B38 Efficient Sampling of Skeletonized Human Crania for DNA Testing

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Learning Overview: After attending this presentation, attendees will understand how to apply an effective cranial sampling protocol in their practices. The results of this study are applicable to any laboratory performing human identification on skeletonized remains.

Impact on the Forensic Science Community: This presentation will impact the forensics community by providing information on how best to sample skeletonized human crania for DNA, allowing for an increased degree of success for Human Identification (HID).

DNA testing of skeletonized human remains continues to be a challenging task in the field of human identification. Efficient selection of elements from the remains tends to provide the best foundation for success, regardless of the extraction technique used or the DNA platform being tested. In mass fatality events involving highly commingled remains, the most numerous element establishes the minimum number of individuals present. In practice, DNA profiles from cranial and post-cranial remains are desired to provide a re-association, as proper re-articulation can be difficult.

This presentation will examine the success rates of each platform tested as compared to each cranial element and provide guidance to the laboratory practitioner on some of the best practices for the sampling of cranial remains for DNA testing. In this study, 2,177 cranial elements were examined during regular casework done at the Armed Forces Medical Examiner-Armed Forces DNA Identification Laboratory (AFMES-AFDIL) from 1990-2018. These samples were provided as part of DNA testing done in partnership with the Defense POW/MIA Accounting Agency (DPAA). Elements processed have postmortem intervals ranging from approximately 45-78 years. Teeth were not included in the survey and will be addressed in a separate study.

The purpose of this project was to develop a recommendation that would be wide-ranging and applicable to remains found in numerous burial conditions, as well as to laboratories that might use different processing techniques. Among the remains tested were those recovered from disinterments, *in situ* locations, and curated remains. Testing involved three different extraction protocols: organic; complete demineralization plus organic purification (Demin1); and complete demineralization plus inorganic purification (Demin2). In addition, multiple types of DNA profiles were generated: mitochondrial DNA (mtDNA) via Sanger sequencing and Next Generation Sequencing protocols (NGS); Y-STR profiles from an enhanced AmpFlSTR® Yfiler™ protocol; and multiple STR kits: AmpFlSTR® MiniFiler™, AmpFlSTR® Identifiler™, and PowerPlex® Fusion.

The occipital is the most commonly sampled element of the cranium, with 687 tests being performed. MtDNA Sanger sequencing of occipital samples extracted with complete demineralization and organic purification have a 91% success rate. While this seems to be a high rate of success, nearly all cranial samples extracted with the same method have a 91-92% success rate, except for cranial fragments of unspecified origin, which have a 66% success rate. If the extraction method is not considered, occipital and temporal fragments are equally successful for mtDNA Sanger sequencing at 85%.

The cranial element that consistently provides results across any platform tested is the temporal. An initial survey of cranial samples tested at AFMES-AFDIL found similar results.[1] However, only mtDNA Sanger sequencing and two types of extractions were evaluated.

Attendees should be able to take this information back to their laboratories and apply an effective cranial sampling protocol to their practices. The results of this study are applicable to any laboratory performing human identification on skeletonized remains.

Reference(s):

1. Suni M. Edson, Alexander F. Christensen, Suzanne M. Barritt, Audrey Meehan, Mark D. Leney, and Louis N. Finelli, "Sampling of the cranium for mitochondrial DNA analysis of human skeletal remains," *Forensic Science International*: Genetics Supplement Series 2, vol. 1 (2009): 269-270, https://doi.org/10.1016/j.fsigss.2009.09.029.

Human Identification (HID), Skeletonized Remains, DNA

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