

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

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The Hague, 28 August 2023

ICMP.SOP.AA.136.3.W.doc

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## **I. Principle**

A comprehensive outline of the procedure for sampling human skeletal and dental remains for DNA-based identification, including the decision on which specimen to sample, will result in optimal sample selection for DNA analysis. Accurate and complete documentation is integral to the procedure; this includes Chain of Custody records.

## **II. Scope**

This Standard Operating Procedure (SOP) outlines best practices in selecting, sampling, and documenting skeletal and dental specimens from human remains for DNA extraction, profiling, matching, and therefore a DNA-based identification.

The sampling procedure should be performed by a forensic expert or technician: forensic pathologist, forensic or biological anthropologist, osteologist, autopsy technician, or other trained and skilled personnel. Sampling is conducted after completion of the forensic examination: autopsy and/or anthropological analysis.

When circumstances preclude adherence to this SOP, a written description should be provided explaining why the standard procedures could not be followed, which procedures were performed, and how the deviation from the SOP could impact the accuracy and reliability of the subsequent processing of samples for DNA analysis. This information must be entered in the ICMP's SOP/Policy Deviation Form (ICMP.ST.QMS.120.doc), which must accompany the samples with the Chain of Custody document.

## **III. References to Related Documentation**

*ICMP.SOP.ST.170.doc – Standard Operating Procedure for the Transfer of Samples, Submission Tables and DNA Match Reports*  
*ICMP.ST.95.doc – Secure Transfer of Items Form*  
*ICMP.ST.98.doc – Request for DNA Analysis - Chain of Custody Form*  
*ICMP.ST.151.doc – Request for DNA Analysis - Presumptive Identifications*  
*ICMP.ST.LS.465.doc – Request for DNA Analysis - Additional & Reserve Samples*  
*ICMP.ST.QMS.120.doc – SOP/Policy Deviation Form*  
*ICMP.ST.LS.1172.doc – PM DNA Sample Envelope*  
*ICMP.ST.AA.1259.doc – Forensic anthropology checklist when collecting PM Samples*

## **IV. Specimen**

Post-mortem (PM) samples of human remains accepted for DNA analysis in ICMP's Human Identification (HID) Laboratory are hard tissue: bone and tooth. The preservation of a case (the deceased person) may vary from a complete body to a single bone or bone fragment. A case may also consist of commingled remains of multiple individuals.

The collected samples can be categorized into primary samples and their reserve samples, depending on the available remains and the preservation and condition of the bones and teeth. Primary samples will be processed upon receipt into the laboratory, and while the reserve samples will be transferred to the HID laboratory, they will only be processed if the primary samples failed to

yield a DNA profile.

## **V. Reagents and Supplies**

### **a. Equipment**

- A table and/or cutting board with an appropriate surface area to photograph and cut the sample;
- Electric autopsy saw – for rapid and efficient specimen cutting;
- Hand saw – backup sample cutting tool;
- Dental pliers – for teeth extraction;
- Table vice or clamp – to hold the specimen in place during cutting;
- Dust extraction system with hood – to remove particulates;
- DSLR camera – to photograph samples;
- Photography scales (e.g. ABFO No. 2 or similar forensic photomacrographic scale) – to include in photographs of the specimen and sample.

### **b. Reagents**

- 1-2% sodium hypochlorite (bleach) solution – to clean and sterilize saw blades to prevent contamination between samples<sup>1</sup>;
- Absolute ethanol – to assist in drying samples;
- 70% ethanol – to clean saw blades to prevent rusting.

### **c. Supplies**

- 50mL conical tubes/sample bags (e.g., ICMP PM DNA Sample Envelopes) – to package and transport samples;
- “Sticky” tape or Evidence Tape – to seal sample container, if the container is not self-sealable;
- Indelible marker and/or pens – to label sample container;
- Container with a seal or lock – to hold and transport a collected batch of samples;
- Waterproof labels or metal tags (e.g., “Aluma-Boss” metal tags) – to tag specimens that have been sampled;
- Paper towels or Kimwipes- to clean the work surface area and equipment between samples.

### **d. Personal Protective Equipment (PPE)**

- Protective gloves – to prevent injury from saws, reagents, and other equipment;
- Respirator (e.g. N95, FFP2, FFP3) – to filter airborne bone dust;
- Safety glasses – to protect eyes from particulates and reagents.

