OSAC PROPOSED STANDARD 2026-S-1035
Isotope Sample Preparation in Forensic Anthropology

Forensic Anthropology Subcommittee
Medicine Scientific Area Committee (SAC)
Organization of Scientific Area Committees (OSAC) for Forensic Science

Draft OSAC Proposed Standard

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Foreword

The Forensic Anthropology Subcommittee of the Organization of Scientific Area Committees (OSAC), under the guidance of the National Institute of Standards and Technology (NIST), recognizes the importance of isotopic analysis as a tool for investigating unidentified human remains. This standard provides requirements for forensic practitioners on isotope sample preparation methods for remains of medicolegal significance prior to the stable isotope analysis of the major chemical elements (i.e., H, C, N, O, and S). Standardizing the preparation methods for remains (i.e., hair, nails, bones, and teeth) is crucial to ensuring comparability of isotopic data in human identification efforts.

Requirements for forensic practitioners on stable isotope analysis methods for the major chemical elements are intended to be provided in a separate standard. Likewise, requirements for the preparation and isotopic analysis of human remains for minor or trace chemical elements, such as Sr and Pb, are intended to be provided in a separate standard.

This standard does not cover the preparation of samples from archaeological remains for stable isotope analysis, nor does it cover the preparation of unidentified human remains for radiocarbon (14C) dating.

All hyperlinks and web addresses shown in this document are current as of the publication date of this standard.

Keywords: *forensic anthropology; isotope analysis; keratin; collagen; bioapatite*

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Standard For Isotope Sample Preparation In Forensic Anthropology

1 Scope

This standard provides requirements for preparing samples of unidentified human remains—specifically, hair, nails, bones, and teeth—for stable isotope analysis. It focuses on sample preparation before measuring the major chemical elements: hydrogen (H), carbon (C), nitrogen (N), oxygen (O), and/or sulfur (S).

2 Normative References

There are no Normative References documents. Annex A, Bibliography, contains informative references.

3 Terms and Definitions

For purposes of this document, the following terms and definitions apply.

3.1
isotope
Each of two or more forms of the same chemical element that contain equal numbers of protons but different numbers of neutrons in their nuclei, hence differing in atomic mass.

3.2
stable isotope
A non-radioactive isotope form of an element; stable isotopes do not undergo radioactive decay.

3.3
isotope ratio (R)
The ratio of the abundances of two stable isotopes of the same element, typically expressed as the abundances of the heavier versus the lighter isotope (e.g., 13C/12C).

3.4
delta (δ) notation
A measure of the isotope ratio of a sample relative to an internationally agreed zero-point (RM) that defines the measurement scale; δ = (Rsample/RRM) – 1 and is often expressed in parts per thousand (‰ “per mil”).

NOTE Isotope δ values are dimensionless quantities.

3.5
hydrogen isotope system
Consists of the stable isotopes 1H and 2H; measured δ2H values reflect hydrological patterns and aid in geolocation.

3.6
carbon isotope system
Consists of the stable isotopes 12C and 13C; measured δ13C values reflect diet.

3.7
nitrogen isotope system
Consists of the stable isotopes 14N and 15N; measured δ15N values primarily reflect the trophic level of the diet.

3.8
oxygen isotope system
Consists of the stable isotopes 16O and 18O; measured δ18O values reflect hydrological patterns and aid in geolocation.

3.9
sulfur isotope system
Consists of the stable isotopes 32S and 34S; measured δ34S values reflect diet and sometimes geolocation.

3.10
major chemical elements
Include the elements H, C, N, O, and S, which comprise the primary building blocks of the biosphere, hydrosphere, and atmosphere.

3.11
sampling
The selective removal of a portion of bone for testing.

3.12
human tissues
Include the biological materials hair, nails, bones, and teeth, which are commonly recovered from skeletal remains.

3.13
maceration
A process involving the removal of soft tissue (flesh) from skeletal remains by soaking in a liquid, such as water.

3.14
internal control standard
A material chemically similar to samples that undergo identical preparation to the samples and helps to capture the uncertainty associated with processing; sometimes called a study standard.

3.15
keratin

A structural fibrous protein that makes up hair, nails, and skin. In humans, hair and nails grow continuously and do not remodel or turnover once formed.

3.16
collagen
The main structural protein in the extracellular matrix that comprises connective tissues.

NOTE: The organic extract from bones is commonly referred to as “collagen,” although it also includes some non-collagenous proteins.

