one amplification from two extracts of that sample. Success rate is standardized by extending the same reporting criteria across all samples. The goal is to determine if the random storage conditions of the unilateral turnovers had a detrimental effect on the mtDNA of the remains versus those of samples recovered in a JRO.

Skeletal elements that have remained *in situ* since the time of the fatal incident are subjected to the ambient regional temperatures and conditions irrespective of whether they are left on the surface or interred by local residents. Internment in these instances typically does not include any type of preservation of the remains, but rather simply the placement of the body in the ground. Soil pH, water levels, temperature, and other environmental conditions are thus free to act upon the remains, potentially damaging *or* preserving them. These effects will also be discussed.

By examining the effects of storage on samples that have a similar time of origin, guidance can be given to other laboratories that specialize in the handling and treatment of skeletonized human remains. Appropriate measures should be taken to preserve osseous remains even if mtDNA testing is not currently being considered as a means of identification. Anticipation of this future need is a conservative step to be taken as failure to sufficiently preserve remains in the present may lead to an inability to identify and return the remains to the families of the missing.

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.

mtDNA, Degraded Skeletal Remains, Storage Conditions

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