

**VALIDATION OF BONE BLEACHING AND SONICATION FOR CLEANING SKELETAL MATERIAL
PRIOR TO EXTRACTION**

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To aid the Central Identification Laboratory in Hawaii (CILHI) with the identification of missing service members recovered from Korean, World War II, and Vietnam battlefields and crash sites, the Armed Forces DNA Identification Laboratory (AFDIL) typically receives skeletal material for mtDNA analysis. However, the environmental and physical elements that the bone samples have been exposed to before CILHI recovery teams retrieve the skeletal remains are not always known. Therefore, environmental and human contaminants can be introduced at any time before receipt. In an effort to reduce the amount of contamination present on the surface of bone samples, AFDIL removes the outer layer of bone from all exposed internal and external surfaces. The bone is then washed with distilled water and 100% ethanol and agitated by shaking to remove residual sanding residue. However, this procedure is not 100 percent effective and low-level contaminants are still present that can possibly lead to mixtures during mtDNA analysis. In order to more effectively remove potential contaminants from bone specimens the bone cleaning procedure was modified to add a single 10% bleach wash before the water and ethanol washes, as well as sonications instead of manual agitation.

For this validation, three individual bone sets were grossly contaminated by rubbing the bone in the palm of an individual's hand before and after sanding and then either washed following AFDIL's current procedure or with the modified ten percent bleach, water and ethanol washes that included a sonication step between each wash, or not cleaned at all. In all instances, results demonstrated that the unwashed contaminated bone gave the contaminant sequence. The contaminated bone washed following AFDIL current procedures gave mixtures of the true sequence and the contaminant and the contaminated bone washed with bleach and sonications generated the authentic bone sequence. It was also observed that sonications substantially increased the amount of residue removed from the bone pores over manual agitation and that by the ethanol wash the solution was no longer turbid.

Finally, the level of contamination used in this validation is extreme and does not typically represent what is commonly seen with AFDIL case samples. Despite the excellent early results, this procedure has only been tested on compact bone primarily the diaphysis (shaft) of the long bones. Future experiments will encompass testing how a 10% bleach wash will affect highly porous bones such as cranial fragments as well as short bones and irregularly shaped bones.

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