## **V. Quality Assurance**

All equipment must be cleaned before sampling. The autopsy saw blade must be rinsed with 1-2%

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<sup>1</sup> Kampmann, M. L., Børsting, C., & Morling, N. 2017. Decrease DNA contamination in the laboratories. *Forensic Science International: Genetics Supplement Series 6*, e577-e578. DOI: <https://doi.org/10.1016/j.fsigs.2017.09.223>.

bleach solution before each sample is cut to prevent DNA cross-contamination. The autopsy saw blades should be rinsed with 70% ethanol to prevent rusting.

If the sample is damp, rinsing it with absolute ethanol and allowing it to air dry completely before packaging can promote dehydration.

Each sample shall be packaged in the assigned and labeled container (a 50mL conical tube, bag, or envelope) immediately after cutting/extraction. Breathable packaging (e.g. paper envelopes) should be used when practical. Damp samples should never be placed in plastic containers, as this encourages mold or bacterial growth; damp samples should never be placed in paper containers as this will compromise the integrity of the container.

Only one sample should be packaged in a container; placing more than one bone sample or tooth in the same container is NOT permissible. Assigning an identical PM DNA Sample Code to multiple samples is NOT permissible.

Only one PM DNA sample shall be cut/extracted at a time; if multiple samples have to be cut/extracted, they must not be cut/extracted simultaneously, but consecutively, by finishing the packaging and tape-sealing of one sample before starting with the next.

All information provided on the Chain of Custody form and the PM DNA Sample Envelope has to be cross-checked before the samples are forwarded to the HID laboratory. It is imperative that all details on these forms are the same. Any discrepancies or error must be amended or noted as soon as they are discovered.

During storage and transport avoid exposing the samples to conditions of elevated heat and humidity, whenever practical.

## **VI. Safety**

A risk assessment of the sampling procedure should be conducted prior to sampling. All staff must be acquainted with risk and health and safety measures, and they must follow the outlined safety rules.

Sound work protection principles should be followed.

Extreme caution must be observed when operating an electric autopsy saw. The manufacturer's operating instructions and safety rules, as well as safety principles in the use of electrical equipment and sharp instruments should be followed.

Protective gloves, safety glasses, and a respirator should be worn during the cutting of the specimens. Gloves and respirators not only protect the staff member but also reduce the risk of DNA contamination by the person performing the sampling. A respirator prevents inhalation of bone dust which is a potential hazard during cutting. In addition, the cutting of bone should be conducted in a well-ventilated area.

Where possible, a Material Safety Data Sheets (MSDS) for bleach and ethanol should consulted.

## **VII. Procedure**

### **8.1. Sample Recording**

1. Photography is integral to the documentation of the sampling procedure; photographs record and establish a visual association between the sampled specimen and the cut or extracted sample. If a photograph cannot be taken, a written description of the sample should be provided, with an explanation of why a photograph could not be produced. Photographs are taken in sequence:
  - Before sampling: a photograph of the complete skeletal element to be sampled with a scale and case designation/number, and the PM DNA Sample Code;
  - After the specimen has been cut or extracted: a photograph of the specimen with the cut/extracted sample, the scale, the case number, and the PM DNA Sample Code;
  - Optional: a close-up photograph of the sample with the scale and PM DNA Sample Code;
  - Optional: immediately after the sample has been packaged: photograph of the sealed container with visible sample information on the container; this photograph records the time of packaging in the image metadata (for digital images) and can be used as a Chain of Custody record.
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 facilitate and standardize information provision ICMP utilizes the following Chain of Custody forms:

*ICMP.ST.98.doc – Request for DNA Analysis Chain of Custody Form;*

*ICMP.ST.151.doc– Request for DNA Analysis - Presumptive Identifications;*

*ICMP.ST.LS.465.doc– Request for DNA Analysis - Additional & Reserve Samples;*

*ICMP.ST.QMS.120.doc– SOP/Policy Deviation Form;*

*ICMP.ST.AA.1259.doc – Forensic anthropology checklist when collecting PM Samples.*

