STANDARD OPERATING PROCEDURE
FOR
SAMPLING BONE AND TOOTH SPECIMENS FROM
HUMAN REMAINS FOR DNA TESTING AT THE
ICMP

Distribution:
Internal
ICMP.SOP.AA.136.2.doc
Sarajevo, 16 February 2015
I. Principle

Comprehensive information on the procedure for sampling skeletal human remains, including the determination of which element to sample, will ensure that the optimum sample for DNA analysis is selected. Accurate documentation ensures that the selection is recorded and that the required Chain of Custody information is completed.

II. Scope

This SOP outlines procedures for best practice in the selection, documentation and sampling of specimens (skeletal and dental) from human remains for DNA testing.

The DNA sample collection should be performed by a forensic expert or forensic technician: physical anthropologist, osteology technician, forensic pathologist, or other skilled and competent individual. The sampling is to be conducted following the forensic examination and/or autopsy.

In situations where unusual circumstances preclude the adherence to this SOP, a written description should be provided explaining why the procedures could not be followed, the alternative procedures performed, and an opinion on how the accuracy and reliability of the resulting analyses was affected using the ICMP’s SOP/Policy Deviation form (ICMP.FSD.QMS.120.doc) which should accompany the samples with the Chain of Custody document.

III. References to Related Documentation

ICMP.SOP.FSD.108.doc – Standard Operating procedure for the Transfer of Samples, Submission Tables and DNA Match Reports within the Forensic Science Department
ICMP.FSD.98.doc – Request for DNA Analysis - Chain of Custody
ICMP.FSD.95.doc – Secure Transfer of Items Form
ICMP.FSD.151.doc – Request for DNA Analysis for Presumptive Identifications
ICMP.FSD.ICD.465.doc – Request for DNA Analysis- Additional & Reserve Samples
ICMP.FSD.QMS.120.doc – SOP/Policy Deviation Form

IV. Specimen

Sampling of specimens for DNA testing can involve two types of hard tissue, bone and tooth (primary samples). Reserve DNA samples (secondary and tertiary samples) may be collected, depending upon the available remains and preservation or condition of the bones and teeth. Specimens are collected within a case of remains. The case of remains may vary from a single bone or bone fragment up to a complete body. A single case may also consist of the commingled remains of multiple individuals.

V. Reagents and Supplies

Required reagents include:

- Solution of water with 10% commercial bleach – to clean and sterilize saw blades to prevent contamination between samples;
- Absolute ethanol – to assist in drying samples;
• Wash grade ethanol (70% ethanol) – to clean saw blades to prevent rusting.

Required supplies include:
• Storage vial/sample bags (e.g., ICMP Bone Evidence Envelopes) – to hold and transport samples;
• “Sticky” tape or Evidence Tape – to seal sample bags and vials;
• Indelible marker and/or pens – to label sample bags and vials;
• Sealable container – to hold and transport all samples collected;
• Waterproof labels or metal tags (e.g., “Aluma-Boss” metal tags) – to label bones that have been sampled;
• Paper towels or Kim Wipes – to clean work areas and equipment between samples.

Personal protective equipment includes:
• Suitable protective gloves – to prevent injury from saws, reagents and other equipment;
• Masks – to protect from particulate inhalation;
• Safety glasses – to protect from particulates and reagents splashing.

Equipment includes:
• Table and vice or clamp – for stable securing of samples for cutting;
• Dust extraction system with hood – to remove particulates;
• Electric autopsy saw – for rapid, efficient sample cutting;
• Hand saw – as a back-up sample cutting tool;
• Dental pliers – to assist in removing teeth;
• Digital or wet film camera – to record sample images;
• Photography scales – for use when taking images.

VI. Quality Assurance

All equipment must be properly cleaned prior to sampling. At a minimum, the blade surfaces of the autopsy saw must be rinsed with 10% commercial bleach solution before each sample is taken. It is highly recommended saw blades are rinsed with wash grade (70%) ethanol, if available, to prevent rusting.

If the sample is moist it can be helpful to rinse with 95% or absolute ethanol and allow it to air dry, to promote dehydration. It is important that the specimen is completely dried before packaging. Breathable (e.g., paper) packaging should be used where practical.

Once the sample has been collected, it is placed in the appropriate vial/sample bag/container/envelope, which is appropriately labeled. Wet samples should never be placed in plastic sample bags as this encourages mould and bacterial growth. These should be allowed to dry, or placed in an appropriate vial/sample bag.

