

FORENSIC BIOLOGY EVIDENCE AND CASE MANAGEMENT MANUAL

Human Remains Examination		
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Human Remains Examination

1 Purpose

- 1.1 For the examination of blood and tissue samples, please proceed to Section 3
- 1.2 For the examination of bone samples, please proceed to Section 4
- 1.3 For the examination of products of conception, please proceed to Section 8
- 1.4 See Appendix A for Summary of Notes based on Sample Type

2 General Guidelines

- 2.1 See the [Evidence Examination](#) manual for general guidelines.
- 2.2 To prepare samples for DNA extraction, submit the appropriate amount of sample for testing by placing samples in the appropriate tube and selecting a suffix for the sample.
- 2.3 See the chart below for appropriate cutting sizes and suffixes. For other sample suffixes see the Evidence Examination manual.

Case Group – _X should be added to every X-Team Case prior to sample type suffix		
Sample type	Amount	Sample Type Suffix
Blood	• Portion of bloodstain • ~3mm square • ~3µL whole blood • May vary based on state of sample submitted. Consult with supervisor for guidance if needed.	_U for unidentified person cases _B for all other cases

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Tissue	• Portion of tissue sample • ~3mm square • May vary based on state of sample submitted. Consult with supervisor for guidance if needed.	<u>U</u> for unidentified person cases
Bone	• Portion of bone sample • Approximately 2-3g. See section 4 for examination of bone samples • May vary based on state of sample submitted. Consult with supervisor for guidance if needed.	<u>U</u> for unidentified person cases
Product of Conception	• Portion of product of conception • ~3mm square	No suffix

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3 Sample Preparation/Examination – Liquid/dry blood, paraffin and tissue samples

3.1 Liquid blood (blood submitted in vial)

- 3.1.1 See [Bloodstain Preparation from Whole Blood manual](#) if a bloodstain card needs to be prepared for vouchered items.
- 3.1.2 See [Processing of Postmortem Specimen manual](#) if a bloodstain card needs to be prepared for non-vouched items.
- 3.1.3 Create a new sub-item number in LIMS for the stain card.
- 3.1.4 Once a bloodstain card is made, follow guidelines for bloodstain card examination.
- 3.1.5 Liquid blood can also be pipetted into 1.5mL microcentrifuge tube for testing. See chart for appropriate sample size and suffix.
- 3.1.6 Case information (case number, ME#, item#, description of item) should be documented in the case notes.

3.2 Dried blood

- 3.2.1 Attach dried blood to a new stain card or put dried blood flake(s) into a 1.5mL screw cap tube for testing. See chart for appropriate sample size and suffix.
- 3.2.2 See Processing of Postmortem Specimens and Creating a bloodstain card in LIMS manual if a stain card needs to be prepared.
- 3.2.3 Case information (case number, ME#, item#, description of item) should be documented in the case notes.

3.3 Bloodstain card

- 3.3.1 Case information (case number, ME#, item#, description of item) should be documented in the case notes.
- 3.3.2 Cut a portion of the bloodstain for testing. See chart for appropriate sample size and suffix.

3.4 Paraffin embedded samples

- 3.4.1 Using a sterile scalpel, cut/scrape a portion of the submitted specimen from the paraffin wax. Make every effort to remove the paraffin wax from the embedded specimen. See chart for appropriate sample size and suffix.

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- 3.4.2 Case information (case number, ME#, item#, description of item) should be documented in the case notes.

3.5 Fresh tissue samples

- 3.5.1 Using a sterile scalpel, cut a portion of the submitted specimen. Make every effort to not include any fatty tissue. See chart for appropriate sample size and suffix.
- 3.5.2 Case information (case number, ME#, item#, description of item) should be documented in case notes.

4 Sample Preparation – Bone

4.1 Cleaning bone samples

- 4.1.1 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.
- 4.1.2 If there is viable tissue on the bone, a portion of the tissue should be cut for testing. Follow fresh tissue sample guidelines from 3.5. Discuss with supervisor for guidance if needed.
- 4.1.3 After removing all soft tissue and dirt from the bone, rinse the cleaned bone with water and place it in a weigh boat with lint free wipes. Cover the bone with additional lint free wipes and another weigh boat.
- 4.1.4 Seal the weigh boat and label it with the following information from the original container:
- Case number
 - ME#
 - Item#
 - Description of item
 - Task performed (i.e. cleaning)
 - Initial and date cleaning was performed
- 4.1.5 Place the sealed and labeled weigh boat in the 56°C incubator for a minimum of 3 hours or until completely dried. The original container should be discarded.
- 4.1.6 Record the following:
- Original container type (i.e. specimen cup, conical, etc.)
 - Package markings
 - Task performed (i.e. cleaning)

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- Initial and date cleaning performed

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

NOTE: If several bones are available, generally compact bone is preferred. Examples of compact bones include the humerus, femur, and tibia.

