

Sample Preparation for Tooth Enamel Sr Analysis

This protocol is designed for the preparation of teeth for strontium isotope analysis. This protocol is divided into two sections: the first being **ENAMEL EXTRACTION** and the second, **ENAMEL PRETREATMENT FOR APATITE EXTRACTION**. This protocol will take approximately 3 days from start to finish.

ENAMEL EXTRACTION

Note: Before drilling, remove loose sediments and organic matter from specimens. Do not allow any sediment to contaminate the samples! Sr and (in particular) Nd content can be much higher in adhering soils than in tooth enamel, so the slightest amount of soil can cause significant contamination. With each batch of teeth, include modern tooth of known value as positive control. The best method for cleaning is dry brushing, followed by ultrasonication in distilled/nanopure water as described below.

EQUIPMENT AND SAMPLE STERILIZATION ~ 15 mins

Equipment needed: 2 Dremel tools, carbide drill tips, ultrasonicator, lab coat

Disposables needed: DI water, isopropyl alcohol, small and large Kimwipes

1. Be sure that Dremel tool mounting assembly and body are clean of all dust before beginning. Wipe down with Kimwipes and isopropyl alcohol.
2. Place tooth in distilled/nanopure water bath in ultrasonicator and sonicate until adhering soil falls off. Wash with isopropyl alcohol and allow to dry.
3. Sonicate all carbide and diamond drill bits in isopropyl alcohol to clean, along with any removable parts of Dremel tool mounting assembly.

ENAMEL EXPOSURE ~ 15 min/tooth

Equipment needed: eye protection, 2 Dremel tools, carbide drill tips, baths, ultrasonicator, lab coat, quartz crystal

Disposables needed: DI water, isopropyl alcohol, gloves, small and large Kimwipes

1. Wearing disposable gloves and eye protection, carefully hold tooth over clean work area. Put down large Kimwipe to catch powder removed for disposal.
2. Use Dremel tool marked "Cleaning" with carbide drill bit to remove outer layer of calculus/plaque over tooth enamel. Do this lightly but thoroughly to expose white enamel over an area larger than the location where enamel samples will be removed, to minimize contamination.
3. Use Kimwipes and isopropyl alcohol to wipe down the tooth; set aside for ENAMEL EXTRACTION.
4. Use Dremel to drill quartz over same work surface for 10 seconds.
5. Dispose of Kimwipe with dust in trash can; wipe down area with isopropyl alcohol and Kimwipes.
6. Reclean Dremel and bits as described in EQUIPMENT AND SAMPLE STERILIZATION before going on to next tooth.
7. Repeat ENAMEL EXPOSURE steps until all teeth are cleaned. Clean all teeth prior to extracting enamel.

ENAMEL EXTRACTION ~ 10 min/sample line

Be sure to remove only enamel and no dentine, which will contaminate the sample

Equipment needed: eye protection, 2 Dremel tools, carbide drill tips, baths, ultrasonicator, balance (measuring to 0.0001 g), extra fine Sharpie, lab coat, quartz crystal

Disposables needed: DI water, isopropyl alcohol, 1.5 ml centrifuge tubes, gloves, small and large Kimwipes, weigh paper, aluminum foil

1. Label a 1.5 ml microcentrifuge tube with Sharpie.
2. Use balance to weigh labeled microcentrifuge tube; record empty weight.
3. Place clean sheet of aluminum foil on work area. Put on new pair of gloves.
4. Tare balance with weighing paper; place weigh paper in center of aluminum foil.
5. Attach clean diamond bit to Dremel tool marked "Enamel". Holding tooth very close to the center surface of the weigh paper, drill a horizontal line just below the occlusal surface of tooth. Accumulate 10-15 mg of tooth enamel powder on the weigh paper; tilt into labeled microcentrifuge tube. Close tightly.
6. Retare balance to zero. Weigh filled tube and record. Calculate and record weight of enamel in the sample.
7. Use Dremel to drill quartz over same work surface for 30 sec.
8. Dispose of weigh paper; recycle aluminum foil. Wipe down work area with alcohol.
9. Reclean Dremel and bits as described in EQUIPMENT AND SAMPLE STERILIZATION before going on to next tooth.
10. Repeat ENAMEL EXTRACTION steps at desired intervals until entire tooth is drilled. Complete single tooth before moving on to next tooth.

