A74 Using Apatite Yield From Bone Sample Preparations for Quality Control in Stable Isotope Analysis Applications

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Learning Overview: After attending this presentation, attendees will have a comprehensive understanding of the value of apatite yield for assessing quality control of bones prepared for isotopic analysis.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by presenting a heretofore unutilized characteristic of bone bioapatite preparations (apatite yield) that can assist with evaluating sample quality prior to isotopic analysis.

Attendees will learn how calculated apatite yield could be used in addition to other quality assessment metrics (i.e., collagen yield, Carbon-to-Nitrogen (C/N), Infrared Splitting Factor (IR-SF), and Carbonate-to-Phosphate (C/P)) to screen bone samples. The importance of how sample quality control measures to ensure viable *in vivo* data are used when applied to forensic interpretations will be discussed.

Since the late 1970s, stable isotope ratios of bone have been a popular method for reconstructing various aspects of human history. In the mineral fraction (i.e., hydroxyapatite, bioapatite, or apatite), carbon isotope ratios of carbonate ions record diet while oxygen isotope ratios record geographical origin. The weight fraction of apatite in fresh bone has been measured at 57%.[1] Preparation of apatite for isotopic analysis requires the removal of collagen and non-collagenous proteins as well as "secondary" carbonates that may have been incorporated during diagenesis. Typically, bone powder is bleached using sodium hypochlorite or hydrogen peroxide to remove collagen, then treated with buffered or weak acetic acid to remove diagenetic carbonates.

Bone apatite preparation methods used in forensic investigations of unknown decedents—such as Jane and John Does, unidentified border crossers, and missing United States service personnel—have developed from the archaeological literature. The literature supplies several quality assessment metrics useful for screening bone samples. For apatite, these include the examination of changes in molecular structure using spectroscopy.[2] One method, Fourier Transform Infrared (FTIR) spectroscopy, is frequently used to assess apatite crystallinity though the measurement of an IR-SF. Additionally, the ratio of C/P in apatite is often measured via FTIR spectroscopy. While helpful for evaluating diagenesis, application of this semi-quantitative method requires a specialized spectrometer and substantial operator training.

This study investigates the utility of bone apatite yield as a quality assessment metric. Bone apatite and collagen fractions were prepared from 288 prehistoric (1,000 to 5,000 years B.P.) human samples from seven Central California sites and 191 "modern" (<100 years B.P.) human samples. The four accepted quality assessment metrics were measured: collagen yield, elemental composition as the atomic C/N ratio, IR-SF, and C/P. Apatite yield was additionally calculated and ranged from 21% to 68% across all samples.

Initial evaluation of apatite yield for quality control focused on prehistoric samples, which included bone in excellent to extremely poor condition. There was no correlation observed between apatite yield and either collagen yield or C/N; only weak correlations were observed between apatite yield and IR-SF (r^2=0.22) or C/P (r^2=0.19). Prehistoric samples were scored based on the traditional quality assessment metrics, with one point assigned for each unacceptable metric (defined as collagen yield <5%, C/N >3.5, IR-SF >3.5, and C/P <0.15). Samples were then categorized into one of four groups, with higher scores indicating poorer quality: 0, 1, 2, or 3-4 combined.

The "best" prehistoric samples, with no flagged quality metrics, had significantly lower mean apatite yield than samples with scores of 2 or higher (one-way Analysis of Variance (ANOVA) with Tukey's multiple comparisons post-hoc test; F=15, p <0.0001). Based on this, the "modern" samples—which are typical of bone used in isotope testing of unidentified decedents—were used to define expected apatite yield, with measurement error included, as 25%–60%.

This study demonstrates that apatite yield is useful in addition to, and potentially as a substitution for, other quality assessment metrics to screen bone samples prior to isotopic analysis. Unlike IR-SF or C/P measures, calculation of apatite yield requires no specialized instrumentation or training and is not determined from a semi-quantitative method. Routine calculation of apatite yield during bone sample preparation could additionally address needs related to necessary quality control monitoring within accredited forensic laboratories, including the demonstration of method repeatability and reproducibility. Given these initial results, it is proposed that bone sample preparations with apatite yield <25% or >60% should be considered unacceptable in forensic investigations.

Reference(s):

1. Wang X. Bank R.A., Tekoppele, J.M., and Agrawal.CM. The role of Collagen in Determining Bone Mechanical Properties. *J Orthop Res*. 2001:19(6):1021-1026.
2. Beasley M.M., Bartelink E.J., Taylor L., and Miller R.M. Comparison of Transmission FTIR, ATR, and DRIFT Spectra: Implications for Assessment of Bone Bioapatite Diagenesis. *J Archaeol Sci*. 2014:46:16-22.

Isotopic Analysis, Bone Apatite, Quality Control (QC)