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Human Remains Examination

1 Purpose

- 1.1 For the examination of blood, bone marrow, oral swab, and tissue, please proceed to Section 2
- 1.2 For the examination of bone samples, please proceed to Section 3
- 1.3 For the examination of products of conception, please proceed to Section 7

2 Sample Preparation – Liquid/dry blood, bone marrow, oral swab and tissue sample

- 2.1 Stained substrates and oral swabs should be cut into small pieces (3 x 3 mm).
- 2.2 Tissues should be minced into small pieces in a weigh boat using a sterile scalpel or razor blade.
- 2.3 Place samples in 1.5mL microcentrifuge tubes or conical tubes when appropriate.
 - 2.3.1 See table below for various sample types:

Sample type	Amount
Liquid blood	100 to 500 µL
Bone marrow	0.5 x 0.5 cm to 1.5 x 1.5cm
Oral swab	1/3 to a whole swab
Blood stain	0.5 x 0.5 cm to 1.5 x 1.5cm
Soft tissue	0.5 x 0.5 cm to 1.5 x 1.5cm
Paraffin embedded tissue	0.3 x 0.3 cm to 1.0 x 1.0 cm

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3 Sample Preparation – Bone

3.1 Cleaning

3.1.1 Before extraction, a bone or tooth specimen should be cleaned entirely of soft tissue and dirt using a range of methods, such as scraping (cut glove required), rinsing and sonication. A combination of sterile scalpels, sterile toothbrushes and running water should be used to clean the specimen.

3.1.2 Rinse bone with water and place in a labeled weigh boat with lint free wipes. Cover with additional lint free wipes and another weigh boat. Seal the weigh boat and place in the 56°C incubator for a minimum of 3 hours (until completely dry). Original container should be discarded.

NOTE: Since the original container is discarded, ensure the FB#, ME#, PM#, and description of the item is noted in the comments.

3.1.3 In comments section of exam sheet, record that cleaning was performed along with initials and date.

NOTE: If necessary, photograph bone or tooth sample after cleaning.

NOTE: If several bones are available, generally compact bone is preferred, such as humerus, femur, or tibia.

3.2 Consumption guidelines

3.2.1 Some bones will be consumed due to weight.

3.2.2 **For bones up to ~1.0g:** Bones will be consumed and must be documented under “*comments*” on exam sheet.

3.2.3 **For bones ~1.0g to 1.50g:** Consumption will be determined by the nature of the bone and whether significant weight will be lost during the processing steps. If the nature of the bone will make the weight drop below the availability to be re-tested (at least 0.50g) then the bone should be consumed and noted in “*comments*” of exam sheet.

NOTE: Factors to consider: spongy, brittle, non-compact bone or where embedded cement and dirt-like material are contributing a portion of the overall weight.

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4 Cutting/ Sonication - Bone

****WARNING****

Protective eyewear, lab coats, cut resistant gloves, sleeve protectors, and HEPA-filtered facial masks should be worn when cutting bone. Avoid breathing bone dust. All cutting of bone must be done under a biological hood.

NOTE: Bones that are too small to be cut should proceed to the sonication step.

- 4.1 Prior to sampling, document on exam sheet: *measurement of the item (inches), appearance, and gross weight (g)*. Initial and date that examination/cutting was performed.
- 4.2 Prepare Tergazyme solution: fill a 50mL conical tube with 3g of Tergazyme powder and fill to the 50mL mark with dH₂O. Suspend the powder with inversion and transfer to Erlenmeyer flask with stir bar. Place on heat/stir plate. Solution is ready for use when reagent has completely dissolved and solution is clear. NOTE: Make sure to keep the temperature under 55°C to prevent inactivation of Tergazyme.

NOTE: Terg-a-zyme is an enzyme-active powdered detergent. A 5% solution should be made fresh prior to bone preparation and cleaning. Refer to Appendix A in the Quality Assurance Manual. Once prepared, the reagent will only be effective for up to 16 hours.

