Bone Histomorphology

1 Scope

Bone histomorphology can assist in determining whether human origin of osseous material can be excluded on the basis of bone microstructure. This document describes how to embed, section, grind, polish, mount, and examine specimens for histomorphological analysis performed by Anthropology Examiners within the Trace Evidence Unit (TEU).

2 Equipment/Materials/Reagents

- Bone saw (Dremel tool or equivalent)
- Sonic cleaner
- Sonic cleaning solution (Ultramet or equivalent)
- Degreaser (Clear-Rite or equivalent, xylene, or acetone)
- Embedding molds (Peel-Away or equivalent)
- Balance
- Resin mixing container (e.g., paper cup)
- Embedding resin (EpoThin resin or equivalent)
- Embedding resin hardener (EpoThin hardener or equivalent)
- Stirring stick (e.g., tongue depressor or wooden rod)
- Vacuum pump
- Sectioning/wafering saw (Isomet Precision Saw or equivalent)
- Cutting fluid (Ultramet or equivalent)
- Cutting blade (high concentration diamond-edged blade or equivalent)
- Blade dressing stick
- Grinder/polisher (Ecomet Grinder/Polisher or equivalent)
- Grinding/polishing discs and cloths in a variety of abrasive grit levels
- Mounting medium or resin (Permount, Crystalbond or equivalent)
- Forceps/tweezers
- Glass microscope slides
- Cover slips
- Transmitted light microscope with 10x and 20x objectives
- Bone histomorphological image exemplars
- Personal protective equipment (e.g., lab coat, gloves, eye protection)

3 Standards and Controls

Not applicable.

4 Sampling

4.1.1 If multiple bone fragments are present, sample selection is dependent upon whether the bone has identifiable cortical bone that could potentially yield a thin section, and whether the fragment needs to be embedded using resin. If DNA analysis may also be performed, consultation with a DNA examiner is recommended prior to finalizing sample selection.

4.1.2 Histomorphological analysis requires a true transverse section, and therefore the determination of anatomical orientation of the specimen is required. In specimens where this orientation cannot be determined, results will be accompanied by an appropriate caveat.

4.1.3 For cases involving a single fragment, a small specimen for histomorphological analysis will be used, leaving some of the original fragment, if possible. The specimen will be cut using a bone saw. If the original fragment is too small, the entire item will be embedded.

5 Procedures

The Forensic Anthropological Examinations procedure will be followed. In cases where the examination requires the determination of whether skeletal material is human or non-human in origin, the following procedures may be used. Depending on the quantity and quality of the material submitted and the nature of the examination request, each of the below steps may not be required. Observations supporting conclusions will be recorded in the case notes.

5.1 Embedding

5.1.1 Embedding is used to stabilize fragments when the specimen is of a small size and/or is taphonomically compromised. Embedding may not be necessary in cases where bone fragments are of sufficient size and quality to be held in the cutting chuck, in which case the examiner may proceed to sectioning.

5.1.2 Prior to embedding, sonic cleaning (with optional 1:20 parts cleaning solution) may be performed at the discretion of the examiner to remove debris. If a sonic bath is used, the specimen will be allowed to dry for 24 hours prior to embedding.

5.1.3 If the skeletal material is fresh/greasy, it may be degreased by placing the sample in a degreaser to remove lipids which will diminish slide quality.

5.1.4 The appropriate embedding mold will be selected such that the specimen will fit within the mold without touching the sides or bottom of the mold. To facilitate this, a thin layer of embedding medium may be poured into the mold and allowed to cure for approximately 24 hours, providing a platform for the sample to ensure that the specimen does not touch the bottom of the mold.

5.1.5 Using a balance, graduated container, or other liquid measuring device, and a mixing container such as a disposable paper cup, an appropriate amount of resin (depending on the mold size and sample size) will be measured and hardener will be added according to the manufacturer’s instructions. The mixture will be stirred using a stirring stick for approximately

2 minutes until the mixture is clear or according to manufacturer's instructions.

5.1.6 The mold and specimen will be labeled with the Laboratory Number and item identifier. This may be achieved using a pencil on paper embedded within the resin with the specimen. The resin/hardener mixture will be poured into the mold(s), allowing a thorough covering of the specimen with epoxy but not overfilling the mold.