It is NOT permissible to place more than one bone or tooth specimen in the same bag/container, or to assign more than one specimen the same specimen number.

During transport and storage of samples avoid exposing the samples to conditions of elevated heat or humidity, to the most practical extent possible.

DNA sampling is a costly and time consuming practice. Optimal samples, as described below, should be taken from each case to ensure the greatest chance of DNA typing success. Less than optimal
samples must not be taken for the sake of sampling expediency, and if taken, the reasons should be justified and documented (using ICMP’s SOP/Policy Deviation Form (ICMP.FSD.QMS.120.doc)).

**NOTE:** ICMP reserves the right to decline to perform DNA analysis if the samples are not suitable or have not been taken according to the priorities and practices listed in this SOP.

**VII. Safety**

Treat all biological specimens as potentially infectious. Gloves, safety glasses, and a mask should be worn at all times when cutting or extracting the specimens. Gloves and mask also reduce the risk of contamination of the sample by the person performing the sampling. Gloves should adequately protect from potential cutting injuries.

The autopsy saw is capable of causing serious injury. Extreme caution must be observed when operating this device. The manufacturer’s operating instruction safety rules and best practice in safe use of electrical equipment and sharp instruments should be followed.

Material Safety Data Sheets (MSDS) for bleach and ethanol should be available and consulted.

The dust caused by cutting bone is a potential particulate hazard. Staff should wear a mask to prevent inhalation of bone dust. Also, the cutting of skeletal material should be conducted in a ventilated area. The use of an enclosed ventilation hood or box with a dust extraction system is recommended for cutting multiple bone samples indoors. Risk assessments should be undertaken and followed when using equipment during sampling.

**VIII. Procedure**

**8.1. Sample Recording**

1. It is standard practice to take a photograph of the complete skeletal element to be sampled with a scale and case number prior to sampling. After the specimen has been cut or extracted, a photographic or digital image is taken including the sampled element, together with the extracted specimen, the sample code and scale. If an image cannot be taken, a written description of the sample should be provided, with an explanation of why an image could not be taken.

2. Additional information concerning the sample may be submitted by authorities that assist ICMP in evaluating samples. This may include details of the site or case context, the properties of the samples, other samples submitted from the same case, or information on possible identifications. To accommodate for this ICMP utilizes the following forms to standardize information provision:
   
   ICMP.FSD.98.doc – Request for DNA Analysis - Chain of Custody
   ICMP.FSD.151.doc – Request for DNA Analysis-Presumptive Identifications
   ICMP.FSD.ICD.465.doc – Request for DNA Analysis- Additional & Reserve Samples
   ICMP.FSD.QMS.120.doc – SOP/Policy Deviation Form

3. The sample code should be documented and definitively associated with both the sample and the element from which it was taken. For samples transferred via the ICMP, bone sample
evidence envelopes are preferred. Document the appropriate information listed on the envelope and follow the procedures listed below regarding the Chain of Custody. Otherwise, samples should be placed in appropriately secure packaging with sample code listed on the container, together with the sample code listed on a separate waterproof label and enclosed with the sample if there is any chance of the container label being rendered unreadable. Label the remaining skeletal element (the part not sampled), with a corresponding label. Metal “Aluma-Boss” type labels may be used, or some other form of durable, indelible labeling retained with the element. This is to ensure the exact location from which the sample was collected can be checked and verified, using labels that do not degrade over time.

4. See Section 3.8. Sample Codes below and Appendix 1 for additional information regarding sample codes.

8.2. Sample Selection

1. The selection of the DNA sample is undertaken by the forensic expert assigned to the case. Appropriate and efficient sample selection is key to effective DNA testing and analysis. The appropriate bone or tooth specimen that is to be submitted for DNA testing is dependent upon the suitability and availability of skeletal elements and the condition or preservation of the human remains. It is the responsibility of the forensic expert to determine the appropriate sample(s) for collection. The forensic expert must use his/her best judgment and expertise in determining the location for sample selection, with reference to Appendix 1 and 3 below. It is NOT recommended that samples be cut near areas of trauma (ante-mortem, peri-mortem, or post-mortem) or the areas of possible degradation and/or contamination.