- 4.3 Using an autopsy saw or a Dremel tool equipped with an EZ456B EZ Lock™ or EZ409 Thin-Cut 1-1/2 Cut-off wheel, cut the bone specimen into approximately 5x5x5mm size pieces. Take enough cuttings for an end weight of approximately 2g to 3g. For older or compromised bones, several aliquots of 2g can be extracted and combined during the Microcon step.
 - 4.3.1 For tooth samples, the whole root should be taken.
 - 4.3.2 For Large Volume Demineralization Extraction, cut the bone specimen into approximately ¼ inch square pieces. Take enough cuttings for an end weight of approximately 0.65g to 0.80g.

NOTE: The cutting wheel should be disposed of after each use and the Dremel and hood should be completely wiped down with bleach and ethanol.

NOTE: 0.50g of dust is optimal for large volume extraction procedure. Due to the nature of each bone, a larger portion may need to be cut to account for loss during the sonication and milling procedures (ex: spongy or brittle bone, non-compact bone and/or bone containing dirt/cement-like material). Bones that do not have enough volume for more than one extraction should be consumed, even if the total bone weight is over the 0.50g recommended for cutting.

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- 4.4 Place the bone pieces in a **new, labeled** 50mL conical tube. Label new conical tube with FB case number, PM item# and sample type.
- 4.5 Cover bone cuttings with 5% Tergazyme solution. Place labeled 50mL conical tubes into a tube rack. Secure tube to tube rack with tape and put tube rack into the sonicator water bath. Place weighted ring over the top of the rack to submerge and sonicate for 30-45 minutes. **Ensure water level in the sonicator is 1-2 inches from the top.**
- 4.6 Bones that are not being consumed should be placed in a new, labeled specimen cup. Label specimen cup with FB case number, ME#, PM item #, sample type.
- 4.7 Once sonication is complete, place a paper towel over the drain of a clean sink and decant the Tergazyme solution. Add water to the tube and gently shake. Decant water from the tube and repeat until the water runs clear and the Tergazyme solution is removed.

NOTE: Some bone pieces may need to go through the sonication process twice. Repeat only when necessary. Bleach out sink when finished.

- 4.8 Place the clean cuttings in a weigh boat on a few small lint free wipes. Cover with additional lint free wipes and another weigh boat. Label the weigh boat with the FB case number, PM item# and sample type. Seal weigh boats with evidence tape.
- 4.9 Dry in a 56°C incubator for a few hours or overnight. After sufficient drying, weigh bone cuttings. **The bone sample must be completely dry before milling.**

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5 Milling - Bone

Sample milling with the SPEX Certiprep 6870 Freezer Mill

NOTE: All freezer mill parts that come into contact with bone specimens, such as the cylinders, metal end plugs and impactors, should be cleaned, dried and sterilized prior to use.

- 5.1 Place forceps, metal bottom, plastic cylinder, impactor, and metal top under UV light for a minimum of 15 minutes.
- 5.2 Assemble specimen vials in the following order: metal bottom, plastic cylinder, impactor, and metal top.
- 5.3 Label metal bottoms with a case identifier using a blue ink Sharpie.
- 5.4 Weigh the dry bone pieces and record weight on exam sheet under “*weight of fragments to be milled (g)*”
- 5.5 Add bone cuttings to specimen vial around impactor using decontaminated forceps. Cover with metal top. The top plug should be placed on to the tube with the rounded side facing out.

NOTE: Shake specimen vial and ensure that the impactor can move back and forth.

- 5.6 Wipe down inside of mill with a labwipe and distilled water. **Do not use bleach or ethanol.**
- 5.7 Plug in mill and switch ON.
- 5.8 Open liquid nitrogen valve on tank and keep the valve open throughout the run, until the run is complete. Be aware that the liquid nitrogen tank may be empty when the detector level reads anywhere from “1/4” to “empty”.

****WARNING****

Liquid Nitrogen can be hazardous. Use cryogenic gloves, protective eyewear/face shield and lab coats when handling. Avoid liquid nitrogen splashes to face and hands.

- 5.9 Open the freezer mill lid.