5.1.7 The specimen will be oriented/aligned within the resin using forceps or a probe if necessary, and the label will be arranged so that it is secure and visible. If large air bubbles are present within the epoxy, they may be removed by placing the mold(s) into a vacuum pump.

5.1.8 The epoxy will be allowed to cure for approximately 24 hours, and the plastic molds will then be removed and discarded. Sharp edges on the top of the epoxy block may be removed using sand paper.

5.2 Thin Sectioning

5.2.1 Thin sections will be made using a wafering saw. The saw will be prepared by filling the reservoir with water and, at the discretion of the examiner, approximately 1:20 parts of cutting fluid. The blade will be mounted and tightly secured on the blade stand. The blade will be dressed as needed using the blade dressing stick.

5.2.2 The bone or epoxy block will be mounted in the chuck and attached to the saw's specimen arm. Specimens will be mounted in a manner that produces an anatomically transverse section (as best as possible, when orientation is known).

5.2.3 The counterweights will be adjusted as necessary to counter balance the weight of the arm, chuck, and sample. A flushing/waste cut will be made by lowering the specimen to the blade, and the measurement point will then be zeroed.

5.2.4 The specimen arm will be positioned to make a section that is 0.8mm (preferable if possible) to 1.5mm thick (if necessary in cases of more friable specimens), at a blade speed of approximately 100rpm. Additional sections will be made as necessary until the desired number of sections have been removed. Three sections are recommended in most cases.

5.2.5 The thin section will be removed from the saw, using forceps if necessary. If the section will be mounted without grinding or polishing, it will be allowed to dry prior to mounting.

5.3 Grinding and Polishing

5.3.1 Grinding and polishing will be performed using a grinder/polisher and removable grinding and polishing discs/platens. Grinding reduces the specimen to a thickness appropriate for analysis, and polishing removes striations on the specimen from the cutting and grinding processes.

5.3.2 Polishing and grinding is best performed immediately after thin sectioning in order to avoid the introduction of water on the specimen multiple times.

5.3.3 Grinding and polishing discs and cloths will be affixed to the rotating platen by magnet or adhesive. Grinding platens will be dressed as needed.

5.3.4 The specimen may be held, applying even pressure, using a manufactured specimen holder, or by holding the specimen to the disc with the aid of a hand-made gripping device (e.g., a microscope slide covered in sandpaper or tape to hold the specimen in place).

5.3.5 Grinding will use successively finer abrasive as needed, until the specimen is reduced to a thickness that allows sufficient transmission of light through the specimen (typically 50-75 micrometers). Water may be added during the grinding process. Thickness and suitability for examination can be periodically tested using microscopy.

5.3.6 Polishing will use successively finer polishing paper/cloths as needed. Suitability for examination can be periodically tested using microscopy.

5.3.7 Grinding and polishing may be repeated/continued as necessary to achieve a section suitable for histomorphological examination. Following grinding and polishing, a sonic bath may be used at the discretion of the examiner to remove particles.

5.4 Slide Preparation

5.4.1 Prior to mounting, the specimen should be allowed to dry. Weight may need to be applied to the specimen to avoid warping.

5.4.2 The microscope slide will be labeled with the Laboratory Number, item identifier, date, and initials of the preparer.

5.4.3 Mounting medium, as needed, will be applied to the center of the slide.

5.4.4 Using forceps, the specimen will be placed on the slide, and additional mounting medium will be applied as necessary. The specimen will be covered with a cover slip to optimize optics for microstructure resolution, and allowed to dry. Weight may be used to even out the coverslip.

5.4.5 The slide will be allowed to dry for at least one day before reading, and two to four weeks before storing in a slide box.

5.5 Examination and Data Collection

5.5.1 The prepared slides will be examined using a transmitted light microscope with 10x and 20x objectives. The total magnification and filter used, typically 100x, polarized, will be recorded in the case notes.

5.5.2 Specimens will be qualitatively examined for the presence of Haversian bone. Haversian bone is characterized by concentric lamellae surrounding longitudinal canals resulting in a circular osteon in transverse section. The presence of histomorphology consistent with Haversian bone supports the conclusion that human origin cannot be excluded.

