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## Dental Calculus Field-Sampling Protocol (Sabin version)

Susanna Sabin<sup>1</sup>, James A. Fellows Yates<sup>1</sup>

<sup>1</sup>Max Planck Institute for the Science of Human History

 Works for me

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 WarinnerGroup

 Zandra Fagernäs 

### ABSTRACT

This protocol describes how to sample dental calculus from skeletal remains for biomolecular analysis. This protocol is particularly recommended for sampling calculus from teeth attached to a jaw bone, skull, or skeleton. The primary use-case is for DNA and proteomic analysis.

### EXTERNAL LINK

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

### 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

NAME	CATALOG #	VENDOR
Microcentrifuge tubes (1.5 or 2 ml, screw-cap or safe-lock)		
Disposable gloves, nitrile		
Aluminum Foil		
Weigh paper	<a href="#">View</a>	Carl Roth

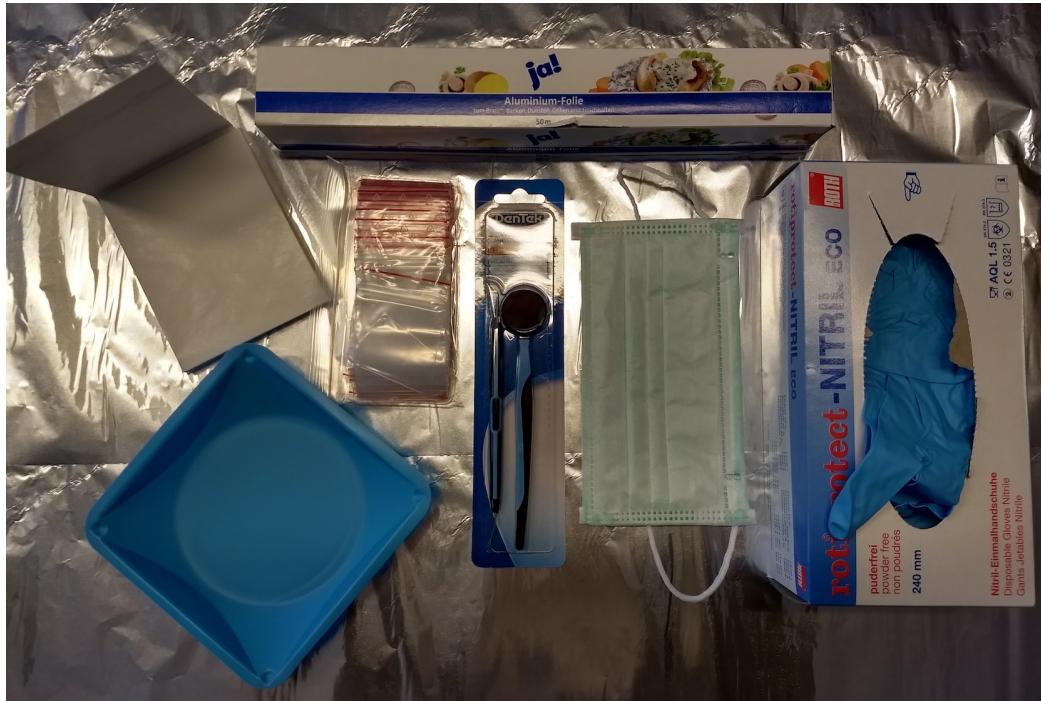
### MATERIALS TEXT

#### Essential

- Alcohol wipes or household Bleach (1:10 dilution)
- 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
- Weigh boats
- Personal protective gear to limit contamination risk (e.g. face mask)
- Tooth ID diagram (with FDI codes)



Example materials (some materials 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.

#### BEFORE STARTING

Decide on a sampling approach e.g. will you 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 replaced after each sampling.

- 2 Cover work surface with two layers of aluminum foil.



**Replacement** The top layer of foil must be replaced after coming into contact with sampling debris. Alternatively, the top layer of foil can be replaced after each sampling.

- 3 Sterilise all utensiles with by wiping with alcohol wipes OR paper towels with some bleach (1:10 dilution with HPLC water).



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

- 4 Replace top layer of your gloves.

#### Sampling Preparation

- 5

Label tube ready for sampling.



**Tubes** Tubes should be closed at all times following removal from packaging before and after depositing the calculus sample. This will limit contamination.

- 6 Fill in your metadata sheet with corresponding tubes IDs.