3.17
bioapatite
Biologically-formed carbonate hydroxyapatite, the calcium phosphate mineral comprising the inorganic component of bones and teeth.

NOTE Carbonate is relatively abundant in bioapatite, constituting approximately 2–5% of tooth enamel and 4–13% of bone bioapatite by weight.

3.18
diagenesis
Postmortem chemical alterations to hard and soft tissues, which have the potential to alter isotope δ values.

3.19
isotopic taphonomy
The consideration of postmortem changes to stable isotope ratios in human tissues as related to taphonomic processes to which the body is exposed (e.g., depositional environment, fluvial processes, soil exposure).

4 Requirements

4.1 General

4.1.1 Forensic practitioners shall be trained in isotope sample preparation procedures prior to preparing samples from unidentified human remains for stable isotope analysis.

NOTE 1 Training ensures the competence of a practitioner to handle/manage evidence, operate specific equipment, and evaluate data.

NOTE 2 The training required depends on the abilities, experience, and qualifications of each trainee. Regardless of the type of training completed, the trainer ensures it instills in the trainee a satisfactory knowledge of the requirements of the operations, investigations, or analyses performed, as well as the ability to make professional judgments. Additionally, training allows the trainee to understand the significance of deviations with regard to normal investigation and/or preparation procedures.

4.1.2 Safety shall be the first consideration in the procedures described below.

NOTE: Practitioner may be exposed to bloodborne pathogens if fresh remains are sampled; examples of safety precautions when working with fresh remains include the use of universal precautions for infection control and vaccinations.

4.1.3 Appropriate attire and personal protective equipment shall be worn when performing or observing hazardous procedures:

- Eye protection, long pants and long sleeves, shoes with fully covered uppers, and gloves shall be worn whenever splatter hazards are present.

- Dust masks and hearing protection shall be used as needed.

4.1.4 Personnel shall be familiar with all safety data sheets (SDS) before working with chemical reagents.

NOTE: Some hazard exposure may be mitigated with engineering controls, such as working with chemical reagents in fume hoods.

4.1.5 An internal control standard shall be used to monitor the effect of variations in preparation processes.

NOTE 1: There are published variations in the chemical reagents, concentrations, times, etc., used in the procedures described below.

NOTE 2: The internal control standard should undergo identical preparation to the samples and is ideally available in sufficient quantity to ensure it can be used for several years. Data on percent yield can be used to assess the uncertainty introduced by variation in processes through time.

4.1.6 Good work practices shall be used to minimize the risk of potential contamination between samples and between a sample and the work environment:

- Workstations shall be clean.

- Sample containers shall be clearly labeled.

- Only one sample container shall be opened at once, when possible.

NOTE 1: More than one sample can be prepared at a time, by working with batches. Good work practices minimize the risk of potential contamination between samples in a batch.

NOTE 2: It is sometimes necessary to open more than one sample container at once – for example, when drying a batch of samples.

4.2 Procedure

4.2.1 General Requirements

4.2.1.1 Reactions shall take place at room temperature, using laboratory-grade chemical reagents.

4.2.1.2 Since preparation is destructive, careful sampling decisions shall be made to preserve material.

NOTE 1: Target sample weights for different tissue types are provided below.

NOTE 2: To minimize risk, a second sample can be collected and stored for a second preparation attempt if the first attempt is not successful.

4.2.2 Hair Preparation

4.2.2.1 A lock of hair from the scalp shall be collected.

NOTE 1: Approximately 90% of human hair is in the growth, or anagen, phase; collecting a lock with multiple (50-100) strands of hair mitigates the impact of strands in the resting, or telogen, phase during which a follicle is dormant before shedding.

NOTE 2: Strands of 10 mm represent approximately one month of growth.

4.2.2.2 Hair samples shall be cleaned:

- Visible surface contaminants shall be removed using water and sonication.
- Adherent lipids shall be removed.

NOTE: One commonly used solution for removing lipids is a 2:1 chloroform/methanol mixture.

4.2.2.3 Samples shall receive a final rinse with water.

4.2.2.4 Clean hair samples shall be dried.

4.2.2.5 Clean and dry hair samples shall be homogenized.

NOTE 1: Hair samples can be homogenized by either cutting into small (<1 mm) pieces or grinding into powder.

NOTE 2: Analysis of serial sections can reveal changes in diet or geolocation over time for an individual based on the known growth rate of hair.

4.2.3 Nail Preparation

4.2.3.1 A fingernail clipping at least 3 mm in length or a toenail clipping at least 1 mm in length shall be collected.

NOTE: Provided lengths represent approximately one month of growth.