3. The PM DNA Sample Code should be documented and definitively associated with both the sample and the sampled specimen. For samples transferred via the ICMP, the PM DNA Sample Envelope (ICMP.ST.LS.1172.doc) is the preferred packaging container. Document the appropriate information listed on the envelope and follow the procedures described in Section 8.16 regarding the Chain of Custody. Otherwise, samples should be placed in secure packaging with the PM DNA Sample Code labeled on the container, together with the PM DNA Sample Code on a separate waterproof label enclosed with the sample if there is a chance of the container label being rendered unreadable. Label the remaining skeletal element with the PM DNA Sample Code on a label or tag, such as metal “Aluma-Boss” labels, or other forms of durable, indelible labeling. This is to record and preserve the association of the sample and the sampled element and to ensure that the association can be checked and verified at a later date.
4. See Section 8.7. PM DNA Sample Codes below and Appendix 1 for additional information regarding PM DNA Sample Codes.

### **8.2. Sample Selection**

1. DNA analysis is a costly and time-consuming procedure. Optimal samples, as described below, ensure the greatest chance of DNA extraction and profiling success. Suboptimal samples or

oversampling should not be practiced for the sake of expediency; if suboptimal samples are taken, the justification should be documented in the ICMP's SOP/Policy Deviation Form (ICMP.ST.QMS.120.doc).

**NOTE:** ICMP reserves the right to decline postmortem sample processing if the samples are not suitable or have not been taken in line with this SOP.

2. Appropriate and efficient sample selection is crucial for effective DNA extraction and analysis. The forensic expert assigned to the cases uses their best judgment in deciding which bone or tooth will be sampled by consulting Appendices 1 and 3 of this SOP. The suitability and availability of skeletal elements and the condition or preservation of the human remains are important factors in sample selection. It is not recommended that bone is cut near areas of trauma (ante-mortem (AM) or peri-mortem) and pathological features, or areas of PM degradation and/or contamination.
3. Samples can be categorized as reserve samples only if there is certainty that the specimens sampled for primary and reserve samples belong to the same individual. The reserve sample will be processed only if the primary sample fails to yield a DNA profile. The reserve and primary samples should be packaged in separate containers and submitted to the laboratory at the same time; this reduces the need for subsequent resubmissions. There is a separate Chain of Custody form for reserve samples (*ICMP.ST.LS.465.doc*) and it is mandatory to note on the form which primary sample the reserve sample is for. Like any sample, reserve samples must have unique PM DNA Sample Codes (see section 8.7. PM DNA Sample Codes below). The practice of taking reserve samples is recommended for cases that will be difficult to access or retrieve after examination, or contain skeletal remains that have been significantly degraded. Taking reserve samples is different than taking multiple samples from the same case to resolve potential commingling; in the latter case, all samples will be designated as primary and processed.
4. The ICMP may request resubmissions for cases where samples are of poor apparent quality, insufficient size, or repeatedly fail to yield sufficient DNA. 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 (*ICMP.ST.LS.465.doc*) that a sample is a resubmission and for which primary or reserve sample the resubmission is for. Like any sample, resubmitted samples must have unique PM DNA Sample Codes (see section 8.7. PM DNA Sample Codes below).
5. Bone weight sufficient for successful DNA extraction is five to 15 grams. If scales are unavailable, a 5-centimeter window section of the femur mid-shaft of an adult individual provides enough bone material for DNA extraction. Larger samples may provide an improved chance for DNA extraction success for highly degraded remains. However, depending on circumstances and preservation, smaller samples of bone down to four grams (and exceptionally less than one 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 entire bone can be submitted. However, if the assigned case consists of only a single tooth, skeletal element, or bone fragment, then in coordination with the ICMP Archaeology and Anthropology Division (AAD) and the HID laboratory, a proper approach to sampling and processing will be determined; the documentation has to be annotated to reflect the fact that the entire specimen was sampled for DNA analysis.
6. DNA analysis results may vary and can be dependent on the bone and tooth type, as well as the location on the skeletal element. Certain skeletal elements are more favorable for DNA

extraction. See Appendices 1, 2, and 3 for recommended elements and their sampling locations.

### **8.3. Body – Single Individual**

1. If the skeletal remains of one individual are determined to be complete or mostly complete, and there is no doubt that commingling of remains of different individuals could have possibly occurred, it is recommended that one sample location on that body be determined by the forensic expert. A reserve sample from a second location on the same bone or a different bone of the body may be taken. The following list shows the elements most likely to yield sufficient DNA for analysis (see also Appendix 1). The order of priority should be followed unless teeth or leg bones are unavailable or otherwise not optimal for DNA sampling:
  - 1) One tooth (healthy, unbroken (root and crown), unmodified);
  - 2) Femur;
  - 3) Tibia;
  - 4) Pelvis (to be sampled if teeth and legs are unavailable or otherwise not adequate for sampling).

