REANALYSIS OF THE NECESSITY TO PROCESS NON-CRIMINALISTIC CASEWORK CONTROLS THAT ARE BELOW THE ARMED FORCES DNA IDENTIFICATION LABORATORY'S DETECTION THRESHOLD AS RECOMMENDED BY SWGDAM GUIDELINES

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The Armed Forces DNA Identification Laboratory (AFDIL) aids the Central Identification Laboratory in Hawaii (CILHI) with the identification of human skeletal remains that have been recovered from past military conflicts by mitochondrial DNA (mtDNA) analysis. Currently, AFDIL uses primer-sets or mini-primer sets to amplify the mtDNA Hypervariable Regions one and two from extracted skeletal remains. Prior to the adoption of the Scientific Working Group on DNA Analysis Methods (SWGDAM) "Guidelines for Mitochondrial DNA Nucleotide Sequence Interpretation[1]" it had been AFDIL's standard practice to visualize all amplicons including reagent blanks, negatives, positives, and samples on a 2% agarose gel stained with Ethidium Bromide (EtBr). Samples yielding a positive result were purified using Amicon Centricon™ filtration columns, sequenced with the ABI Prism® Big-Dye™ Terminator Cycle Sequencing Ready Reaction Kit (v 1.0) and loaded on an ABI 377 Automated DNA Sequencer.

Subsequent to the adoption of SWGDAM's "Guidelines for Mitochondrial DNA Nucleotide Sequence Interpretation", all controls were sequenced regardless of whether they were below AFDIL's designated contamination threshold (observable band on a 2% EtBr stained agarose gel). This procedural change increased AFDIL's workload by approximately 25% at a cost of $37.00 per-sample, which does not include the cost for increased labor and review time. Because this is a substantial increase in workload, reagent costs, and labor, a 60-day evaluation period was selected to determine whether sequencing all PCR amplification controls had any scientific impact or affected the integrity of mtDNA casework results. During this evaluation period all negative controls and reagent blanks, as well as their corresponding samples, were sequenced whether they were positive or not on an EtBr stained gel. Results demonstrated that none of the 1306 Primer Set reagent blank and negative controls yielded reportable sequence, and only 10 out of the 791 (1.2%) Mini Primer Set amplification controls yielded reportable sequence. However, the generated control sequences did not negatively impact the integrity of the corresponding case samples.

In conclusion, the reagent blank and negative controls that do not yield an observable band on an EtBr agarose gel will no longer be processed for AFDIL non-criminal mtDNA casework. Nevertheless, to remain compliant with SWGDAM guidelines, AFDIL will process all reagent blanks and negative controls along with their corresponding criminal casework samples regardless of whether or not the controls yield a positive result on an EtBr agarose gel.

1. Scientific Working Group on DNA Analysis Methods (SWGDAM), "Guidelines for Mitochondrial DNA (mtDNA) Nucleotide Sequence Interpretation," *Forensic Science Communications,* Vol. 5, No. 2-April