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Dental Calculus Field-Sampling Protocol (Warinner Version)

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ABSTRACT

This protocol describes how to sample dental calculus from individual teeth for biomolecular analysis. The primary use-case is for DNA and proteomic analysis. For a printable handout, see:

<http://christinawarinner.com/resources/archaeologists/>. Note, if you are sampling from intact jaws, we recommend using the Sabin protocol: <https://dx.doi.org/10.17504/protocols.io.bqecmtaw>

EXTERNAL LINK

<http://christinawarinner.com/resources/archaeologists/>

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Velsko, I.M. & Warinner, C., 2017. Bioarchaeology of the Human Microbiome. *Bioarchaeology International*, 1(1–2), pp.86–99. Available at: <http://journals.upress.ufl.edu/bioarchaeology/article/view/169>.

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KEYWORDS

ancient DNA, dental calculus, sampling, archaeogenetics, archaeology, biomolecular archaeology, skeleton, proteomics

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GUIDELINES

All reagent manufacturers are optional. You can replace with manufacturers of your choice. Sterility is the most important factor here to reduce contamination.

Gloves should be nitrile and powder free. Latex gloves are not compatible with proteomic or archaeobotanical analyses.

MATERIALS TEXT

MATERIALS

☒ Microcentrifuge tubes (1.5 or 2 ml, screw-cap or safe-lock) **Contributed by users**

☒ Disposable gloves, nitrile **Contributed by users**

☒ Aluminum Foil **Contributed by users**

Essential

- 2ml Eppendorf(-like) Tube Rack Block
- Alcohol wipes or household Bleach (1:10)
- Lab-grade water (HPLC) (*note*: only if using bleach)
- Paper towels (*note*: only if using bleach)
- Dental scalar
- Sharpie (Tube/Bag labelling)
- Biro Pen (Metadata sheet writing)
- Metadata sheet (if not pre-supplied: should have Sampling ID; Collection ID; Collection Storage ID (Optional); Tooth FDI Code; Surface; Pathologies?; Consolidants?; Other Notes)
- Camera

Optional

- Copy of picture-based short-version sampling protocol (<http://christinawarinner.com/resources/archaeologists/>)
- Sample Bags
- Tooth ID diagram (with FDI codes)



Example set up (some objects not shown)

SAFETY WARNINGS

- Bleach can be hazardous to eyes. Ensure a source of clean water/eye wash is available.
- Bleach can be hazardous to skin. Ensure a source of clean water/eye wash is available.
- Point of dental scalar can be sharp.
- Tough to remove calculus deposits can suddenly flick off at high speed. Careful of getting it into eyes.

ABSTRACT

This protocol describes how to sample dental calculus from individual teeth for biomolecular analysis. The primary use-case is for DNA and proteomic analysis. For a printable handout, see:

<http://christinawarinner.com/resources/archaeologists/>. Note, if you are sampling from intact jaws, we recommend using the Sabin protocol: <https://dx.doi.org/10.17504/protocols.io.bqecmtaw>

BEFORE STARTING

Decide on a sampling approach e.g. whether you will pool calculus from multiple teeth or surfaces (increasing yield, but loss of bio-geographic information)

Make sure you have a bin available. You will produce a lot of waste to ensure minimal contamination.

Workstation Preparation

- 1 Put on two pairs of gloves

Replacement The outer layer will be replace after each sampling.

- 2 Wrap your tube rack in foil.

- 3 

Sterilise all utensils with by wiping with alcohol wipes or paper towels with some bleach.

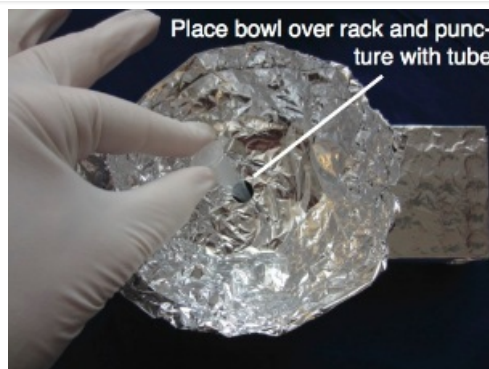
Bleach If you use bleach, ensure that you wipe down the metal utensils with (preferably HPLC) water afterwards to remove residual bleach and prevent corrosion.

Sampling Preparation

- 4 Label tube ready for sampling.
- 5 Fill in your metadata sheet with corresponding tubes IDs.
- 6 Label all bags for placing corresponding tubes in.
- 7 Construct a foil bowl as in the picture. The purpose of the bowl is to catch any calculus that fragments unpredictably or "jumps" due to static electricity.

Specifications Ensure the foil bowl is wide enough to insert the sample, your hands and the dental scalar so that you can comfortably orient everything without knocking the bowl, and see what you are doing.

Ensure the foil bowl has a smooth internal surface and so that the calculus will fall directly into the tube.