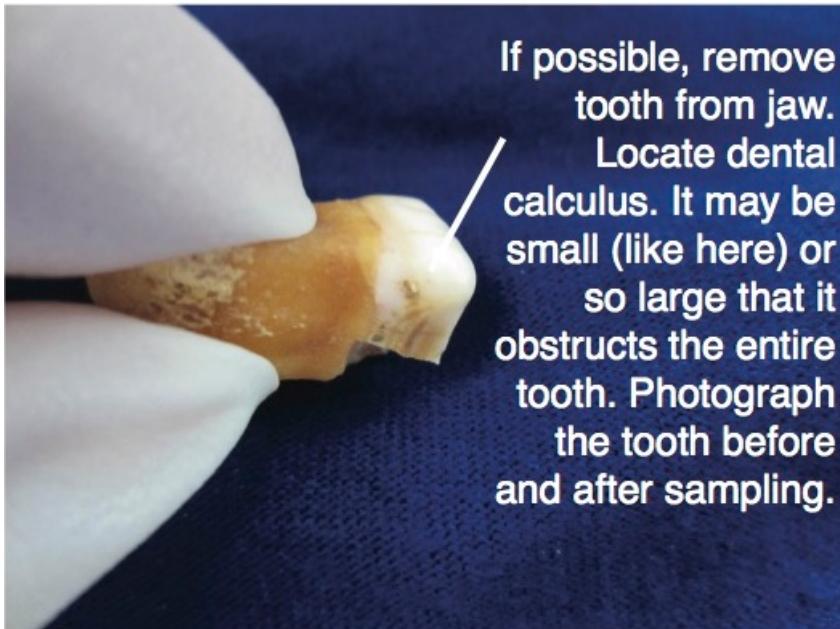
- 7 Label all bags for placing corresponding tubes in.

- 8 Lay out fresh sheet of weighing paper. Create a gentle crease in the weigh paper to ease specimen collection and handling.



**Recommendation** Place the weighing paper inside a weighing boat for better sample containment during the sampling process, or create a foil barrier surrounding the weighing paper for the same purpose.

- 9 If possible, remove the tooth from the jaw. Locate the dental calculus.



Example of small deposit of calculus



**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.



Avoid touching the calculus 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.

- 10 Write on a piece of paper the name of the sample and/or label on the tube. Place the label next to the weighing paper.

- 11 Place the tooth on the weighing paper, and take photographs of the tooth as a whole. If the tooth is still attached to a larger specimen, take photographs of the tooth from as many angles as possible. Include close ups of the calculus at different angles. Ensure the sample label is clearly visible.