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5.10 Programming and use of the 6870 freezer mill

5.10.1 Adjust mill settings as follows:

Cycle	set to # of samples + 2	
Time	T1 (milling)	3.0 min
	T2 (pause)	2.0 min
	T3 (pre-cool)	15.0 min
Rate	Bones – 8-10	
	Teeth – 6-8	

- 5.10.2 Place mill tubes into the mill with four in the chamber.
- 5.10.3 Slowly close the mill to avoid splashing.
- 5.10.4 Lock the mill shut and turn on the power switch located in the back left side of the mill.
- 5.10.5 Look over the settings; freezer mill settings should be as outlined in the table from Step 10 above. If the settings need to be changed press the settings button on the screen and make changes.
- 5.10.6 Change cycle number to match total number of samples plus two (N + 2).
- 5.10.7 When mill has been programmed, press the start button. The screen should change and status should change from “Standby” to “Pre-cool”. Allow the mill to run the cycle. You will hear the mill running when on the “run” status. When the sound ends, the cycle is over and the mill needs to be opened and samples removed.
- 5.10.8 Place the next 4 mill tubes in the mill chamber.
- 5.11 Inspect each sample after removal from the mill. If sample is sufficiently pulverized, remove the metal top using the Spex Certi-Prep opening device. **Samples may be reinserted into the mill for additional grinding, if necessary.**
- 5.12 Using decontaminated tweezers, remove impactor from vial and submerge in a 4L Nalgene bucket of 10% bleach.
- 5.13 Transfer the bone dust to a tared and labeled 50mL conical tube (label conical tube with FB case number and sample name). Ensure complete dust transfer by tapping bottom of cylinder. Record the weight of the dust under “*dust weight (g).*” Print out cutting label and affix label to conical tube.

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- 5.14 Place remaining mill parts in the 4L Nalgene bucket of 10% bleach, all parts should be submerged.
- 5.15 Wrap each sample tube in parafilm.
- 5.16 Place tubes of bone dust in designated area for pending extraction.
- 5.17 When finished milling, flip mill switch off and leave mill open for liquid nitrogen to evaporate.

6 Cleaning mill parts:

****NOTE: Mill parts must be cleaned immediately after processing.****

- 6.1 Separate all mill parts and scrub individually with toothbrush using 10% bleach.
- 6.2 Rinse with water and place mill parts in a bucket containing 0.1% SDS.
- 6.3 Brush parts with a new toothbrush in the SDS solution.
- 6.4 Rinse parts with water again and place in a bucket containing 10% bleach.
- 6.5 Rinse all parts with water.
- 6.6 Separate the plastic cylinders from the metal parts.
- 6.7 Rinse metal parts in 200 proof ethanol. **DO NOT** rinse the plastic cylinder in ethanol as it will cause the plastic cylinder to degrade.
- 6.8 Expose all the parts to UV light for a minimum of 2 hours-overnight. The UV light in a biological hood or a StrataLinker can be used. All parts exposed to bone dust need to be placed face up towards the UV light. The mill tubes need to be standing up.

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7 Sample Preparation – Products of Conception

- 7.1 The term *product of conception (POC)* refers to either an embryo (up to the formation of organs in the first 8 weeks of gestation) or a fetus (up to approximately 30 millimeters and weighs approximately 4 grams).
- 7.2 The *placenta* is a temporary organ of pregnancy. Anatomically, the placenta has two parts: decidua (D), genetically identical to the mother, and chorionic villi (CV), genetically identical to the POC. Decidua appears as a compact tissue, while chorionic villi look more incoherent and loose. Morphological differentiation between D and CV can be made by observation:

7.2.1 By naked eye (Figure 1a and 1b):



Figure 1a: CV by naked eye Figure 1b: CV by naked eye - detail

7.2.2

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7.2.3 Using stereo-microscopy (Figure 2a and 2b):



Figure 2a:
Stereo-microscopic (MIDEO) image of
chorionic villi.



Figure 2b:
Stereo-microscopic (MIDEO) image of
Decidua.

