B104 Lessons Learned From the DNA Analysis of More Than 11,000 Skeletal Samples: More Than 20 Years of Process Improvements

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After attending this presentation, attendees will better understand how rapid adoption and implementation of new procedures within their laboratories will increase DNA reporting success rates for skeletonized remains that are more than 40 years of age.

This presentation will impact the forensic science community by providing a framework of adaptations implemented by the Armed Forces DNA Identification Laboratory (AFDIL) and the Joint POW/MIA Accounting Command Central Identification Laboratory (JPAC-CIL) that can serve as a model for any laboratory seeking to improve DNA recovery from skeletonized human remains.

Since the creation of the laboratory in 1992, the AFDIL has striven to provide world-class DNA analysis to aid the JPAC-CIL in the identification of missing United States service members. Over the past 22 years, procedures have been constantly adapted and improved in order to stay at the cutting edge of DNA technology. This presentation will show how changes to mitochondrial DNA (mtDNA) and autosomal Short Tandem Repeat (auSTR) amplification strategies, as well as DNA extraction and general laboratory protocols, have significantly increased results over time. The increase in DNA reporting success rates will be of particular use to any laboratory performing work on missing persons or cold cases due to the similarity in degradation patterns over time.

Thoughtful application of modifications to standard operating procedures can reap enormous benefits when applied within practicing laboratories. In 1999, the introduction of mini-primer sets allowed for mtDNA sequence data to be generated from previously unreportable samples due to amplicon size. In 2001, a customized LIMS was introduced, which allows for the tracking of samples from cradle (arrival) to grave (final report). Specially designed modules provide for automated comparisons of casework mtDNA profiles to thousands of family references samples in a matter of seconds, allowing analysts to spend more time in the laboratory rather than manually calculating comparisons. Introduction of capillary electrophoresis in 2002 further improved the speed by which samples could be processed within the laboratory.

While all of these previous modifications showed a positive impact on sample processing, the sampling procedure at JPAC-CIL remained essentially the same: remove a 5.0g fragment of compact bone to send to AFDIL for DNA processing of which 2.0g was needed for extraction. This technique generated successful results nearly 85% of the time, but cases that had much smaller bone fragments or fragments of poor quality were unable to be sampled. In 2007, the demineralization extraction protocol was implemented. The success rate for mtDNA processing jumped to more than 92% and the sample size for extraction was reduced ten-fold. In the years following implementation of this extraction technique, JPAC-CIL has been able to slide their sampling strategy to increasingly smaller samples. Over 24% of samples submitted currently weigh less than 2.0g. Cases that would have previously been shelved due to size of available bone fragments can now be processed with positive results. A simple improvement in the extraction procedure removed the restrictions of both sample size and quality, allowing almost any skeletal element to be sampled successfully.

In the summer of 2013, AFDIL incorporated an inorganic purification step into the extraction procedure, completely eliminating phenol, chloroform, and butanol from use. While this modification has had only a moderate impact on mtDNA reportability, autosomal success has more than doubled. AFDIL has used commercially available auSTR and Y-chromosomal Short Tandem Repeat (Y-STR) kits since 2007 to support mtDNA matches or separate individuals with common mitotypes. Both auSTR and Y-STR success rates have jumped from 25% to more than 45%, thus making the use of nuclear DNA testing on aged skeletal samples a more consistently viable option.

Progressive improvements will only provide positive results to any forensics laboratory working on missing persons or cold cases, as examination of changing mtDNA, auSTR, and YSTR success rates from more than 11,000 osseous samples demonstrates. The adaptations described above and implemented by AFDIL and JPAC-CIL serve as a model for any laboratory seeking to improve DNA recovery from skeletonized human remains, to include laboratories performing identification of Unknown Human Remains (UHR).

The opinions or assertions presented are the private views of the authors and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the United States Army Medical Research and Materiel Command, the Armed Forces Medical Examiner System, or the Joint POW/MIA Accounting Command-Central Identification Laboratory.

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