B112 Re-Evaluation of a Contamination Threshold: Mixture and Contamination Detection Limits Redefined During the Validation of the ABI 3100® Genetic Analyzer

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After attending this presentation, attendees will learn the applicability of re-defining their laboratories own contamination thresholds when imple- menting new and potentially more sensitive techniques.

Each forensic laboratory must have a contamination threshold that is deemed sufficient to control for extraneous profiles. This presentation will impact the forensic community and/or humanity by demonstrating why re- evaluation of such thresholds should occur when new technologies are implemented into standard procedures to insure that the standards set for sample quality are still being met and that the integrity of the reported profile is not called into question.

Contamination in forensic DNA laboratories is a constant threat. Each laboratory must examine its own guidelines and requirements for gener- ating DNA profiles in order to ensure that the authentic sequence is being reported. In 'ancient' or low-copy number (LCN) DNA labs, this is espe- cially critical as the target DNA can be difficult to acquire and is easily overwhelmed by 'modern' DNA.

At the Armed Forces DNA Identification Laboratory (AFDIL), numerous steps are taken to ensure the reporting of an authentic mtDNA profile. These steps are covered quite thoroughly in Edson, et al. (2004) and will not be revisited here. What is of import to this current presentation is the usage and detection of extraction and amplification controls. Negative controls are commonly used in a forensic setting to evaluate potential contamination in amplification. At AFDIL, it is standard oper- ating procedure for non-criminalistic casework to not carry these controls through sequencing unless a visible product band is produced on a 2% agarose gel stained with ethidium bromide (EtBr). To evaluate the Scientific Working Group on DNA Analysis Methods' (SWGDAM) 2003 recommendation that all laboratories handling DNA samples process all amplification controls, AFDIL undertook at two-month trial period. During this time all controls were sequenced irrespective of results on a product gel. Over these two months, 2097 controls were processed on the ABI PRISM® 377 DNA Sequencer from hypervariable region, primer set and mini-primer set amplifications. Results demonstrated that zero out of 1306 hypervariable region primers or primer sets controls produced readable sequence; and that 10 out of 791 (1.26%) mini-primer sets controls pro- duced readable sequence. In no instance did the contamination negatively affect the results of the cases. This study cost the laboratory an additional
$77,000, or $37.00 per sample, not including the cost of manpower.

However, in the spring of 2005, the ABI 3100® Genetic Analyzer and a novel sequencing approach were validated for processing ancient skeletal remains at AFDIL. As this system is purported to be more sensitive to low concentrations of DNA, part of the validation included defining the thresholds of the 3100 for mixture and contamination detection. To evaluate contamination detection thresholds, a total of 47 negative and reagent blank controls generated during the course of normal casework were examined. One out of the 47 controls produced a visible band on an EtBr stained agarose gel, and was not sequenced. After sequencing and analysis on the 3100, two out of the 46 other controls (4%) produced low quality data. This suggests that the ABI 3100® Genetic Analyzer may be more sensitive than the older 377 platforms.

Further supporting this hypothesis was the evaluation of the 3100 for mixture detection thresholds. To evaluate these, sample extracts from both high and low quality DNA amplicons were mixed in various ratios (9:1, 7:3, 5:5, 3:7, and 1:9), then sequenced and analyzed on the 3100. Mixtures were detected at a 1:9 ratio.

Based on the initial results garnered from the validation, it was decided that for the first six months of processing casework samples on the ABI 3100®, all negative controls and reagent blanks irrespective of agarose gel product results will be processed in order to reset the contami- nation threshold. This presentation will discuss the contamination and mixture results obtained during the validation of the 3100 as well as the contamination thresholds defined during the 6-month evaluation of all amplification controls on the 3100.

Each forensic laboratory must have a contamination threshold that is deemed sufficient to control for extraneous profiles. Re-evaluation of such thresholds should occur when new technologies are implemented into standard procedures to insure that the standards set for sample quality are still being met and that the integrity of the reported profile is not called into question.

The views expressed herein are those of the authors and not neces- sarily those of the Armed Forces Institute of Pathology, the U.S. Army Surgeon General, nor the U.S. Department of Defense.

Contamination Threshold, mtDNA, Validation

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