2. “Reserve” samples may be taken in cases where there is certainty that all samples come from the same individual. The reserve sample will be DNA tested only if the primary sample fails DNA testing. The reserve and primary samples should be packaged in separate envelopes and sent together. The forensic expert taking the sample should indicate on the packaging and Chain of Custody document which is the primary sample and which should be considered a reserve. Reserve samples must have unique sample identifiers, distinct from other samples in the case (see section 8.3. Sample Codes below). This practice is particularly recommended for cases that will be difficult to access or retrieve after examination, or are assessed as being of poor bone quality. Submitting reserve sample(s) at the time of initial sampling reduces the need for resubmissions. The taking of reserve samples is not to be confused with the situation where multiple samples are taken from the same case in order to resolve potential commingling, when all samples are to be DNA tested.

3. The ICMP may request resubmissions for cases where samples are of poor apparent quality, insufficient size or repeatedly fail to yield sufficient DNA results. Resubmission is contingent on the adequacy and suitability of the remaining biological material in a case. The forensic expert requested to provide a resubmission sample should indicate on the packaging and Chain of Custody document that a sample is a resubmission to differentiate it from primary or reserve samples. Resubmission samples must have unique sample identifiers, distinct from other samples in the case (see section 8.3. Sample Codes below).

4. Desirable bone sample weight is 5 to 15 grams. A 5 centimeter window-section of femur mid-shaft (adult male) provides an adequate amount of sample weight. Larger samples may provide an improved chance for DNA testing success with cases of highly degraded remains. However, depending on circumstances and preservation, smaller samples of bone down to 4 grams (and
exceptionally less than 1 gram), can provide DNA results and may be taken if there is no alternative. For smaller skeletal elements, such as hand or foot bones, the forensic expert may submit the entire bone, however if the assigned case consists of only a single tooth, skeletal element or bone fragment, then coordination with the DNA laboratory and/or the Identification Coordination Division should determine the proper sampling and processing approach.

5. DNA testing results may vary and can be dependent on the bone and tooth type as well as the location within the skeletal element. Certain skeletal elements are more favorable for DNA testing. See Appendices 1, 2 and 3 for recommended elements and sampling locations on bones.

8.2.1. Body – Single Individual

6. If an individual is determined to be complete or mostly complete and represented as one case, then it is recommended that one sample location be determined by the forensic expert. A reserve sample from a second location or bone on the body may be taken. The following list shows the most suitable elements for sample selection (see also Appendix 1). It is based on elements most likely to yield suitable DNA. The order of priority should be followed unless teeth or leg bones are unavailable or otherwise not optimal for DNA sampling:
   1) One healthy tooth;
   2) Femur;
   3) Tibia;
   4) Pelvis (to be sampled if teeth and legs are unavailable or otherwise not optimal for DNA sampling).

8.2.2. Body Part – Single Individual

7. If a body part from a single individual is represented as a case, then it is recommended that one sample location be determined by the forensic expert. A reserve sample from a second location on the same bone or body part may be taken (see 8.3. Sample Codes below). The selection of samples for DNA testing will vary depending upon available human remains. See Appendices 1, 2 and 3 for recommended elements and bone locations, depending upon available skeletal elements.

8.2.3. The Arm and Hand – Single Individual

8. Current success rates for DNA testing of the arm bones (humerus, ulna, and radius) are relatively poor compared to the hand bones (metacarpal, carpal, proximal phalanx). Sampling arm bones is not recommended unless the availability of more suitable samples in restricted. If skeletal elements from the hand are available and these hand bones are conclusively associated with the arm, then it is recommended that the hand bones be sampled as a priority over sampling an arm bone.

9. The following list shows, in order of preference, sample selection for a complete or partial arm with hand:
   1) Metacarpal (4th Metacarpal preferred);
   2) Carpal (Capitate preferred);
   3) Humerus.

10. The following list shows, in order of preference, sample selection for arm bones:
   1) Humerus;
   2) Ulna;
3) Radius.

11. The following list shows, in order of preference, sample selection for a hand:
   1) Metacarpal (4th Metacarpal preferred);
   2) Carpal (Capitate preferred);
   3) First Proximal Phalanx.

   NOTE: See Appendices 1 and 2 for recommended elements and sample locations on bones by priority.

8.2.4. Commingled Remains – Single and/or Multiple Individuals

12. If sampling from cases with multiple bones and/or bone fragments of an unknown number of individuals, then the forensic expert must select the appropriate number of samples and sample locations based on assessment of the number of discreet sets of remains that can be recognized. It is NOT recommended to sample a complete bone or bone fragment that is not paired/associated/articulated with another skeletal element or to sample teeth that are loose or separate from the mandible or maxilla, unless there is justification for doing so. Should the forensic expert choose to deviate from the recommendations, the reasoning should be justified and documented (using ICMP’s SOP/Policy Deviation Form (ICMP.FSD.QMS.120.doc)).