7.2.4 Using light microscopy of formalin fixed, paraffin embedded, and stained tissue (Figure 3a and 3b):

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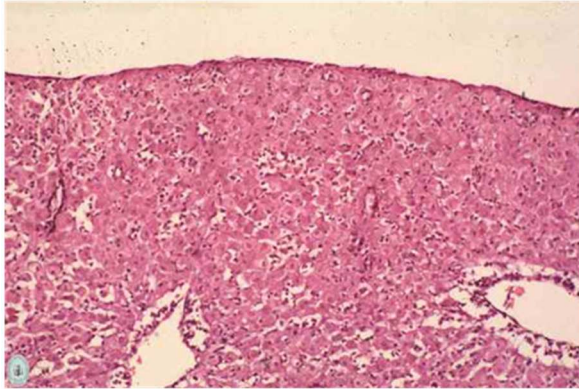


Figure 3a:
Microscopic image of formalin fixed,
paraffin embedded and routinely stained
decidua

7.3



Figure 3b:
Microscopic image of formalin fixed,
paraffin embedded and routinely stained
chorionic villi

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- 7.4 It is possible for tissues of POCs to lack uniformity, be of different gestational ages, or be differently preserved. Therefore, besides general guidelines for evidence examination, examination of POCs requires that some specific scenarios be taken into consideration.
- 7.5 Follow the Note taking – general guidelines and Evidence Examination general guidelines in the [Evidence Examination](#) manual when examining POC. Use a Product of Conception (POC) Packaging and Exam Worksheet for initial documentation of each POC item.
- 7.5.1 Describe the general condition of the item (full embryo/fetus, fragments, unrecognizable tissue parts, etc.).
- 7.5.2 Take one overview photograph of each item. Each photograph **must** have a ruler visible in the frame, either a plain straight ruler or an x, y axis ruler.
- 7.5.3 Weigh each item and document the tissue weight.
- 7.5.4 Determine if the POC is more or less than 24 weeks of gestational age (weight of \geq 500g is considered $>$ 24 weeks of gestational age).
- 7.5.5 Sampling of the item depends on the general condition of the item.
- 7.5.6 If the POC is **morphologically well defined**, take a sample from it for DNA typing; the sample should be approximately 3x3x3 mm in size.
- 7.5.7 If the POC is $<$ 24 weeks of gestational age and/or it is **not morphologically well defined**, rinse it several times in dH₂O using Petri dish and observe it wet under a stereo microscope.
- 7.5.8 Referring to Figure 2a and 2b for guidance, take a chorionic villi sample for DNA typing; the sample should be approximately 3x3x3 mm in size. If an exemplar from the mother/victim is not available, take a decidua sample as well.
- 7.5.9 If the POC is $>$ 24 weeks of gestational age, retain a sample for further testing. Inform OCME Identification Unit and keep the POC in a freezer, properly packed, until a permit for city burial is obtained by OCME Identification Unit. Return the empty packaging to the OCME Evidence Unit.
- 7.5.10 Submit samples for DNA extraction on an **Exemplar** test batch, using the notation “D” for decidual tissue and ACV@ for chorionic villi as appropriate.

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7.5.11 Depending on the outcome of the DNA testing, the disposition of the POC varies:

Testing outcome	Procedure
No mother/victim exemplar, and DNA profile of the POC is female	<ul style="list-style-type: none"> - Retain a sample of POC for further testing; - Dispose the remainder of POC in the red waste trash (If the POC is >24 weeks old, follow step 5d); - Return the empty packaging to the OCME EU
No mother/victim exemplar, and DNA profile of the POC is male	<ul style="list-style-type: none"> - Retain a sample of POC for further testing; - Dispose the remainder of POC in the red waste trash (If the POC is >24 weeks old, follow step 5d); - Return the empty packaging to the OCME EU
No mother/victim exemplar and DNA profile of the POC is a mixture	<ul style="list-style-type: none"> - Repeat testing (See Step 5 above)
There is a mother/victim exemplar and DNA profile of the POC is foreign to the victim (mother), having expected allele sharing	<ul style="list-style-type: none"> - Retain a sample of POC for further testing; - Dispose the remainder of POC in the red waste trash (If the POC is >24 weeks old, follow step 5d); - Return the empty packaging to the OCME EU
There is a mother/victim exemplar and DNA profile of the POC is a deducible mixture	<ul style="list-style-type: none"> - Retain a sample of POC for further testing; - Dispose the remainder of POC in the red waste trash (If the POC is >24 weeks old, follow step 5d); - Return the empty packaging to the OCME EU
There is a mother/victim exemplar and DNA profile of the POC is an undeducible mixture	<ul style="list-style-type: none"> - Repeat testing, following Step 5a or 5b

7.5.12 For the return of empty packaging, bleach each container in which POC have been submitted using 10% bleach prior to return to the Evidence Unit.