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RE-EVALUATION OF A CONTAMINATION THRESHOLD: A NEW LOOK AT THE CONTAMINATION DETECTION LIMITS OF THE ABI 3130® GENETIC ANALYZER

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The Armed Forces DNA Identification Laboratory (AFDIL) processes 800 individual skeletal remains and over 2000 blood references every year in the generation of mitochondrial DNA (mtDNA) profiles. This work supports the Joint POW/MIA Accounting Command – Central Identification Laboratory (JPAC-CIL) with the identification of United States service members missing from past military conflicts. In April 2003, the Scientific Working Group on DNA Analysis Methods (SGWDAM) released mtDNA guidelines specifying that all mtDNA amplifications including negative controls and reagent blanks were to be sequenced regardless of whether the controls were below a laboratory's designated contamination threshold. For AFDIL, this threshold was visibility on an Ethidium Bromide (EtBr) stained 2% agarose gel. A two month study was done to determine whether sequencing controls had any scientific impact or affected the results of casework when using the ABI PRISM® 377 DNA Sequencer. It was determined that processing all controls elevated the cost and time required to process degraded skeletal remains for mitochondrial DNA and that processing all controls did not reveal contamination events that would have otherwise gone undetected. This study determined that sequencing these controls cost the laboratory an additional $77,000.00 which breaks down to $37.00 per sample not including the cost of manpower. At that time, AFDIL ceased processing amplification controls that did not produce a positive result on the product gel. However, during the summer of 2005, AFDIL began to process casework on the ABI 3100® Genetic Analyzer and the later upgrade to the 3130-xl platform. With the shift to a more sensitive instrument, all amplification controls were sequenced to complete a study similar to that done for the ABI 377®. The contamination threshold of the 2% agarose gel was re-evaluated in a five week survey. In this study, each control that failed to yield a visible band on the product gel was given a specific designation in the laboratory information management system, LISA, to enable tracking. These amplifications were evaluated on a weekly basis to see if sequence data was generated. Results of the most recent control survey and its impact on casework at AFDIL will be discussed. The views expressed in this abstract are those of the author and do not reflect the official policy of the Department of the Army, the Department of Defense or the U.S. Government