   NOTE: See Appendices 1 and 3 for recommended elements and sample locations on bones.

8.3. Sample Codes

1. Every sample designated for DNA testing must be assigned a unique code. A standard coding protocol should be agreed with the designated authority before sampling begins. The requirements for a suitable sample code are described below.

2. The sample code must be legible and neatly written in capital letters or printed. For clarity, and to distinguish characters from other numbers or letters, a zero must have a diagonal slash through it (e.g. Ø) and the number seven must have a dash through it (e.g. 7).

3. Proper labeling of the original skeletal element and the extracted sample itself is vital in the DNA identification process. The sample codes aid in overall reviews of DNA testing results, which identify patterns of success rates by skeletal element, which in turn direct the sampling selection process. Furthermore, differentiation of primary, reserve and resubmission samples through proper labeling assists in the quality control and coordination of the sampling and case management process.

8.3.1. Determination of a Sample Code

4. The sample code should not have blank spaces. The sample code consists of the site code, case number, and sample side and element. The following example shows the sequence and appropriate characters for separation:

   THAI-132B-LF

   (Site Location THAI, Case Number 132B, Skeletal Element Left Femur)
8.3.2. Site Code

5. This designation is assigned by the investigating authority and should be unique. This may include a court order number.

8.3.3. Case Number & Letter

6. This number is assigned by the investigating authority or an authorized individual. Often, cases may include a letter code which describes the case: for example B for Body; BP for Body Part; I for Isolated Bones; and GBP for General Bone Bag. The case numbers and letters are usually assigned during recovery operations or designated within the mortuary.

8.3.4. Sample Side and Element Code

7. The codes for the specific tooth or element usually include a letter for the side of the body: L for left; R for Right; and U for Un-sided or side could not be determined. See Appendix 1 for full list of sample codes for teeth and bones.

8.3.5. Element Number

8. Some cases may have several duplicate skeletal elements, each of which may be sampled. The element number is used to distinguish between the same elements in a commingled assemblage. For example, two left femora (LF) are distinguished as:

THAI-Ø86BP-LF1
THAI-Ø86BP-LF2

8.3.6. Determination of a Sample Code for a Resubmission (second) Sample from the Same Bone

9. If the first (primary) DNA sample produced an unsuccessful or insufficient DNA result then another sample may be selected from the same skeletal element. This a resubmission sample. To differentiate it from the primary sample the identifier – RSB – should be added to the existing sample code. For example, for a right tibia (RT):

THAI-199BP-RT1 → THAI-199BP-RT1-RSB

10. If there is repeat sampling of the same skeletal element as a resubmission then a numerical identifier should be added, for example, for a right tibia (RT):

THAI-199BP-RT1 RSB → THAI-199BP-RT1-RSB2

8.3.7. Determination of a Sample Code for a Resubmission (second) Sample from the Same Body Part, but Different Bone

11. Follow the same procedure as for a new sample, with the additional identifier RSB. For example, for a right femur (RF):

THAI-199BP-RF1-RSB
8.3.8. Determination of Sample Code for a Sample Submitted as a Reserve

12. Specimens from cases that are additional to the primary sample and submitted to the DNA laboratory that DO NOT require immediate processing for DNA testing, are required to be designated as RESERVES. Reserve specimens will be retained for an agreed designated period by ICMP and processed only if the primary, most desirable sample fails to yield a DNA profile. Samples should be listed as reserves only in cases where there is no possibility that they originate from different individuals. If there is doubt samples should be given a new sample code. Examples of the circumstances where taking reserve samples are appropriate include taking tooth and femur samples from the same fully articulated body which is to be reinterred or difficult to access in the future, or where remains from a single case are highly degraded and DNA extraction is expected to be problematical. The reserve samples should have the identifier – RES – added to the existing sample code. For example, for a left tibia (LT):

THAI-234BP-LT1-RES

8.4. Transfer of Samples to Bone Unit at ICD for DNA analysis

1. The chain of custody procedures outlined in the SOP for the Transfer of Samples, Submission Tables and DNA Match Reports within the Forensic Science Department (FSD) (ICMP.SOP.FSD.108.doc) should be followed when preparing samples for DNA analysis.

2. In general, the ICMP pre-printed envelopes should be used for packaging where possible. Irrespective of the type of sample packaging, the sample code must be written on the packaging in indelible ink. The opening of the packaging should be tape-sealed, signed and dated over the seal. This protects the integrity of the sample as any attempts to tamper with the contents will become apparent.

