A56 Choose Your Own (Accreditation) Adventure in the Defense POW/MIA Accounting Agency (DPAA) Isotope Testing Program: Part II–Sample Analysis

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Learning Overview: After attending this presentation, attendees will learn about the work completed to accredit isotope sample analysis under ISO/IEC 17025:2017 for Geographic Profiling (i.e., isotope testing) of human remains.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by illustrating the process used by the Defense POW/MIA Accounting Agency (DPAA) Laboratory to prepare for accreditation for isotope testing—specifically sample analysis—of unidentified individuals.

In 2017, the Defense POW/MIA Accounting Agency (DPAA) Laboratory in Hawaii began developing a series of forensic laboratory tests ("isotope testing") for determining the "geographic profile" of unidentified remains. This is possible because the isotopic signatures of an individual's diet and drinking water are recorded in their tissues—bone, teeth, hair, nail, etc. Preparation of tissue samples for isotope testing was accredited in 2019; another presentation describes that completed work program. This study presents the work completed in 2020 to accredit isotope sample analysis. It primarily focused on the measurement of bone collagen via Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS) for nitrogen, carbon, and sulfur.

The initial step was to procure light isotope analytical instrumentation, which was ordered in January 2019 and included an EA Isolink™ attached to a DELTA V™ Plus Mass Spectrometer via a Conflo IV™ interface (all from Thermo Fisher Scientific™). The instrumentation was officially accepted in October 2019, after all specifications preset by Thermo Fisher Scientific™ were met by the field service engineer. Certified reference materials for normalization (scaling) and quality control were also ordered from the United States Geological Survey's Reston Stable Isotope Laboratory, the International Atomic Energy Agency, and Elemental Microanalysis. Collagen powders available as commercial products were purchased to serve as in-house standards.

Following instrument installation, a triple-gas EA-IRMS method to measure collagen was developed in-house, based on recommendations from Sayle et al.[1] The method was validated following a ten-point plan recommended by the Forensic Isotope Ratio Mass Spectrometry Network.[2] The isotopic ranges of the method are governed by available scaling reference materials and cover the expected variation of isotope delta (δ) values for human bone collagen: +4 to +15‰ for δ^{15}N values, −23 to −9‰ for δ^{13}C values, and −3 to +14‰ for δ^{34}S values. Sample mass limits were determined by analyzing varying masses of the in-house collagen standards, ranging from 0.7mg to 1.3mg. Based on the variability of measurement results, the ideal mass range was determined to be 1.0mg ± 5%. The carryover, or memory, of the method was determined by analysis of blanks between reference materials, quality control materials, and collagen standards; no carryover was observed for the ideal mass range.

Next, precision, accuracy, and uncertainty of the method were determined. To assess precision, 18 replicates of three in-house collagen standards were analyzed seven times over five months, varying analysts and instrument operating conditions. Accuracy of the method was determined using quality control reference materials that were measured as "unknown" samples 16 times with the measured δ value for a material compared to its certified value. Precision and accuracy data were used to calculate uncertainty, using the square root of the sum of squares rule and a coverage factor of 2 to approximate a 95% confidence interval.[3] Uncertainty was determined to be 0.34‰ for δ^{15}N values, 0.30‰ for δ^{13}C values, and 1.3‰ for δ^{34}S values.

The EA-IRMS method was externally validated by re-analyzing 60 collagen samples previously tested by an external provider for carbon and nitrogen only. The mean difference (± standard deviation) between measurement results from the external provider and the DPAA Laboratory was −0.13 ± 0.06‰ for δ^{15}N values and +0.19 ± 0.08‰ for δ^{13}C values; both mean differences were below calculated uncertainty. Data provided by the external provider were also used to validate a Microsoft® Excel® workbook developed in-house for data processing.

Finally, the DPAA Laboratory's Standard Operating Procedure (SOP) for isotope testing, which was initially developed during accreditation of sample preparation, was updated to encompass sample analysis. Personnel were competency certified and annual proficiency exams were implemented. Copies of the method validation report, updated SOP, and training records were provided to American National Standards Institute National Accreditation Board (ANAB). An assessment by ANAB is scheduled for November 2020, and accreditation is anticipated for 2021.

Reference(s):

1. Sayle K.L, C.R. Brodie, G.T. Cook, W.D. Hamilton. 2019. Sequential measurement of δ^{15}N, δ^{13}C and δ^{34}S values in archaeological bone collagen at the Scottish Universities Environmental Research Centre (SUERC): A new analytical frontier. *Rapid Communications in Mass Spectrometry* 33:1258-1266.
2. Dunn P.J.H., H. Salouros, J.F. Carter, S.P. Doyle. 2017. Forensic application of stable isotope delta values: Proposed minimum requirements for method validation. *Rapid Communications in Mass Spectrometry* 31:1476-1480.
3. Dunn P.J.H. and J.F. Carter (editors). 2018. *Good practice guide for isotope ratio mass spectrometry*, 2nd Ed. FIRMS Network.

Method Validation, Isotope Analysis, Quality Assurance (QA)

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