### **8.4. Body Part – Single Individual**

1. If a body part recovered from the field was articulated *in situ*, and therefore all bones from that body part conclusively belong to a single individual, 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 a different bone of the body part may be taken. The selection of samples will vary depending on available skeletal elements that form the body part; see Appendices 1, 2, and 3 for recommended elements and bone locations.

### **8.5. Disarticulated and/or Commingled Remains – Multiple Individuals**

1. When sampling cases with multiple bones and/or bone fragments of an unknown number of individuals, the forensic expert must select the appropriate number of samples and sample locations based on the assessment of the MNI (minimum number of individuals) and the number of discrete 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.ST.QMS.120.doc)*).

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

### **8.6. Human Remains Exposed to Fire**

1. Burnt human remains undergo taphonomic changes including increased fragmentation and loss of collagen that may render DNA extraction impossible. When selecting samples for DNA analysis from burnt human remains, forensic experts should use their best judgment and consult relevant and updated literature and best practice standards regarding sampling of burnt remains for DNA analysis. Burnt human remains should be inventoried, weighed, and documented

before sampling for DNA analysis.

2. Bone/tooth color indicates the temperature and time of exposure, and can guide the selection process<sup>2</sup>:
  - **Partially burnt bone:** ranges in color ranging from yellowish-brown (unburnt) to deep brown (indicating exposure to fire); these remains have the potential for successful DNA extraction; the sampling area should be far from the partially burnt area, and the selected sample should not weigh less than one gram;
  - **Charred bone:** ranges in color ranging from dark gray to deep black; charred bone is typically unsuitable for DNA extraction;
  - **Partially mineralized bone:** ranges in color ranging from dark gray, blue-gray to gray-black; partially mineralized bone is typically unsuitable for DNA extraction;
  - **Mineralized bone:** ranges in color ranging from light gray to white, and is extremely fragile; mineralized bone is unsuitable for DNA extraction.

### 8.7. PM DNA Sample Codes

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

**NOTE:** By default, the PM DNA Sample Code should be written in the Latin alphabet without diacritical marks. However, based on agreement with the designated authority, provisions can be made in ICMP's Chain of Custody and the Integrated Data Management System (iDMS) for data formatted in other alphabets; see the Ukrainian translation of this SOP, for example).

2. The PM DNA Sample Code must be legible: 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. 0̄) and the number seven must have a dash through it (e.g. 7̄).
3. Proper labeling of the original skeletal element and the extracted sample itself is vital to the integrity of the Chain of Custody. The PM DNA Sample Codes aid in overall reviews of DNA analysis results, which identify patterns of success rates by the skeletal element, which in turn direct the sampling selection process. Furthermore, the differentiation of primary, reserve, and resubmission samples through proper labeling assists in the quality control and coordination of the sampling and the case management process.

### 8.8. Determining a PM DNA Sample Code

1. The PM DNA Sample Code should not have blank spaces. The PM DNA Sample Code consists of the site location (Site Code or otherwise abbreviated location name), the case number, and the sided skeletal 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**)

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<sup>2</sup> Schwark, T., Heinrich, A., Preuße-Prange, A. & Von Wurmb-Schwark, N., 2011. Reliable genetic identification of burnt human remains. *Forensic Science International: Genetics* 5: 393-399.

## 8.9. Site Code

1. This designation is assigned by the investigating authority and should be unique; the judicial order number may be appended in the PM DNA Sample Code as well.

## 8.10. Case Number

1. The field numbering and tracking system for human remains is assigned by the investigating authority or authorized personnel during the recovery operation, or exceptionally, in the mortuary. Often, case numbers may include a letter code that describes the remains, for example:
  - B for Body (complete or incomplete);
  - BP for Body Part (two or more in situ articulated skeletal elements);
  - I for Isolated Bones;
  - GBP for General Bone Bag (scattered or concentrated assemblage of disarticulated, fragmented, and commingled remains).

