A109 Assessing Isotope Data Comparability: An Example From the Application of Isotope Testing to Unidentified Human Remains From Past Conflicts

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Learning Overview: After attending this presentation, attendees will have learned a straightforward method for determining a real interpretative difference that should be used to compare isotope data generated by different laboratories.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing an example of assessing comparability between isotope datasets from different laboratories; the recommended practice can be easily implemented by research groups collecting and using isotope data to provenance unknown individuals.

Isotope testing has proven to be a useful tool for provenancing unidentified human remains. However, application of the technique requires reference datasets of potential source origins or populations that are sometimes compiled from remains prepared and analyzed at different laboratories. It is critical that scientists assess comparability between datasets, especially when drawing conclusions about an unknown decedent.

Inferences about similarities between a sample and available reference data are questionable when variations in isotope measurement results are not well-defined. To address this, Pestle et al. designed an inter-laboratory comparison to quantify variability in the isotopic analysis of ancient human remains; they found that approximately half the variation was due to differences in sample preparation methods, while the other half was due to differences in analytical techniques.[1] This led to the development of a Minimum Meaningful Difference (MMD) metric. The MMD was calculated by taking the mean pairwise inter-laboratory difference and adding four times the mean of the standard deviation of results from each participating laboratory; differences between laboratories were considered real if they exceed the MMD threshold. While the MMD represents a useful metric for assessing isotope data comparability, it also represents a worst-case scenario due to the number of participants in the inter-laboratory comparison.

A more straightforward method for assessing isotope data comparability focuses on paired samples, whereby a set of samples is prepared and/or analyzed twice to investigate practical differences between two laboratories by calculating a Real Interpretative Difference (RID).[2] To demonstrate this, 30 samples were collected from long bones and split for preparation as collagen and bioapatite at the Defense POW/MIA Accounting Agency (DPAA) Laboratory in Hawaii and California State University (CSU), Chico. Extracts of collagen from both laboratories were submitted to the University of California, Davis for measurement of carbon and nitrogen isotope values, while the bioapatite extracts were submitted to IsoForensics, Inc. for measurement of carbon and oxygen isotope values. Based on collagen yields, nine samples were affected by diagenesis and were not used in subsequent statistical evaluations.

Mean differences in the collagen prepared at the DPAA and CSU, Chico were 0.10‰ for $\delta^{13}C$ values and 0.19‰ for $\delta^{15}N$ values; while small, these differences were significant when compared to a theoretical mean of 0 (one-sample *t*-test; $p<0.001$ for both). There was no significant difference in the $\delta^{13}C$ values of the bioapatite extracted at the DPAA and CSU, Chico. In contrast, there was a significant difference in bioapatite $\delta^{18}O$ values ($p<0.0001$), with pairwise differences as large as 1.3‰. RID thresholds were calculated by adding three times the Standard Deviation (SD) of the mean pairwise differences (representing ~99% confidence) to the long-term SD of check standards used during sample analysis. Check standards included bovine liver (SD=0.07 and 0.08 for $\delta^{13}C$ and $\delta^{15}N$ values, respectively) and powdered marble (SD=0.09 and 0.16 for $\delta^{13}C$ and $\delta^{18}O$ values, respectively). Final RID values were 0.40‰ for collagen $\delta^{13}C$ values, 0.74‰ for collagen $\delta^{15}N$ values, 1.42‰ for bioapatite $\delta^{13}C$ values, and 1.61‰ for bioapatite $\delta^{18}O$ values. No pairwise difference exceeded RID thresholds for either collagen $\delta^{13}C$ or $\delta^{15}N$ values. Likewise, no pairwise difference exceeded the bioapatite RID values for either carbon or oxygen isotope values.

This study demonstrates a method for assessing comparability between isotope datasets, focusing on modern human bone collagen and bone bioapatite. Results show that collagen prepared by the DPAA today can be reliably compared to previously published datasets of collagen prepared by CSU, Chico.[3] Comparison of bone bioapatite prepared at the two laboratories suggests that carbon isotope values are reliable, but the location of preparation has a significant impact on oxygen isotope values. More work is needed to identify and potentially control for this variable as it could have a serious impact on provenance predictions based on the measured $\delta^{18}O$ value.

Reference(s):

1. Pestle, W.J., Crowley, B.E., and Weirauch, M.T. (2014) Quantifying Inter-Laboratory Variability in Stable Isotope Analysis of Ancient Human Remains. *PLoS ONE*. doi:10.1371/journal.pone.0102844.
2. Chesson, L.A., Kenyhercz M.W., Regan L.A., and Berg G.E. (2019) Addressing Data Comparability in the Creation of Combined Data Sets of Bioapatite Carbon and Oxygen Isotopic Compositions. *Archaeometry*. doi:10.1111/arcm.12480.
3. Bartelink, E.J., Berg G.E., Beasley M.M., and Chesson L.A. (2014) Application of Stable Isotope Forensics for Predicting Region of Origin of Human Remains From Past Wars and Conflicts. *Annals of Anthropological Practice* doi:10.1111/napa.12047.

Bone Apatite, Bone Collagen, Quality Control (QC)

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