ENAMEL PRETREATMENT FOR APATITE EXTRACTION

This protocol uses pretreatment with bleach (removes organics) and acetic acid (removes adsorbed carbonates). Always run a full procedural blank (empty tube) through this protocol.

COLLAGEN REMOVAL ~ Overnight procedure

Equipment needed: eye protection, lab coat, microcentrifuge tube rack, vortexer, pipetters, microcentrifuge tube rack lid

Disposables needed: gloves, 50% bleach solution (in wash bottle), aluminum foil, pipette tips

1. Wear gloves, lab coat, and eye protection. Place sample tubes in microcentrifuge rack and transport into fume hood in wet lab. Turn fume hood on. Do all work in fume hood.
2. Open each tube and fill with ~1.5 ml of 50% bleach solution to remove collagen. Close tube before moving to next sample.
3. Vortex for 3 sec. Open tubes and let them stand overnight in fume hood, covered loosely with a sheet of aluminum foil.

CARBONATE REMOVAL ~ 9 hrs, + overnight

Equipment needed: eye protection, lab coat, microcentrifuge tube rack, vortexer, pipetters, microcentrifuge, vacuum desiccator, microcentrifuge tube rack lid, ultrasonicator, freezer

Disposables needed: gloves, DI water (in bottle), pipette tips, 0.1 M acetic acid

1. Close tubes. Vortex, then place in microcentrifuge for 5 mins at 5000 rpm for (with tube hinge pointing down); decant bleach. If the sample does not readily vortex, place tube in ultrasonicator and sonicate for 10 sec, then vortex for 3 sec.
 2. Centrifuge 5 mins at 5000 rpm and decant; add distilled water to fill. Rinse samples 4 times total.*
 3. Add 0.1 M Acetic acid (0.1 ml per 1 mg of original sample weight); vortex briefly. Let tube stand open exactly 4 hrs.
 4. Vortex, then place tubes in vacuum desiccator and slowly evacuate air until samples achieve a low boil for 5 mins.
 5. Return to atmospheric pressure, and repeat evacuation and repressurization twice.
 6. Depressurize, then wait 4 hours.
 7. Centrifuge tubes for 5 minutes and decant acetic acid. Rinse samples 4 times total.* Decant and pipette out last bit of liquid, recentrifuging as needed to keep sample solid. Leave tubes open.
 8. Place open tubes in a microcentrifuge tube box in the freezer for 30 mins. Open the vacuum desiccator before taking samples from freezer.
 9. Place open tubes immediately in the desiccator. Do not allow samples to melt first. Samples dry in ~ 12-15 hrs.
- *To rinse samples: Fill tube with DI water. Vortex for 5 sec. Centrifuge for 2 min at 2500 rpm. Remove supernatant with pipette and discard.

APATITE YIELDS ~ 30 mins

Equipment needed: eye protection, lab coat, analytical pan balance, notebook, computer with Excel

Disposables needed: gloves

1. Close dried centrifuge tubes. Reweigh tubes, which now contain only apatite. Record weights into lab notebook. Then use spreadsheet to calculate apatite yield from wet chemistry. Enter calculated data in lab notebook.
2. Samples are ready to be taken to TIMS clean lab for strontium extraction.

1 M Acetic acid recipe: 1000 ml H₂O + 57.2 ml acetic acid.

0.1 M Acetic acid recipe: 900 ml H₂O + 100 ml 1.0 M acetic acid.

Revision History:

Created by S. Ambrose: 11/06

Revised by John M. Marston, E. Baxter, J. Bethard, D. Honn: 9/13

Standardized by K. Wade, Boston University: 1/17/18

References:

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2002. The Seasonal Mobility Model for Prehistoric Herders in the South-western Cape of South Africa Assessed by Isotopic Analysis of Sheep Tooth Enamel. *Journal of Archaeological Science* 29(9): 917–932.