## 8.11. Sided Specimen Code

1. The codes for the specific element usually include a letter for the side of the body:
  - L for Left;
  - R for Right;
  - U for Unsided, when the side could not be determined.
2. See Appendix 1 for a complete list of codes for teeth and bones.

## 8.12. Element Number

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

THAI-Ø86GBP-LF1  
THAI-Ø86GBP-LF2

## 8.13. Determination of the PM DNA Sample Code for a Resubmission Sample from the Same Bone

1. If the primary sample failed to yield a DNA profile, then another sample may be selected from the same skeletal element; this is a resubmission sample. To differentiate it from the primary sample the identifier – **RSB** – should be added to the existing PM DNA Sample Code of the primary sample. For example, for a right tibia (RT):

THAI-199BP-RT1 → THAI-199BP-RT1-**RSB**

2. If there are repeat resubmissions from the same skeletal element, then a numerical identifier should be added. For example, for a right tibia (RT):

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

#### **8.14. Determination of the PM DNA Sample Code for a Resubmission Sample from the Same Body or Body Part, but Different Bone**

1. 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**

#### **8.15. Determination of the PM DNA Sample Code for a Sample Submitted as a Reserve**

1. An additional sample taken from the same skeletal element or same Body or Body Part as the primary sample and sent to the HID laboratory alongside its primary sample, is designated as a “reserve sample”. Reserve samples will be retained for a designated period by ICMP and processed only if the primary 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 as their primary samples; *if there is any doubt, samples should be treated as primary and given their own PM DNA Sample Code*. The reserve samples should have the identifier **–RES** – added to the existing PM DNA Sample Code of the primary sample. For example, for a left tibia (LT):

THAI-234BP-LT1-**RES**

2. 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 challenging.

#### **8.16. Sample Packaging and Chain of Custody**

1. The Chain of Custody procedures outlined in the *Standard Operating procedure for the Transfer of Samples, Submission Tables and DNA Match Reports (ICMP.SOP.ST.170.doc)* should be followed when preparing samples for DNA analysis.
2. Failure to comply with the recommended Chain of Custody procedures may result in a delay in the processing of samples for DNA analysis.

#### **8.17. PM DNA Sample Envelope**

1. In general, ICMP’s *PM DNA Sample Envelope (ICMP.ST.LS.1172.doc)* should be used for packaging where possible; the envelope should be tape-sealed, signed, and dated over the seal. Official rubber stamps can also be used in lieu of signatures or on the seal of the *Envelope*. If another packaging will be used, sample recording procedures as described in Section 8.1., paragraph 3 and VI. Quality Assurance should be followed regardless.
2. The ICMP *PM DNA Sample Envelope* contains essential information on the PM DNA Sample provenance and other information crucial for maintaining the integrity of the Chain of Custody. All information on the *Envelope* is mandatory. In instances where information is unknown or unavailable, ‘N/A’ (not available) should be placed in the relevant cell and the explanation noted in the *SOP/Policy Deviation Form*, which must accompany the samples with the Chain of Custody document.
3. The information required on the PM DNA Sample Envelope is the following:
  - **PM DNA Sample Code** – unique sample code assigned to the sample;

- **Sample Type** – short description of the sampled specimen (type and side of the sampled skeletal element, or FDI notation number of the extracted tooth or teeth);
- **Time/Date Seized/Produced** – exact time/date the sample was cut or extracted;
- **Location/Facility** – the exact (official) name of the field location, or official name of the Facility/Mortuary where sampling was conducted;
- **Town/Municipality/Country** – the exact names of places, related to the location of the mortuary where the skeletal remains were examined and sampled;
- **Seized/Produced By** – names of persons cutting/extracting and physically placing the PM DNA sample inside the envelope or container;
- **Signature** – signatures of persons that seized/produced the sample;
- **Forensic Expert/Legal Authority** – name of the person in charge of the examination of skeletal remains (person named on the judicial order with jurisdiction over the respective case, usually a forensic pathologist, coroner, or court-appointed medicolegal expert);
- **Signature and Date** – the signature of the Forensic Expert/Legal Authority;
- **Source Case Number** – the complete Case Number assigned by the investigating authority (see 8.10.);
- **Location/Grid & Date** – exact location and GPS coordinates, if available, of the location where skeletal remains were found and date of the exhumation/recovery;
- **Court or Judicial Order number:** number of the document or judicial order number provided by a designated authority in charge of the excavation and/or examination of the human remains.