4.2.3.2 Preparation of nail clippings shall follow that described above for hair.

4.2.4 Bone Preparation

4.2.4.1 Soft tissue shall be removed from fleshed bone for sampling.

NOTE 1: The use of detergent for maceration is generally not recommended as it can affect isotope δ values.

NOTE 2: Carrion insects, such as dermestid beetles, can be used for cleaning fleshed bones.

4.2.4.2 Bone shall be sampled for the extraction of either collagen or bioapatite.

NOTE 1: Preferred starting weight is 1.0 g.

NOTE 2: Bone tissue continually remodels during life with remodeling rates that vary between cortical and trabecular bone tissues, and between bones of different densities. Therefore, samples from different bones may reflect different time intervals of an individual's life. Guidance is available in the literature on carefully designing and completely describing bone sample collection location (e.g., "full transverse section of medial-midshaft").

4.2.4.3 Bone samples shall be physically abraded to remove surface contaminants.

NOTE: Physical abrasion is particularly important for skeletal remains that were buried or exposed to the environment.

4.2.4.4 Abraded bone samples shall be solvent cleaned using ethanol and water.

4.2.4.5 Bone samples shall be degreased (delipified) using a 2:1 chloroform/methanol mixture.

NOTE 1: Lipids can have different $\delta^{13}C$ values than proteins (like collagen) or minerals (like bioapatite); their presence can therefore impact measured δ values.

NOTE 2: Degreasing can take place either before or after the demineralization of bone for collagen extraction. For bioapatite extraction, degreasing takes place prior to powdering the bone.

NOTE 3: Elemental composition data collected during stable isotope analysis can be used to confirm that samples were sufficiently degreased (e.g., ratio of carbon to nitrogen atoms); for details, see the separate standard on measurement procedures.

4.2.5 Bone Collagen Extraction

4.2.5.1 Collagen shall be extracted from bone samples for stable isotope analysis, which requires the complete removal of the bioapatite (mineral) fraction.

NOTE: Either bone chunks or bone powder can be used for demineralization.

4.2.5.2 Bone samples shall be soaked in a dilute solution of hydrochloric acid (HCl) for demineralization.

NOTE: Concentrations in the literature used for demineralization range from 0.2 M to 1 M HCl, with concentrations of 0.2-0.6 M most used.

4.2.5.3 Any humic acids that may be present shall be removed via treatment with sodium hydroxide (NaOH).

NOTE 1: Humic acid treatment is particularly important for skeletal remains recovered from outdoor environments.

NOTE 2: The most common concentration used in the literature is 0.125 M NaOH.

4.2.5.4 Collagen shall be homogenized and dried prior to being weighed for stable isotope analysis.

NOTE 1: Acid and heat can be used to gelatinize collagen to homogenize it. If collagen cannot be gelatinized, it can be dried and then ground to a homogeneous powder.

NOTE 2: Gelatinization may be followed by filtration to remove contaminants with relatively low molecular weights; however, this is not generally a concern for forensic samples.

4.2.5.5 The collagen percent (%) yield shall be calculated.

4.2.6 Bone Bioapatite Extraction

4.2.6.1 Bioapatite shall be extracted from bone samples for stable isotope analysis, which requires the complete removal of the collagen (organic) fraction.

4.2.6.2 Bone samples shall be ground into a powder for bioapatite extraction.

NOTE: Preferred starting weight is 0.5 g of bone powder.

4.2.6.3 Bone powder shall be "bleached" to remove organic material.

NOTE: Either hydrogen peroxide (H_2O_2) or sodium hypochlorite (NaOCl) can be used to bleach samples. Concentrations of NaOCl used in the literature range from 1% to 4%, while H_2O_2 is most used at a concentration of 30%.

4.2.6.4 Labile carbonates shall be removed using an acetic acid (CH_3COOH) solution.

NOTE: The CH_3COOH solution can be either weaker (i.e., 0.1 M) and unbuffered or stronger (i.e., 1.0 M) and buffered with Ca/Na acetate.

4.2.6.5 Bioapatite shall be dried.

4.2.6.6 Bioapatite percent (%) yield shall be calculated.

4.2.6.7 Bioapatite shall be homogenized via grinding prior to being weighed for stable isotope analysis.

4.2.7 Tooth Dentinal Collagen Extraction

4.2.7.1 Dentin shall be collected for collagen extraction:

- A whole tooth shall be bisected to access the dentin.
- Enamel/cementum shall be removed via physical abrasion.