4.2 Cleaning teeth samples

- 4.2.1 All teeth should be cleaned with a toothbrush to remove all dirt and grime.
- 4.2.2 Proceed directly to the sonication step on the same day. See step 5.3 for Tergazyme preparation and 5.8 onwards for sonication instructions
- 4.2.3 If there is a filling on the tooth, do not remove it at this time.

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5 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.

5.1 Prior to sampling, document the following:

- measurement of the item (inches),
- appearance (shape, color, bone composition, etc.)
- gross weight (grams).
- Initial and date cutting was performed

5.2 Bones that are too small to be cut should proceed to the sonication step. Other factors to consider include bone composition (spongy, brittle, compact, etc.)

5.2.1 Some bones will be consumed due to weight. Consult with supervisor before proceeding with consumption.

5.2.1.1 For bones up to ~1.0g: bones will be consumed, and consumption must be documented.

5.2.1.2 For bones ~1.0g to <2g: 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 retested, then the bone should be consumed, and consumption must be documented.

5.3 Prepare Tergazyme solution: fill a 50mL conical tube with distilled water up to the 50mL mark. Weigh 3g of Tergazyme powder into a weigh boat and transfer the powder to an Erlenmeyer flask with a stir bar. Pour the distilled water into the Erlenmeyer flask. Place the Erlenmeyer flask on a heat/stir plate. The Tergazyme solution is ready for use when the powder has completely dissolved and solution is clear.

NOTE: Make sure to keep the temperature under 55°C to prevent inactivation of Tergazyme.

NOTE: Tergazyme is an enzyme-active powdered detergent. A 5% solution should be made fresh prior to bone preparation and cleaning. Once prepared, the reagent will only be effective for up to 16 hours.

5.4 Clean bone holding forceps with 10% bleach followed by 70% ethanol.

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- 5.5 Place bone holding forceps under UV light for a minimum of 15 minutes.
- 5.6 Using a Dremel tool and bone holding forceps, cut the bone specimen into approximately 5x5x5mm size pieces. Take enough cuttings for an end weight of approximately 2g to 3g. Due to the nature of each bone, the end weight may need to account for weight loss during the sonication and milling procedure.
- 5.6.1 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.
- 5.7 Place the bone pieces in a new, labeled 50mL conical tube. Label new conical tube with the case number, item# and sample type.
- 5.8 Cover bone cuttings with 5% Tergazyme solution. Wrap conical tubes with parafilm and place labeled 50mL conical tubes into a tube rack. Secure tube to tube rack using weighted rings over the top of the rack as the rack is placed into the sonicator bath.
- 5.8.1 Ensure water level in the sonicator is 1-2 inches from the top.
- 5.9 Sonicate for 30-45 minutes.
- 5.10 Bones that are not being consumed should be placed in a new, labeled specimen cup. Label specimen cup with the case number, ME#, item #, sample type. Seal the specimen cup with evidence tape and initial and date the seal.
- 5.11 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.
- 5.12 Place the clean cuttings in a weigh boat with lint free wipes. Cover the bones with additional lint free wipes and another weigh boat.
- 5.13 Seal the weigh boat and label it with the following information:

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- Case number
- ME#
- Item#
- Description of item
- Task performed (i.e. cutting)
- Initial and date cutting was performed

- 5.14 Dry in a 56°C incubator for a few hours or overnight. The bone sample must be completely dry before milling.
- 5.15 Place bone holding forceps into a bucket of 10% bleach. When cleaning forceps, take them apart and follow the same protocol as cleaning mill parts – See section 6.
- 5.16 Clean cut resistant gloves by soaking and hand washing the gloves in a bucket of 10% bleach (separate from the bucket with the bone holding forceps). Rinse gloves thoroughly with water and allow to dry before next use.

6 Milling

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.

- 6.1 Clean forceps with 10% bleach and 70% ethanol.
- 6.2 Place forceps, metal bottom, plastic cylinder, impactor, and metal top under UV light for a minimum of 15 minutes.
- 6.3 Assemble specimen vials in the following order: metal bottom, plastic cylinder, impactor, and metal top.
- 6.4 Label metal tops and bottoms with a case identifier using a blue ink Sharpie.
- 6.5 Weigh the dry bone pieces and record weight of fragments to be milled (g).
- 6.6 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.