#### 8.18. Checklist

1. In contexts where it is not possible to undertake a complete forensic anthropology examination, a checklist form *ICMP.ST.AA.1259.doc – Forensic anthropology checklist when collecting PM Samples* should be completed in order to provide as much detail as possible about the context of the case.

**Appendix 1: List of teeth and skeletal elements to sample with priority ranking and coding system**

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

Region	Bone/Tooth Element	Priority	Suffix	Left	Right	Unsided
HEAD	Mandibular Tooth	1	MNT			
	Maxillary Tooth	1	MXT			
	Petrous Part of Temporal Bone	1	PT	LPT	RPT	UPT
	Mandible	2	MN	LMN	RMN	UMN
	Occipital Bone	3	OCP			
	Parietal Bone	4	PR	LPR	RPR	UPR
	Skull – Other – Cranial Vault	4	SK			
	Facial – Cranial Bones	6	FC			

TORSO	Innominate Bone	1	P	LP	RP	UP
	Cervical Vertebra	2	CV			
	Thoracic Vertebra	2	TV			
	Lumbar Vertebra	2	LV			
	Scapula	3	SC	LSC	RSC	USC
	Rib	4	RB	LRB	RRB	URB
	Clavicle	5	CL	LCL	RCL	UCL
	Sacrum	6	SAC			
	Manubrium/Sternum	6	ST			

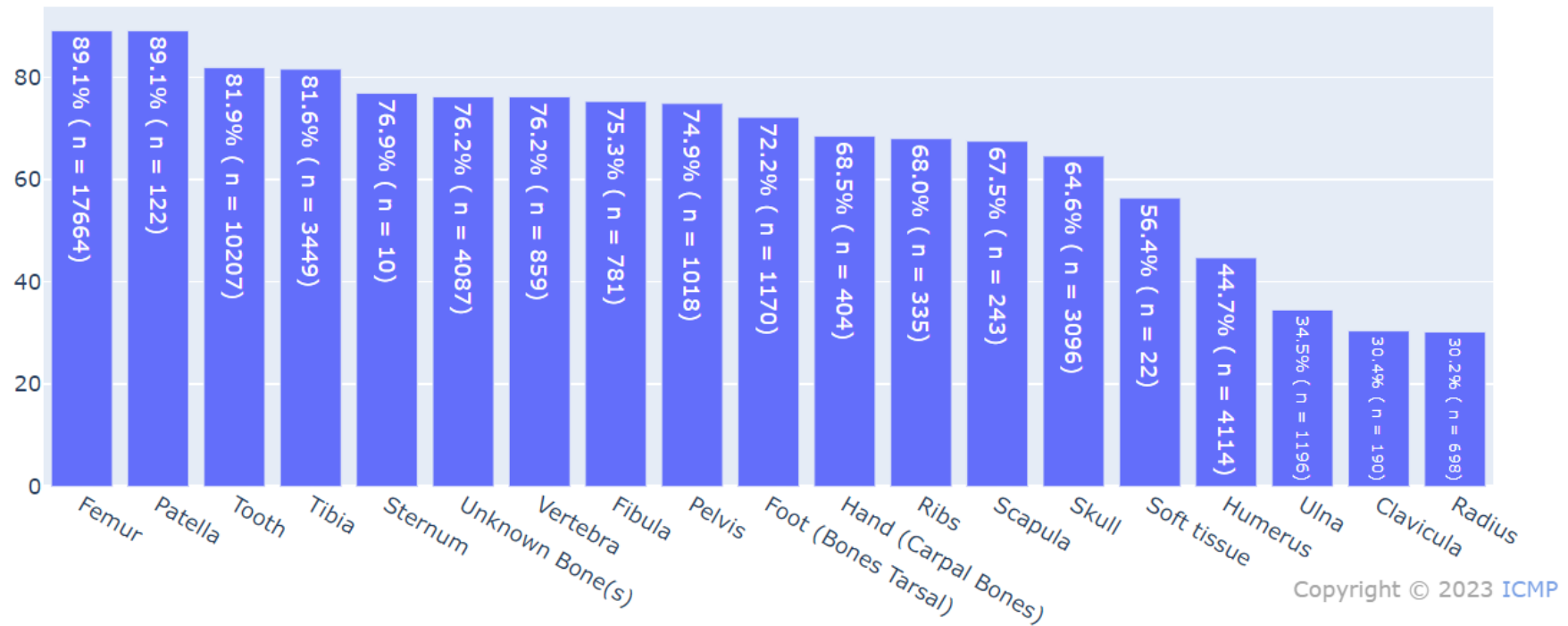
ARM	Humerus	4	H	LH	RH	UH
	Radius	5	R	LR	RR	UR
	Ulna	5	U	LU	RU	UU