**Measurements** If possible, include a scale for measurement.

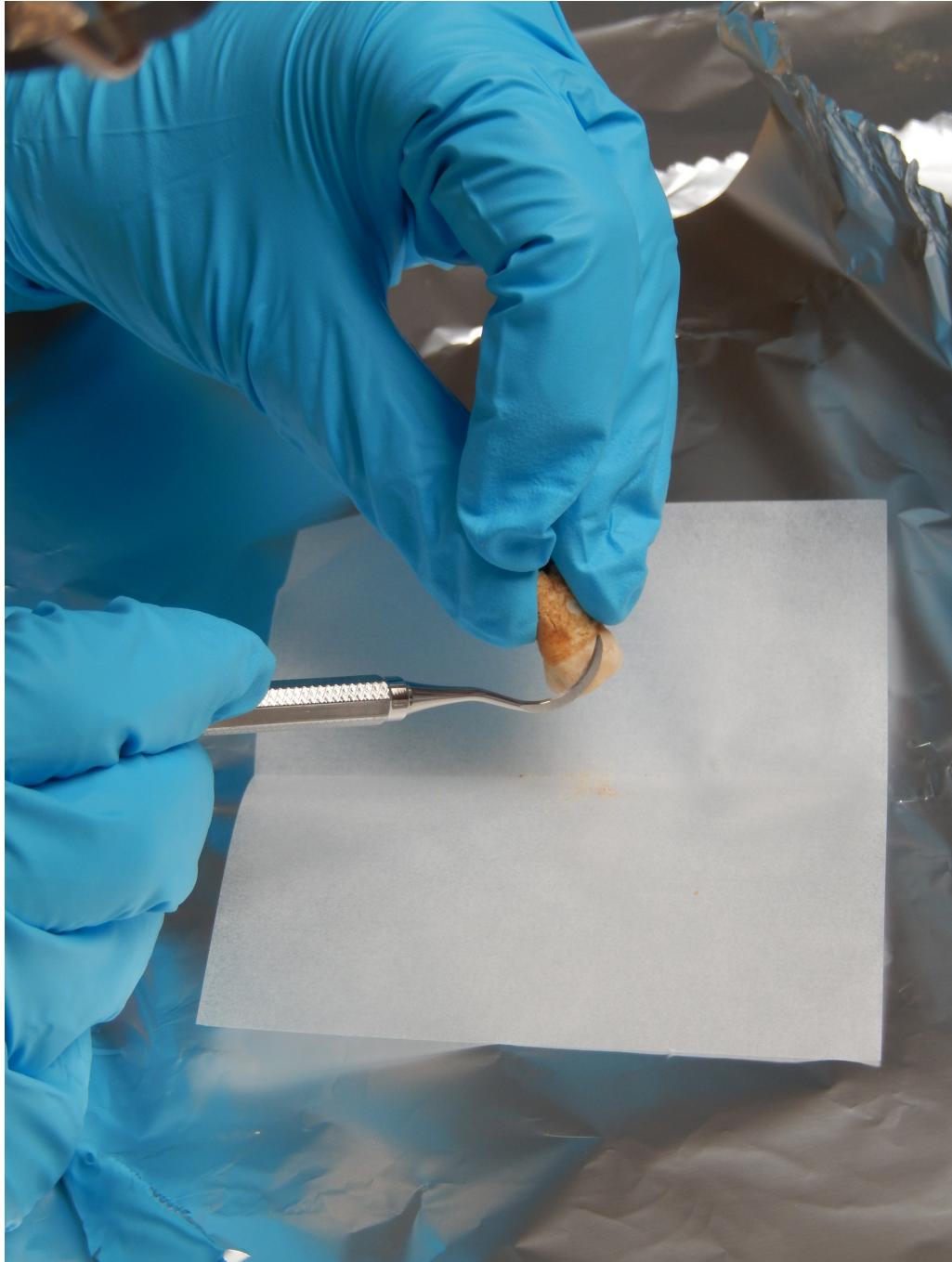
- 12 Record the location of the selected calculus deposit on your metadata sheet (e.g. lingual, buccal surface etc). Also add other information such as whether there is evidence of disease or consolidants.

#### Sampling Procedure

- 13 Position the specimen such that you can scrape the calculus from the tooth with a downward motion towards the fold in the weighing paper.
- 14 Take the sterile dental scalar. Scrape the broad flat edge against the calculus deposit only, **without** touching the enamel, along the surface of the tooth in a downward motion towards the fold in the weigh paper.



Example: Calculus sampling from molars embedded in mandible. Creased weighing paper with aluminum foil barrier.



Example: Calculus sampling from single tooth into creased weighing paper.



Example: Calculus sampling from tooth as part of articulated museum specimen over a creased weighing paper and weighing boat.



**Powdery** If the calculus deposit is coming off in a powdery form, try to go deeper into the bowl and scrape directly into the crease of the weighing paper. This will minimise loss from static and airflow, as well as reduce contamination of your workstation.



**Force** Each deposit of calculus have different adhesion to the tooth. Try gentle pressure at first and increase if necessary. Don't be surprised if it suddenly pops off. The foil or weighing boat should catch this.



Do not use the pick of the scalar, as you may scratch or damage the enamel surface. This will confound microwear and other scientific analysis.

- 14.1 Gently tap the collected calculus from the weighing paper into the labelled tube, using the crease as a guide.
- 14.2 If some calculus has not fallen directly onto the weighing paper, use sterile forceps/tweezers to place the calculus deposit(s) directly in the tube

15 Close the cap of the tube securely. Place tube in pre-labelled sample bag.

#### Post-Sampling Procedure

16 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 to prevent corrosion.

17 Replace outer layer of gloves.

18 Photograph the tooth again, focusing on the sampling site. Again ensure the sample ID is visible in the picture.

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

20 Return tooth to appropriate storage. Discard the weighing paper and weighing boat (if used). If necessary or desired, discard the top layer of foil from the work surface and lay out a fresh sheet of foil for continued sampling.

#### Sampling Preparation

21 Replace gloves and repeat sampling preparation [go to step #5](#)

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