- 6.7 Wipe down inside of mill with a lint free wipe and distilled water. Do not use bleach or ethanol.

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- 6.8 Plug in mill and switch ON. The power switch is located on the back left side of the mill.
- 6.9 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 “¼” 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.

- 6.10 Open the freezer mill lid.
- 6.11 Place the mill tubes into the mill chamber.
- 6.12 Slowly close the mill to avoid splashing of the liquid nitrogen.
- 6.13 Lock the mill shut.
- 6.14 Programming and use of the 6870 freezer mill.
- 6.14.1 Freezer mill settings should be as outlined in the table below. If the settings need to be changed, Press the settings button on the screen and make changes. Cycle number should match the total number of samples plus two (N+2).

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	

- 6.15 When mill has been programmed, press the START button. The status should change from “STANDBY” to “PRE-COOL”. Allow the mill to run the cycles. You will hear the mill running when on the “RUN” status. When the sound ends, the cycle is over. When the cycles are completed, the status will change to “RUN COMPLETED”. Slowly open the lid of the mill and remove the samples.
- 6.16 If additional samples need to be milled, place the new samples in the mill chamber and repeat step 6.11-6.14.
- 6.17 Inspect each sample after removal from the mill. If sample is sufficiently pulverized, remove the metal top from the plastic cylinder. Samples may be reinserted into the mill for additional milling, if necessary.

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- 6.18 Using decontaminated forceps, remove the impactor from the vial and submerge the impactor and forcep in a bucket of 10% bleach.
- 6.19 Transfer the bone dust to a tared and labeled 50mL conical tube (label conical tube with case number and sample name). Ensure complete dust transfer by tapping bottom of cylinder.
- 6.20 Record the weight of the dust (g) and initial and date of milling.
- 6.21 Print out item label and affix label to conical tube containing bone dust.
- 6.22 Place remaining mill parts (plastic cylinder, still attached bottom, and top) in the bucket of 10% bleach. All parts should be submerged.
- 6.23 Wrap each conical tube containing bone dust in parafilm.
- 6.24 Repeat steps 6.16-6.22 for all milled samples.
- 6.25 When finished milling, flip mill switch off, close the liquid nitrogen valve and leave the mill open for liquid nitrogen to evaporate.

7 Cleaning mill parts:

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

- 7.1 Separate all mill parts and scrub individually with toothbrush using 10% bleach.
- 7.2 Rinse with tap water and place mill parts in a bucket containing 0.1% SDS.
- 7.3 Brush parts with a new toothbrush in the SDS solution.
- 7.4 Rinse parts with water again and place in a bucket containing 10% bleach.
- 7.5 Rinse all parts with distilled water.
- 7.6 Separate the plastic cylinders from the metal parts.
- 7.7 Briefly rinse metal parts and plastic cylinders in 200 proof ethanol. **CAUTION: DO NOT** leave mill parts sitting in ethanol as it will cause the plastic cylinder to degrade and the metal parts to rust.
- 7.8 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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8 Sample Preparation – Products of Conception

NOTE: It is possible for tissues of products of conception to lack uniformity, be of different gestational ages, or be differently preserved. Therefore, besides general guidelines for evidence examination, examination of products of conception requires that some specific scenarios be taken into consideration. Discuss with a supervisor for guidance.

NOTE: The product of conception may need to be defrosted a day prior to examination. Follow general guidelines for evidence exam and retain innermost product of conception packaging in the fridge for defrosting, if needed.

- 8.1 Before examination, prepare the biological hood and necessary tools for examination. Make sure the tools and hood are cleaned with 10% bleach and then 70% ethanol.
- 8.2 Turn on the UV with all the necessary tools inside for at least 15 minutes. Turn on the ventilation/fan in the hood for another 15 minutes prior to use.
- 8.3 The product of conception can be received in many ways. Some examples are listed here:
 - 8.3.1 Product of conception received in a **specimen cup/container**. The specimen could be submerged in liquid, with transport medium or blood.
 - 8.3.1.1 Pipette or decant the liquid into a beaker, to be discarded. Make every effort to avoid picking up the product of conception when transferring the liquid to the beaker.
 - 8.3.2 Product of conception received in **paraffin block**
 - 8.3.2.1 Using a sterile scalpel, cut/scrape a portion of the submitted specimen from the paraffin wax. Make every effort to remove the paraffin wax from the embedded specimen.
 - 8.3.3 Product of conception received in **suction aspiration collection jar**
 - 8.3.3.1 Retrieve sample by picking up every piece in the jar with cleaned forceps.
 - 8.3.3.2 If needed, use distilled water to rinse tissue from suction aspiration collection jar for collection.
- 8.4 When examining a product of conception, ensure the following are documented and well described:
 - 8.4.1 General condition of the product of conception – full embryo/fetus, body fragments, tissue, any other pertinent notes
 - 8.4.2 Color of product of conception