5.5.3 The presence of histomorphology consistent with non-Haversian bone (e.g., fibrolamellar, laminar, plexiform bone) supports the conclusion that the bone is non-human in origin (i.e., that human origin can be excluded).

5.5.4 The presence of osteon banding (i.e., multiple areas of linear osteonal organization) supports the conclusion that the bone is non-human in origin (i.e., that human origin can be excluded).

5.5.5 In some cases, histomorphological material can be compared with image exemplars appearing in published literature. Referenced literature or exemplars will be recorded in the case notes.

5.6 Reporting

5.6.1 In the event that the remains are determined to be non-human, this will be stated in the FBI *Laboratory Report* (7-1, 7-1 LIMS). When possible or appropriate, the origin(s) of the remains will also be stated. For example: "*The submitted item(s) is/are skeletal material of non-human origin (or human origin can be excluded). No further anthropological examinations were conducted.*"

5.6.2 In the event that human origin cannot be excluded, this will be stated in the *Laboratory Report*. For example: *"The submitted item(s) cannot be excluded as human in origin."* Where possible or appropriate, examinations will proceed according to the examination request following appropriate Standard Operating Procedures.

5.6.3 In the event that the examination is inconclusive, the *Laboratory Report* will state this. For example: *"The submitted item(s) is/are of undetermined origin. No further anthropological examinations were conducted."*

6 Calculations

Not applicable.

7 Measurement Uncertainty

Not applicable.

8 Limitations

8.1 The conclusions that can be reached regarding human or non-human origin are dependent on the condition and completeness of the skeletal remains. Results based on fragmentary or poorly preserved material may be inconclusive.

8.2 Human versus non-human origin of bone cannot always be determined on the basis of

bone microstructure.

9 Safety

9.1 While working with physical evidence, Laboratory personnel will wear at least the minimum appropriate protective attire (e.g., laboratory coat, eye protection, protective gloves).

9.2 Universal precautions will be followed.

9.3 Exposure to physical, biological and chemical hazards may be associated with the examination techniques performed. Safety procedures related to specific materials, instruments or equipment (e.g., wafering saws) will be followed. Refer to the FBI Laboratory Safety Manual for guidance.

10 References

- Forensic Anthropological Examinations, Trace Evidence Procedures Manual (current version)
- FBI Laboratory Safety Manual (current version)
- Crowder C, Stout S. Bone Histology: An Anthropological Perspective. CRC Press: Boca Raton, 2012.
- Mulhern DM, Ubelaker DH. Differences in osteon banding between human and nonhuman bone. Journal of Forensic Sciences 2001; 46(2):220-222.

Rev. #	Issue Date	History
2	02/07/2018	Updated throughout removing references to TEU where appropriate; added forensic anthropologists to the Scope in Section 1 and throughout document. Added 'or Sample Selection' to Section 4 title. Moved former Sections 5.1.1, 5.1.2, and 5.1.3 to Section 4. Updated Sections 5.1.4 and 5.1.8 to specify approximate curing time. Provided materials to use in Section 5.1.5 as well as adding manufacturer's instructions. Added discretion of forensic anthropologist to Section 5.2.1. Section 5.2.4 added three sections are recommended in most cases. Updated Section 5.3.4 to add tape as option. Section 5.3.5 revised to provide guidance of sufficient transmission of light. Removed reference to glossy surface in Section 5.3.6. Added Laboratory Report to Sections 5.6.1, 5.6.2, and 5.6.3. Updated references in Section 10.
3	02/10/2020	'Sample Selection' removed from Section 4 title. Added reference to DNA analysis Section 4.1.1. Updated wording in Sections 5.1.4, 5.1.6, and 5.6.1. Added examples to Sections 5.5.3 and 5.5.4. Changed 'forensic anthropologist' to 'Anthropology Examiner' in Scope and 'examiner' throughout document.

Redacted - Signatures on File

Approval

Trace Evidence Unit Chief — Date: 02/07/2020

Anthropology Technical Leader — Date: 02/07/2020