CASE REPORT

Identifying Missing U.S. Servicemembers from the Korean War—Do Storage Conditions Affect the Success Rate of mtDNA Testing?

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Between the years of 1993 and 1994, the Democratic People's Republic of Korea (DPRK) repatriated the remains of United States servicemembers missing during the Korean War (1950–1953). Two hundred and eight coffins were returned, each presumably containing the remains of a single individual. When anthropologists of the Joint POW/MIA Accounting Command Central Identification Laboratory (JPAC-CIL) began identifying these remains, they found this to be not true. Each coffin contained the remains of at least two individuals, many of which showed evidence of curation or other handling. The task now became, not only identification, but the puzzle of separating the commingled remains of an undetermined number of individuals.

The identification of missing U.S. servicemembers is typically undertaken using a combination of techniques such as archaeology, anthropology and DNA analysis. DNA analysis is performed at the Armed Forces DNA Identification Laboratory (AFDIL) using mitochondrial DNA (mtDNA), which is compared to profiles acquired from maternal relatives to support and confirm identification. With this set of remains returned from the DPRK, colloquially known as the K208, the number of maternal relatives for comparison could be in the hundreds. Anecdotally, the scientists of AFDIL believed that the K208 samples did not work as well as other samples from Korea or other conflicts. The hypothesis was that the K208 were most likely stored in less-than-optimal conditions (i.e., room temperature); therefore, the mtDNA present would be more degraded and tend to produce partial or no sequence data.

When partial sequences are generated, comparisons are made even more difficult in that a partial reportable profile may not cover the section of mtDNA containing diagnostic polymorphisms. MtDNA is not a positive means of identification, with multiple individuals potentially sharing the same maternal line and therefore the same mtDNA profile. The selection of skeletal samples that will give reliable sequences is important.

As of November 2005, 5,021 individual osseous fragments had been submitted to AFDIL from JPAC-CIL for mtDNA analysis. Of these, 72.8% were successful in producing a reportable sequence of at least 100bp. To be considered a reportable sequence, AFDIL's criteria require verification by at least two individual amplifications. Breaking these results down by bone type (Figure 1), compact weight-bearing bones such as the femur and tibia were the most successful (87.8% and 86.3%, respectively). Bones comprised of thin layers of compact bone or large amounts of trabecular bone have a greater surface area that exposes the DNA to increased impact from taphonomic effects, thereby causing excessive damage and inhibiting successful sequencing.

When the initial analysis was done, samples were not separated by military conflict. Each conflict impacts the quality of the samples, not only because of differences in time since death, but the environment in which the samples were recovered. These conditions vary widely even within the same conflict. Analyzing the samples

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according to military conflict of loss reveals significant differences between each grouping (Table 1). The Korean War samples were not the poorest performers.

The Korean War samples are noteworthy, not only because of the K208, but because of a second set of remains recovered under different circumstances. Collected by JPAC-CIL anthropologists under Joint Recovery Operations (JROs) until 2005, this second set of remains has presumably seen little disruption since time of death and has been stored in climate-controlled circumstances upon return to the lab, providing a unique opportunity to compare results from samples that were presumably left in situ and those that were removed from burial and stored under unknown conditions. Dividing and analyzing the Korea samples gave the expected result: the K208 samples performed significantly worse than the JRO samples as a whole. However, when individual skeletal elements were examined independently, there was no significant difference between the two groups.

The key does not lie in individual skeletal elements per se or even in how samples were stored, but rather in how the remains were handled during disinterment. Remains recovered by professional anthropologists, such as those in the JROs, have a certain amount of information recovered with them. There is a context to the recovery, which allows sampling of the best elements to provide identification or reassociation of cranial and post-cranial elements. Remains returned by an outside source have no extra information other than that provided by that source. This loss of context increases the number of skeletal elements that must be sampled for mtDNA analysis. No longer can long bones and teeth be sampled preferentially, but all elements that cannot be reassociated anthropologically may be submitted for analysis, as the commingling calls into question the identity of them all. Of the samples submitted to AFDIL from the K208, 22.4% were smaller bones or those that typically give less successful results, whereas 8.3% were submitted for the JRO. Overall, JRO samples were successful 84% of the time, while the K208 were successful 73% of the time. Implicit here is the impact of the loss of context. It is how the samples are procured, not the temperature at which the samples are stored, that is important in determining whether a mtDNA profile will be generated.

While individuals from the Korean War, specifically those from the K208, have been identified in recent years, the identification puzzle remains. As new technologies emerge to increase mtDNA yield during extraction, these identifications will increase despite the sampling of often less-than-optimal skeletal elements.

Table 1. Success rate of samples as divided by military conflict.

Conflict	Total Specimens Submitted	Number of Successful Specimens	% Success
Civil War	23	21	91.3
Cold War	55	49	89.1
Korean War	2,133	1,633	76.6
Vietnam War	1,544	942	61.0
World War II	1,153	949	82.3
Unspecified	113	59	52.2

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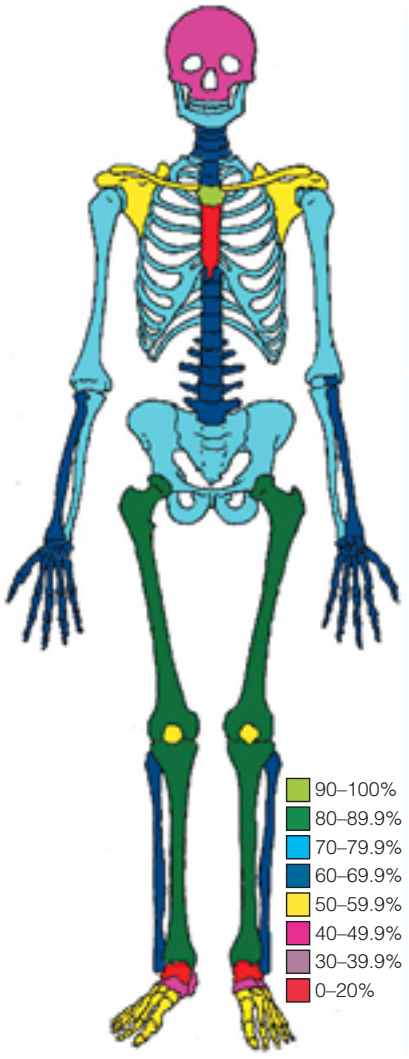

Figure 1. Percent success of mtDNA analysis for each skeletal element submitted to AFDIL. "Success" is generating 100bp of reportable mtDNA sequence. The manubrium is an outlier as only one was submitted, and data were reported; hence the 100% success.