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- 8.4.3 Weight of product of conception
- 8.4.4 Gestational age – paperwork can state approximate gestational age of product of conception. Gestational age per exam could be <24 weeks or >24 weeks.
- 8.5 All submitted products of conception must be documented by notes and photography. An overview of the product of conception must have a ruler visible in the frame, either a plain straight ruler or an x, y axis ruler.
- 8.6 Sampling of the product of conception should be approximately 3x3x3mm in size (fit into the tip of the bottom of a 1.5mL screw cap tube):
 - 8.6.1 Sampling is dependent on condition of the item:
 - 8.6.2 If a full embryo/fetus or body fragments are visible (morphologically well defined), take a sample directly from the specimen. Document what was sampled in exam notes.
 - 8.6.3 If the submitted product of conception is not morphologically well defined (unrecognizable tissue, potential placental tissue), place a portion of the sample in a weigh boat and submerge the sample with distilled water. Observe the sample for small finger-like projections/branching tree-like structures. These finger-like projections/branches are potential chorionic villi.
 - 8.6.3.1 **If small finger-like projections from the submerged tissue are observed**, take a sample of the finger-like projection/branches. Document in exam notes.
 - 8.6.3.2 **If small finger-like projections from the submerged tissue are NOT observed**, and there are multiple pieces submitted for the product of conception, examine all pieces submitted by submerging piece by piece in distilled water. Take a sample of the finger-like projection/branches if observed, otherwise, take a sample of the specimen submitted. Document in exam notes.
 - 8.6.3.3 **If small finger-like projections from the submerged tissue is NOT observed**, and only one piece was submitted for the product of conception, take a sample of the specimen submitted. Document in exam notes
- 8.7 Print out item label and affix label to 1.5mL screw cap tube containing product of conception for testing.
- 8.8 After sampling the product of conception, the submitted product of conception should be retained based on the condition of the item (fit into a 1.5mL microcentrifuge tube or 50mL conical tube):
 - 8.8.1 If a full embryo/fetus or body fragments are visible (morphologically well defined), depending on the size of the specimen, take a sample directly from the specimen, retain the entire specimen, or retain body fragment(s). Document retention in exam notes.

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- 8.8.1.1 **If the product of conception gestational age is >24 weeks** (full embryo/fetus) and is intact, discuss with a supervisor the appropriate way to handle the product of conception.
- 8.8.1.2 **If a sample or a body fragment was retained, and the remainder of the product of conception is to be returned to the OCME Evidence Unit**, repackage the remaining specimen in the original container. Document that the remaining specimen was repackaged in the original container in exam notes. Return packaging with product of conception to the OCME Evidence Unit.
- 8.8.1.3 **If the entire specimen was retained**, document that the entirety of submitted specimen was retained. Clean the original packaging that contained the product of conception with 10% bleach prior to returning the empty packaging to the OCME Evidence Unit.
- 8.8.2 **If the submitted product of conception is not morphologically well defined** (unrecognizable tissue, potential placental tissue), the entire submitted specimen should be retained. Document retention in exam notes.
- 8.9 Retained product of conception should be placed in a KPAK for storage.

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9 Appendix A: Summary of Exam Notes based on Sample Type

9.1 Examination notes for **stain cards/blood/tissue samples**:

- ME# (if applicable)
- Item # (if applicable)
- Description of item

9.2 Examination notes for **bone samples**:

- Type of bone (i.e. spongy, compact, etc.)
- Measurement of bone (inches)
- Appearance (i.e. shape, color, etc.)
- Gross Weight (grams)
- Weight of Bone Fragment(s) to be milled (grams)
- Weight of Bone Dust from milling (grams)
- Initial of examining analyst – cleaning, cutting, milling
- Date of cleaning, cutting, milling

9.3 Examination notes for **products of conception**:

- General condition of the product of conception – full embryo/fetus, body fragments, tissue, any other pertinent notes
- Color of product of conception
- Weight of product of conception (grams)
- Gestational age – paperwork can state approximate gestational age of product of conception. Gestational age per exam could be <24 weeks or >24 weeks.
- Sampling from product of conception (i.e. body fragment, tissue, etc.)
- Retention/disposal of product of conception