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## **Protocol for sectioning human dentine: expanded from Methods 1 and 2 (Beaumont *et al.* 2013)**

**Select your tooth:** take into account the developmental ages of the tooth, the incisal/occlusal wear and whether the tooth is still forming (pay close attention to the apex, is it closed completely?). Record the state of the tooth: e.g. is the enamel perfect, cracked, eroded? Is the root surface intact, eroded? Is the tooth friable? If caries is present, what surfaces are affected and is the pulp chamber involved? Before any preparation, ensure that the tooth is recorded by photograph: mesial, distal, buccal, lingual/palatal, occlusal and tips of roots, with a specimen label and scale. Avoid sampling any carious tissue.

### **1) Preparing the root:**

Using a hand-held diamond saw in a dental handpiece, take the selected tooth and section longitudinally from crown to root apex:

- 1) single-rooted incisor or canine tooth: section longitudinally to produce a mesial and distal section.
- 2) Single-rooted premolar tooth: section longitudinally to produce a buccal and lingual/palatal section.
- 3) Two rooted premolar: section the tooth to remove the selected root.
- 4) Molar tooth: section longitudinally to remove the longest root (mesial on a lower molar, palatal on an upper).

Remove as much of the enamel as possible from the resulting section, without breaching the enamel/dentine junction (EDJ). This can be achieved using the diamond saw to “shave” the enamel. Retain enamel and calculus for other/future analyses.

### **2. Cleaning the root:**

If air abrasion is available, use this to remove surface contamination and the cementum.

If air abrasion is not available, use a large rose-head dental bur in a hand-held dental handpiece to gently abrade the cementum from the root surface.

### **3. Sectioning the root.**

For a single analysis for each section, go to Method 1 or 2

If each section is to be divided for duplicate analysis (e.g. isotopes of carbon and nitrogen and also phosphate oxygen) Method 1 is recommended.

#### **3.1. Method 1: Demineralising small sections**

##### **3.1.1. Embedding the root in plaster**

This method is advised where the dentine is of poor quality, very abraded or crumbly: it is also to be used when each section will be measured not only for carbon and nitrogen, but also for additional isotopes.

- 1) Prepare a mould using firm-bodied dental impression putty, by taking an impression of an Isomet blank and leaving the upper surface uncovered. Allow this to set (the ideal shape is 1cm x 1cm x 10cm). (Figure 1)
- 2) Prepare 6cm x 1cm strips of Parafilm
- 3) Prepare microtubes for each section with duplicate labels
- 4) Prepare a mixture of 50% plaster and 50% dental stone to embed the root(s): using a dental alginate mixing bowl and spatula, take equal quantities of dental stone and dental plaster, and mix using de-ionised water to achieve a “dropping” consistency which can be poured into the mould
- 5) Dip the root into the plaster/stone mix. Using the Parafilm strip, suspend the root in the mould with the root apex (the tip of the root, furthest from the crown) as near as possible to the end of the mould, with space around the tooth for an even volume of the plaster/stone mix.
- 6) Pour the plaster/stone mix into the mould. Depending on the size of the root, it may be possible to embed two roots in the same mould but ensure the moulds are labelled and the position of each is recorded.
- 7) Agitate the mould by tapping gently to allow any bubbles in the plaster to rise to the surface.
- 8) Place the filled mould on an absorbent surface such as a paper towel to dry overnight.
- 9) The next day, gently remove the plaster block from the mould and label the dry block with the specimen number(s). (Figure 2)



Figure 1 creating a mould using dental impression material

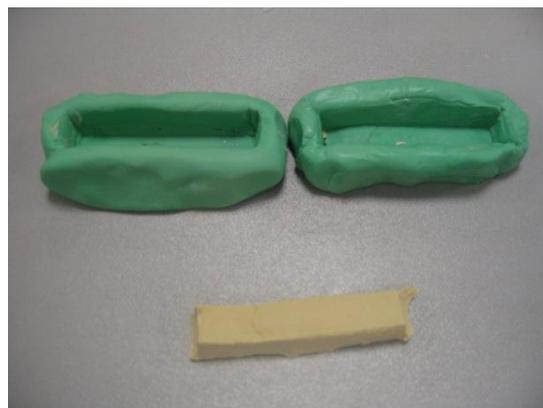


Figure 2 plaster block removed from mould

### 3.1.2 Sectioning using a Buehler Isomet.

- 1) Prepare the Isomet with the finest (thinnest) diamond blade available, and with de-ionised water in the trough.
- 2) Position the plaster block in the Isomet with the root end towards the blade, and wind the micrometer so that the plaster block is touching the blade at zero on the micrometer.
- 3) Using the setting on the Isomet, ensure that when the saw has ALMOST passed through the block, the motor will be switched off.
- 4) Remove a small section of the plaster using the saw to reveal the tip of the root.
- 5) Set the micrometer on the Isomet to the required section size (in mm) and then position the block: start the blade at the required speed to section the block/root. (Figure 3)
- 6) Observe the block during sectioning to ensure that there is no slippage of the block, and when the section is almost complete, check that the saw switches off.
- 7) Collect the plaster/root section (Figure 4) and put on an absorbent surface: gently remove the root from the plaster block and place in a microtube. Keep these in order in a rack, but label when the last section has been cut (see 11).
- 8) Repeat from stage 5-stage 7, until there is no dentine left in the plaster block.
- 9) Weigh each dentine section using a micro-balance and record.
- 10) If each section is to be divided, this should be carried out prior to demineralization and the half section not intended for carbon and nitrogen isotope analysis retained in a labelled, dry microtube.
- 11) Ensure that the LAST section of dentine obtained is labelled "1" and each section is then labelled in reverse order. Because the tip of the root is the first to be cut in the sequence this will ensure that section 1 refers to the earliest FORMING dentine, and the tip of the root is the last FORMING dentine.
- 12) Introduce the 0.5M HCl into each microtube to about  $\frac{3}{4}$  of the tube depth. Seal each tube and place in a refrigerator at 4°C.
- 13) Check each section daily until there is no fizzing of the acid and the section is soft and pliable. For such small dentine sections this should take about 5-7 days. Once this has occurred, wash with de-ionized water x 3. The sections can be stored in water at this stage prior to gelatinization
- 14) Proceed to Method 2, step 4.