3. There may be incidences where the individual submitting samples to the Identification Coordination Division is different to the individual that cut or extracted the samples. In such situations, this should be clearly documented on the accompanying paperwork and on the sample packaging (where practical).

4. Failure to comply with the chain of custody procedures recommended may result in a delay in the processing of samples for DNA analysis.
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<th>Date of Implementation</th>
<th>Prepared By</th>
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<sup>1</sup> This is a re-write of the previous version.
Appendix 1: List of the teeth and skeletal elements with priority ranking and coding system

Note that each region of the skeleton has sample locations with multiple priorities. Priority 1 is the most preferred and Priority 6 is the least preferred.

<table>
<thead>
<tr>
<th>Region</th>
<th>Bone/Tooth Element</th>
<th>Priority</th>
<th>Sample Codes</th>
<th>Left</th>
<th>Right</th>
<th>Un-sided</th>
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Appendix 2: Success Rates of DNA Extraction from Individual Skeletal Elements

![Success rates of DNA extraction from individual skeletal elements](image-url)
Appendix 3: Sampling descriptions of preferred teeth and preferred sample locations on the bone

The sampling location on the bone and the selection of particular teeth is based on various factors. Each skeletal element has specific recommendations and is considered individually.

In general, avoid sampling areas where the bone is discolored as this may indicate an increased concentration of certain metals in the soil or high humidity in the grave environment that may have resulted in DNA degradation of the bone.

Avoid certain sampling areas, which show individualizing characteristics that may be used for identification purposes, for example the mandibular angle of the mandible or teeth that exhibit dental work.

Avoid sampling areas that indicate trauma, such as along fracture edges, as these areas may contribute to future re-association efforts.

**TEETH**

Intact and well-preserved teeth with completely formed root apices are required where possible. However, the presence of completely formed root apices is not an absolute requirement for submission.

Select a tooth from the following order:

1. First molar;
2. Second molar;
3. Third molar;
4. First or second premolar;
5. Canine; and
6. Incisor.

If several teeth articulate within a dental arcade, one healthy tooth should be submitted for DNA testing. Record the tooth number submitted. Ensure that all information concerning the tooth is recorded as part of standard examination procedures before sampling. This may include assessment of calcification, eruption, or tooth transparency used in age at death estimations.

When possible, teeth with extensive carious destruction, and/or peri-mortem or post-mortem fractures should be avoided.

Since teeth with dental restorations have an increased risk of crown fracture which may minimize the DNA yield, these should not be submitted for sampling. In addition, these may be essential for a radiographic comparison should ante-mortem radiographs become available at a later date.

If a distinctive anterior tooth needs to be selected for sampling, such as one that contributes to a diastema or displays a cultural modification, a photographic or digital image of the dental arcade must be taken, with scale and case number, before sampling.

In a highly commingled situation, avoid sampling a loose tooth or teeth that have been replaced in the alveolar process. These are generally single-rooted teeth and should be considered least desirable in order of tooth sampling preference.
If the quality of the teeth has been compromised and does not fulfil the above stated criteria, it is recommended that two teeth from either the maxilla or the mandible are submitted for sampling. A tooth from the maxilla and a tooth from the mandible should never be submitted together to ensure only one person is represented by the sample.

**SKELETAL ELEMENTS**

The following sections provide recommendations for first and second priorities for the collection of samples from the individual skeletal elements. A second priority location (indicated in gray) is provided, in case part of the skeleton or skeletal elements display possible identifying features, trauma, or other indicator of degraded DNA material. The forensic expert may choose to select the left or the right sided element, subject to the preservation or availability of the skeletal material.

**Occipital Bone**
A relatively large sample should be taken that includes the external occipital protuberance. Avoid cutting through the cranial sutures or fractures as this may compromise the integrity of the cranium and are used to confirm physical re-associations in cases of disarticulated and/or commingled remains. Be sure to record occipital protuberance sex determination values before taking the sample.

Owing to the relatively large sample size, an occipital bone is sampled only once.

**Parietal Bone**
A relatively large sample should be taken. Avoid cutting through the cranial sutures or fractures as this may compromise the integrity of the cranium and are used to confirm physical re-associations in cases of disarticulated and/or commingled remains.

Owing to the relatively large sample size, a parietal bone is sampled only once.
**Temporal Bone**
Cut the endocranial aspect of the petrous portion of the temporal bone.

In the case of a subadult cranium with thin vault bones, sampling of the petrous portion of the temporal bone is preferred, rather than sampling the occipital or parietal bones.