4.2.7.2 Dentin powder shall be used for preparation.

NOTE: Preferred starting weight is 1-2 mg of dentin powder.

4.2.7.3 Preparation of tooth dentinal collagen shall follow that described above for bone collagen.

4.2.8 Tooth Enamel Bioapatite Extraction

4.2.8.1 The tooth shall be physically abraded to remove surface contaminants.

4.2.8.2 The abraded tooth shall be solvent cleaned using ethanol and water.

4.2.8.3 Enamel powder shall be used for preparation.

NOTE 1: Preferred starting weight is 25 mg of enamel powder.

NOTE 2: Tooth enamel does not remodel and thus retains an in vivo signature from the time interval of tissue formation. A collection of enamel from different teeth may reflect different time intervals of an individual's life.

4.2.8.4 Preparation of tooth enamel bioapatite shall follow that described above for bone bioapatite.

NOTE 1: The strength of solutions used in tooth enamel bioapatite preparation is often weaker and the treatment times are shorter than that used for bone bioapatite preparation; this is due to the proportion of organic material in enamel (~1%) versus bone (~30%).

NOTE 2: Recent isotopic studies have suggested that the stable isotope analysis of enamel powder that did not undergo chemical processing is optimal, especially for forensic samples.

4.3 Diagenesis

4.3.1 The impact of diagenesis, especially the context of deposition, upon the "endogenous" isotope δ values shall be considered when preparing human remains samples for stable isotope analysis.

4.3.2 Prior to preparation, hair and nail shall be microscopically examined for structural changes that may impact isotope δ values.

NOTE: Keratinous proteins are primarily impacted by the moisture and temperature of the environment. Fungal organisms are the main decomposers of keratin.

4.3.3 Following preparation of bone and teeth, material extracts shall be assessed for compositional changes that may impact isotope δ values.

NOTE 1: The target weight fraction (or, percent yield) for extracted collagen is > 2–5%.

NOTE 2: The molecular structure of extracted bioapatite is commonly evaluated via microscopic histological analysis, infrared spectroscopy, and x-ray diffraction; however, these methods may not reliably assess isotopic alterations. An additional quality metric collected during bioapatite extraction is weight fraction (or percent yield).

4.4 Reporting

4.4.1 A practitioner shall choose a published method and follow it or use published methods to create a standard operating procedure that is then followed.

4.4.2 A complete description of the procedures used in isotope sample preparation shall be included/referenced when reporting isotope δ values.

NOTE: This is especially important as there are published variations in the chemicals, concentrations, times, etc., used in the procedures described above.

4.4.3 Data shall be reported on the context of sample preservation.

NOTE: See section on diagenesis for more details.

Annex A

(informative)

Bibliography

The following bibliography is not intended to be an all-inclusive list, review, or endorsement of literature on this topic. The goal of the bibliography is to provide examples of concepts and publications addressed in the standard.

1) Ambrose, Stanley H. "Preparation and characterization of bone and tooth collagen for isotopic analysis." *Journal of Archaeological Science* 17, no. 4 (1990): 431-451.

2) Crowley, Brooke E., and Patrick V. Wheatley. "To bleach or not to bleach? Comparing treatment methods for isolating biogenic carbonate." *Chemical Geology* 381 (2014): 234-242.

3) France, Christine AM, Nawa Sugiyama, and Esther Aguayo. "Establishing a preservation index for bone, dentin, and enamel bioapatite mineral using ATR-FTIR." *Journal of Archaeological Science: Reports* 33 (2020): 102551.

4) Garvie-Lok, Sandra J., Tamara L. Varney, and M. Anne Katzenberg. "Preparation of bone carbonate for stable isotope analysis: The effects of treatment time and acid concentration." *Journal of Archaeological Science* 31, no. 6 (2004): 763-776.

5) Jørkov, Marie Louise S., Jan Heinemeier, and Niels Lynnerup. "Evaluating bone collagen extraction methods for stable isotope analysis in dietary studies." *Journal of Archaeological Science* 34, no. 11 (2007): 1824-1829.

6) Koch, Paul L., Noreen Tuross, and Marilyn L. Fogel. "The effects of sample treatment and diagenesis on the isotopic integrity of carbonate in biogenic hydroxylapatite." *Journal of Archaeological Science* 24, no. 5 (1997): 417-429.