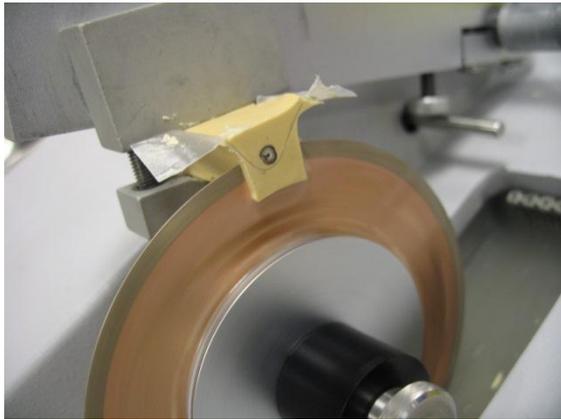


Figure 3 plaster block and root positioned to allow cutting of consecutive measured sections

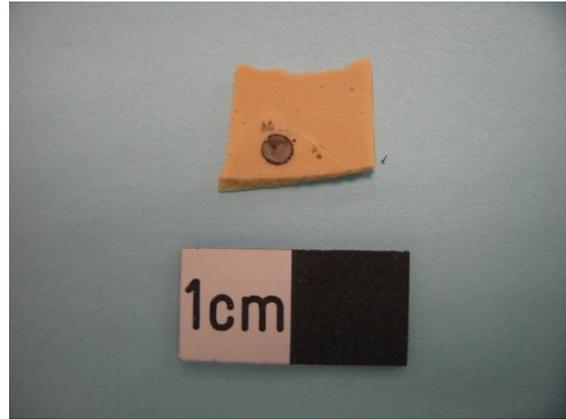


Figure 4 plaster containing embedded, measured root section

### 3.2 Method 2: Demineralizing whole teeth prior to sectioning

This method is recommended when the root is resorbing, developing or is very curved, as it allows the sectioning process to follow the natural root morphology. In addition, any well-preserved root can be sectioned using this method.

Prepare the longitudinal section of tooth as described in 1 and 2 above. After removal of enamel and surface cleaning, weigh the whole tooth section and record.

- 1) Demineralization: place the whole section in a labeled test tube, using 0.5M HCl, in a refrigerator at 4°C. Check the samples every 2 days. Allow the tube and contents to come to room temperature, and then check for bubbles resulting from the action of the acid on the sample, and test the flexibility of the dentine. If the dentine is still hard, but no bubbles can be seen, replace the acid. Well preserved dentine may take 3 weeks or more to demineralize.
- 2) Once demineralized, prepare the tooth for sectioning. Use a metal ruler to measure the dentine, and a sharp scalpel to cut. Estimate the number of sections you will cut from the length of the tooth from apex to EDJ and the width of the sections (e.g. for a 19mm tooth, you will achieve 19x1mm sections). Prepare labelled microtubes.
- 3) Remove the demineralized tooth from the test tube, and remove excess moisture. Place the tooth on a non-slip surface (such as cardboard or a silicon cutting board) alongside the metal ruler (Figure 5) and cut the required section using the scalpel: because the root will contain a pulp chamber or root canal, it will tend to squash flat as you press, take care that you are cutting across the same portion of root, and that the two sides do not slip against each other (Figure 6). Start from the section nearest to the EDJ and label this as section 1 which refers to the earliest FORMING dentine. Place one section in each tube.
- 4) Gelatinization: to each tube and section, add weak HCl (about pH3) filling about ¾ of the tube.

- 5) Heat the sealed microtubes at  $\sim 70^{\circ}\text{C}$  for 24 hours to produce soluble collagen.
- 6) Centrifuge the microtubes so any remaining debris is at the tip of the tube.
- 7) Transfer the microtubes to a freezer at  $-35^{\circ}\text{C}$  for at least 3 hours.
- 8) Transfer the microtubes to a freeze-dryer, open the lids, and leave for 24 hours.
- 9) Remove the microtubes containing the collagen from the freeze-dryer, close the lids and transfer to weighing room to weigh and record the collagen yield for each section (method 1) or a calculated combined yield for the entire root (method 2)



Figure 5 measure the demineralised root against a metal ruler

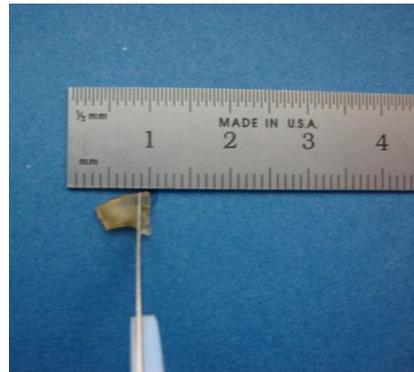


Figure 6 cut measured sections using a sharp scalpel on a non-slip surface

#### 4 Measurement by IRMS.

Depending on the instrument used, small samples of collagen can be weighed in duplicate into tin capsules. If insufficient sample is present from any section, it may be combined with an adjacent section.

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