*Location and relative size of a petrous portion sample (A right temporal is illustrated, endocranial view)*

**Mandibular Body**
A window of the inferior mandibular body is sampled leaving the alveolar process intact.

*Location and relative size of a mandibular body sample (A right mandible is illustrated)*

**Scapula**
The first priority is the acromion and spine (black) of the scapula. The second choice is the axillary border (gray). Also, the glenoid cavity and coracoid may also be used.

*Location and relative size of a scapula sample (A right scapula is illustrated)*

**Clavicle**
The clavicle is cross-sectioned and the acromial and sternal ends are bagged together.

*Location and relative size of a clavicle sample (A right clavicle is illustrated)*

**Manubrium and Sternum**
A complete or mostly complete manubrium or sternum may be sampled. Select the area(s) best preserved with the most cortical bone.
Vertebrae (Cervical, Thoracic, and Lumbar)
The transverse and spinous processes (black) are first priority for the thoracic and lumbar vertebrae. The vertebral body (gray) is second priority. A complete cervical vertebra may be submitted, if possible.

Location and relative size of a vertebral samples
(The superior aspect is illustrated)

Sacrum
The medial sacral crest and articular surfaces (black) are priority.

Location and relative size of a sacral sample
(The posterior aspect is illustrated)

Ribs
The vertebral half (black) of one the middle ribs are priority. A complete or mostly complete upper or lower rib (gray) is second priority for selection of rib samples.

Upper (1st to 3rd)  Middle (4th to 9th)  Lower (10th to 12th)
**Humerus**

First priority is a window of bone from the distal medial shaft approximately 2 cm above the plane of the trochlea (black). Second priority is a window section at or near the proximal mid-shaft (gray).

*Location and relative size of a humerus sample*  
* (A right humerus is illustrated)

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**Ulna and Radius**

The first priority is window of bone removed from the proximal shaft along the interosseous crest (black). Second priority is a cross-section of the distal mid-shaft (gray).

*Location and relative size of samples for a radius and ulna*  
* (Both right arm bones are illustrated)

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**Hand Bones**

The metacarpal and capitate are the preferred bones, when sampling a complete hand. However priorities will vary, depending upon the available elements and the preservation.

**Hand Phalanges**

The first priority is the 1st proximal phalanx (black). Second priority is the 2nd proximal phalanx (gray) or another large hand phalanx.

**Metacarpals**

The first priority is a 4th metacarpal (black). Second priority is a 3rd metacarpal (gray).

**Carpals**

The first priority is a complete capitate (black). Any available larger carpal bones are second priority.
**Pelvis/Innominate**

The first priority is rectangular section is cut from the greater sciatic notch area (black). The superior border of the cut is beneath the preauricular surface and into the bone toward the arcuate line. The inferior border is approximately mid-point of the sciatic notch to avoid damaging the acetabulum. Be sure to record sciatic notch sex determination values before taking the sample. The second priority is a rectangular section from the iliac crest near the anterior area (gray).

When an innominate sample is excised, use a fantail blade.

*Location and relative size of an innominate sample (A right innominate is illustrated)*

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**Femur**

First priority is a window of bone is removed from the proximal anterior shaft below the plane of the lesser trochanter (black). Second priority is a window of bone is removed from the distal anterior shaft (gray).

*Location and relative size of a femoral sample (A right femur is illustrated)*

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**Patella**

A complete or mostly complete patella may be sampled.

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**Tibia**

The first priority is a window of bone removed from the anterior proximal shaft (black). Second priority is a window of bone is removed from the distal anterior shaft (gray).

*Location and relative size of a tibia sample (A right tibia is illustrated)*
Fibula
A window of bone is removed from the distal shaft. In some cases, a cross-section of the shaft is necessary. If so, the proximal and distal ends are bagged together.

Location and relative size of a fibular sample
(A right fibula is illustrated)

Foot Bones
The metatarsal and talus are the preferred bones, when sampling a complete foot. However priorities will vary, depending upon the available elements and the preservation.

Foot Phalanges
The first priority is the 1st proximal phalanx (black). Second priority is the 2nd proximal phalanx (gray) or another large foot phalanx.

Metatarsals
The first priority is a 5th metatarsal (black). Second priority is the 1st metatarsal (gray).

Smaller Tarsals
The first priority is a complete navicular (black). Second priority is a cuboid (gray).

Talus
A complete or mostly complete talus may be sampled.

Calcaneus
The posterior area or the body of the calcaneus (black) is first priority for the calcaneus.