7) Meier-Augenstein, Wolfram. *Stable Isotope Forensics: Methods and Forensic Applications of Stable Isotope Analysis*. John Wiley & Sons, 2017.

8) Meier-Augenstein, Wolfram, and Helen F. Kemp. "Stable Isotope Analysis: Bone and Teeth." *Wiley Encyclopedia of Forensic Science* (2009).

9) Meier-Augenstein, Wolfram, and Helen F. Kemp. " Stable Isotope Analysis: Hair and Nails." *Wiley Encyclopedia of Forensic Science* (2009).

10) Pestle, William J., Brooke E. Crowley, and Matthew T. Weirauch. "Quantifying inter-laboratory variability in stable isotope analysis of ancient skeletal remains." *PLoS One* 9, no. 7 (2014): e102844.

11) Roberts, Patrick, Ricardo Fernandes, Oliver E. Craig, Thomas Larsen, Alexandre Lucquin, Jillian Swift, and Jana Zech. "Calling all archaeologists: Guidelines for terminology, methodology, data handling, and reporting when undertaking and reviewing stable isotope applications in archaeology." *Rapid Communications in Mass Spectrometry* 32, no. 5 (2018): 361-372.

12) Sealy, Judith, Malia Johnson, Michael Richards, and Olaf Nehlich. "Comparison of two methods of extracting bone collagen for stable carbon and nitrogen isotope analysis: Comparing whole bone demineralization with gelatinization and ultrafiltration." *Journal of Archaeological Science* 47 (2014): 64-69.

13) Snoeck, Christophe, and Maura Pellegrini. "Comparing bioapatite carbonate pre-treatments for isotopic measurements: Part 1—Impact on structure and chemical composition." *Chemical Geology* 417 (2015): 394-403.

14) Pellegrini, Maura, and Christophe Snoeck. "Comparing bioapatite carbonate pre-treatments for isotopic measurements: Part 2—Impact on carbon and oxygen isotope compositions." *Chemical Geology* 420 (2016): 88-96.

15) Stantis, Chris, Lesley A. Chesson, Daniel L. Johnson, Thuan H. Chau, Kirsten A. Verostick, Gregory E. Berg, and Gabriel J. Bowen. "Improving inter-laboratory comparability of tooth enamel carbonate stable isotope analysis ($\delta^{13}C$, $\delta^{18}O$)." *Forensic Chemistry* (2025): 100679.

16) Szpak, P., K. Krippner, and M. P. Richards. "Effects of sodium hydroxide treatment and ultrafiltration on the removal of humic contaminants from archaeological bone." *International Journal of Osteoarchaeology* 27, no. 6 (2017): 1070-1077.

17) Szpak, Paul, Jessica Z. Metcalfe, and Rebecca A. Macdonald. "Best practices for calibrating and reporting stable isotope measurements in archaeology." *Journal of Archaeological Science: Reports* 13 (2017): 609-616.

18) Vaiglova, Petra, Nicole A. Lazar, Elizabeth A. Stroud, Emma Loftus, and Cheryl A. Makarewicz. "Best practices for selecting samples, analyzing data, and publishing results in isotope archaeology." *Quaternary International* 650 (2023): 86-100.

19) Van Klinken, Gert J. "Bone collagen quality indicators for palaeodietary and radiocarbon measurements." *Journal of Archaeological Science* 26, no. 6 (1999): 687-695.

20) Yoder, C. J., and Eric J. Bartelink. "Effects of different sample preparation methods on stable carbon and oxygen isotope values of bone apatite: A comparison of two treatment protocols." *Archaeometry* 52, no. 1 (2010): 115-130.

This is not meant to be an all-inclusive list, as the group recognizes other publications on this subject may exist. At the time these standards were drafted, the following publications were

available to the working group members for reference. Additionally, any mention of a particular software tool or vendor as part of this bibliography is purely incidental, and any inclusion does not imply endorsement by the authors of this document.

1] Bright J.-A., I.W. Evett, D. Taylor, J.M. Curran, and J.S. Buckleton. “A series of recommended tests when validating probabilistic DNA profile interpretation software.” Forensic Sci. Int.: Genetics 14, 2015, pp. 125-131.

2] Coble M.D., J. Buckleton, J.M. Butler, T. Egeland, R Fimmers, P. Gill, et al. “DNA Commission of the International Society for Forensic Genetics: Recommendations on the validation of software programs performing biostatistical calculations for forensic genetics applications.” Forensic Sci. Int.: Genetics 25, 2016, pp. 191-197.