## H12 Field Contamination of Archaeological Bone Samples Submitted for Mitochondrial DNA (mtDNA) Analysis

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After attending the presentation, attendees will understand the importance of field and laboratory procedures that minimize the potential for DNA contamination, and will be briefed on analysis procedures to enable detection of contamination that does occur.

This presentation will impact the forensic community by illustrating how DNA samples may be contaminated in the field even when stringent laboratory procedures are followed to avoid such contamination. It will also demonstrate how such contamination may be detected.

The Armed Forces DNA Identification Laboratory (AFDIL) has been generating mitochondrial DNA (mtDNA) profiles from the osseous remains of missing U.S. service members and civilians in support of the mission of the Joint POW/MIA Accounting Command–Central Identification Laboratory (JPAC-CIL) since 1992. Extensive precautions are taken in order to assure that the sequences generated are authentic and not that of an outside or modern contaminant.

Once remains are accessioned at the JPAC-CIL, they are housed in secure storage and checked out to a specific analyst for each stage of analysis. All analysts wear personal protective equipment (PPE) during analysis to reduce the risk of contamination. Remains are sampled for mtDNA analysis as soon as possible after accessioning. Samples are obtained using single use rotary blades that have been sterilized in 20% bleach solution and with an ultraviolet crosslinker, and bleach and ultraviolet light are applied to the interior of the sampling hood prior to each sample being cut. Samples are individually bagged and sent to AFDIL for analysis.

At AFDIL, samples are similarly accessioned and checked out for analysis. Details of internal protocols at AFDIL can be found in Edson, et al., 2004. Methods taken to avoid contamination include, but are not limited to, the following: the removal of the outside surface of the bone by sanding and subsequent washing in di$H_2O$ and EtOH, preparation and extraction of the samples in “clean” rooms using individual hoods, and the wearing of multiple layers of PPE. Profiles are generated from the extracts using a redundant amplification strategy of overlapping primers and a subsequent comparison to an internal database of staff profiles, including both AFDIL and JPAC-CIL staff, in addition to any laboratory visitors. Profiles generated from remains are not reported until they meet the internal criteria of multiple, consistent amplifications and are proven not to be consistent with any staff members at either laboratory who had a potential exposure to the samples (as tracked by the laboratories’ computerized accession records).

While extensive care is taken once the remains are received at JPAC and AFDIL, there is limited control of possible contamination events in the field while remains are being recovered. On average, over 750 osseous samples are processed at AFDIL each year, and such field contamination events have proven to be rare. However, in recent years, two field contamination events have occurred. Both of these cases involved historical aircraft crash sites, in which the anthropologist directing the recovery contaminated a bone fragment in the field. In the first case, five samples were submitted to AFDIL for processing. Three of the samples were reported and were consistent with the two individuals presumed to be on the aircraft. No data was generated from one sample; but the fifth sample showed a mixture of the endogenous sequence (consistent with one crewmember) and that of the field anthropologist. The sample was exceptionally small, only 1.1 g, and cut into two pieces upon submission. In the second case, many of the bone samples were burned. One of the samples produced a high-quality sequence that appeared to be endogenous and met the reporting criteria. However, upon comparison to the sequence database, it was found that this profile was consistent with the field anthropologist and inconsistent with references for any of the crewmembers, and the sample was not reported.

These two cases demonstrate the importance of selecting samples of good quality and of sufficient size for cleaning. While the cleaning protocols are certainly more than adequate under normal conditions, both of these samples had limitations which prevented the cleaning protocol from being sufficiently applied; the first sample being very small and difficult to hold during sanding and the second having been subjected to burning and therefore having an extremely friable surface. Smart sample selection, coupled with reduced handling of samples in the field will help to eliminate possible contamination events and/or false reporting of results.

The above described events occurred in 2006 and 2007 respectively. While other contamination events have occurred at a low-level, these are the only two instances of the field anthropologist introducing modern DNA to the entire sample, a failure rate of 0.14% and 0.12%, respectively. This presentation will focus on the details

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of the protocols used at AFDIL and JPAC-CIL for the processing of degraded osseous remains. Attendees will learn how to perhaps implement these protocols into their own laboratory use for prevention and detection of field and in- house contamination of mtDNA samples.

The views expressed herein are those of the authors and not necessarily those of the Joint POW/MIA Accounting Command, the Armed Forces Institute of Pathology, the US Army Surgeon General, nor the US Department of Defense.

**Reference:**

Edson, S.M., J.P. Ross, M.D. Coble, T.J. Parsons, and S.M. Barritt (2004). Naming the Dead: Confronting the Realities of the Rapid Identification of Degraded Skeletal Remains. Forensic Science Review **16(1)**: 63-90.

**Mitochondrial DNA, Contamination, Human Skeletal Remains**

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