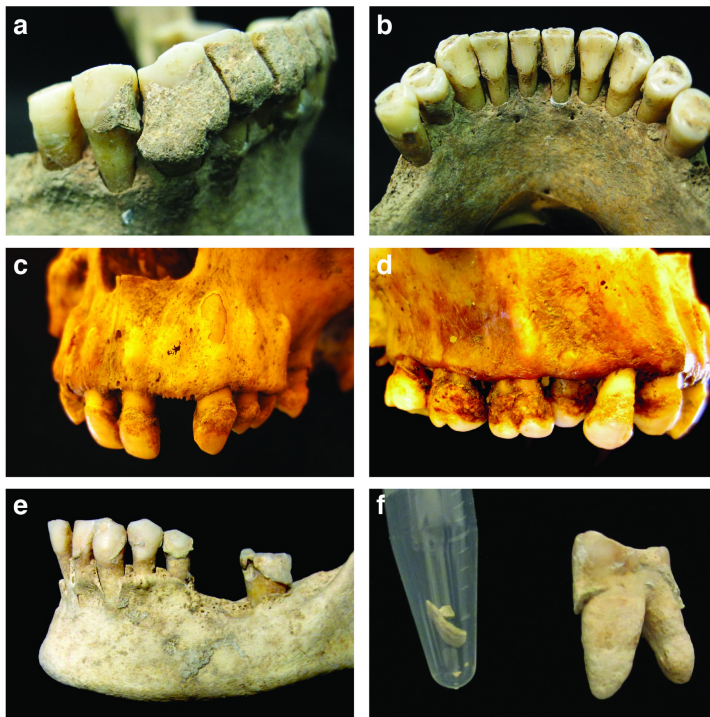
- 8 Place the foil bowl on the tube rack, and press tube through the bottom of the bowl and through the foil of the tube rack. The fit between the tube and the foil should be snug.
- 9 Replace top layer of your gloves.

- 10 Grasp tooth root and locate the dental calculus. Surface dirt can be removed by gently brushing with a dental scalar, away from the bowl or tube.



Example of large calculus deposit

Identification Dental calculus deposits can vary a lot in size and morphology. Large deposits should be relatively clear to identify. Smaller deposits will often have a rough surface, and form a ridge that would follow the gum line. Very small deposits may also be patch round shapes.



Originally published in: Warinner, C. (2016). Dental Calculus and the Evolution of the Human Oral Microbiome. *Journal - California Dental Association*, 44 (7), 411–420. <https://www.ncbi.nlm.nih.gov/pubmed/27514153>



Avoid touching the calculus with anything other than a dental scaler as much as possible, to reduce the risk of contamination.



Check that there are no fractures or other indications of a weakened structural integrity of the tooth. If this is identified, please consult with the collection curator or manager whether they are comfortable in proceeding with sampling. In some cases, the calculus may be what is holding the tooth together, and removing it may result in the fracturing of the tooth.

- 11 Write on a piece of paper the name of the sample and/or label on the tube.

- 12 

Place the tooth on or next to the labelled paper, and take photographs of the tooth as a whole, including a close up of the calculus at different angles. Ensure the sample label is clearly visible.

Measurements If possible, include a scale for measurement.

- 13 Record the location of the selected calculus deposit on your metadata sheet (e.g. lingual, buccal surface, etc). Also add other information such as whether there is evidence of disease or consolidants.

Sampling Procedure

- 14 Hold tooth by the root, with the crown orientated so the calculus will fall directly down into the bowl. Ensure the tooth is positioned in the centre of the bowl, and slightly below the rim.



Example of how to hold tooth, and positioning of scaler

- 15 

Take the sterile dental scaler. Using the broad flat edge of the scaler, apply downward pressure to the calculus deposit. Ideally, this will result in the entire calculus deposit breaking off cleanly and falling into the tube. In some cases, however, the calculus is more difficult to remove. If difficulty is encountered, the scaler can be used to scrape against the calculus deposit (try to avoid touching the enamel) along the surface of the tooth in a downward motion towards the bowl, so that the calculus falls directly into the tube.

Powdery If the calculus deposit comes off in a powdery form (rather than in fragments), try to go deeper into the bowl and scrape directly into the tube (rather than allowing it to fall). This will minimize loss due to static and

airflow, as well as reduce contamination of your workstation.

Force Each dental calculus deposit has a different adhesion to the tooth. Try gentle pressure at first and increase if necessary. Don't be surprised if it suddenly pops off. The bowl should catch this if it fragments suddenly in an unanticipated direction.



Be sure to use a dental scalar and not a dental pick. It is difficult to control calculus sampling with a dental pick.



Avoid scratching or damaging the occlusal surface of the crown. Damage to this surface will confound future microwear analysis.

15.1 If your calculus has not fallen directly into the bowl, use sterile forceps/tweezers to move the calculus deposit(s) directly into the tube.

16 Close the cap of the tube. Place tube in pre-labelled sample bag. Remove and discard foil bowl apparatus.

Post-Sampling Procedure

17 

Photograph the tooth again, focusing on the sampling site. Again ensure the sample ID is visible in the picture. Return tooth to its sample bag.

18 

Record any other observations from sampling, such as an approximate weight of the calculus sample.

19 

Sterilise all utensils used by wiping with alcohol wipe or bleach on a paper towel.

Bleach If you use bleach, ensure that you wipe down the metal utensils with (preferably HPLC) water afterwards to remove residual bleach and prevent corrosion.

20 Replace gloves and repeat sampling preparation ↻