HAND	Metacarpal	3	MC	LMC	RMC	UMC
	Carpal	4	CR	LCR	RCR	UCR
	Hand Phalanx	4	HPH	LHPH	RHPH	UHPH

LEG	Femur	1	F	LF	RF	UF
	Tibia	1	T	LT	RT	UT
	Fibula	2	FB	LFB	RFB	UFB
	Patella	3	PAT	LPAT	RPAT	UPAT

FOOT	Metatarsal	1	MT	LMT	RMT	UMT
	Talus	1	TAL	LTAL	RTAL	UTAL
	Tarsal	3	TR	LTR	RTR	UTR
	Calcaneus	3	CAL	LCAL	RCAL	UCAL
	Foot Phalanx	4	FPH	LFPH	RFPH	UFPH

**Appendix 2: Success Rates of DNA Extraction from Teeth and Individual Skeletal Elements<sup>3</sup>**



<sup>3</sup> The real-time update on sample success rates per sample type is available on ICMP's IDMS Dashboard; the chart supplied here is from August 2023.

### **Appendix 3: Sampling descriptions of preferred teeth and 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 the degradation of the DNA of the bone.

Avoid sampling areas that exhibit individualizing characteristics that may be used for identification purposes, for example, teeth that exhibit dental work, antemortem trauma, or pathological features. In addition, avoid sampling along fracture edges (peri- or postmortem), as these areas may contribute to future reassociation efforts.

#### **TEETH**

Intact and well-preserved teeth with completely formed root apices are preferred. 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 analysis. Record the tooth number submitted; ICMP prefers that FDI World Dental Federation notation<sup>4</sup> is used for this purpose; if another dental notation system is used, this should be noted in the *SOP/Policy Deviation Form*. 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 PM 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 antemortem 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.

Avoid sampling loose 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 fulfill the above-stated criteria, it is recommended that two teeth from either the maxilla or the mandible are submitted for sampling. A

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<sup>4</sup> See ISO 3950 "Dentistry — Designation system for teeth and areas of the oral cavity, <https://www.iso.org/standard/68292.html> [ACCESSED May 2023]

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.

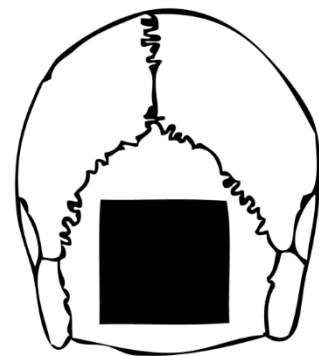
## SKELETAL ELEMENTS

Based on Hines *et al.* 2014<sup>5</sup>, 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; sutures and fractures 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.

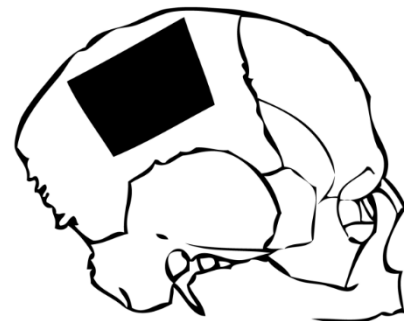
Owing to the relatively large sample size, an occipital bone is sampled only once.



### 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; sutures and fractures 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 part of the temporal bone.

In the case of a subadult cranium with thin vault bones, sampling of the petrous part 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)*



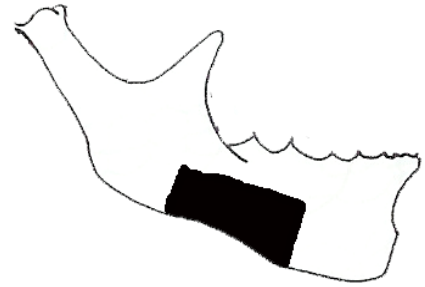
<sup>5</sup> Hines, D.Z.C., Vennemeyer, M. Amory, S., Huel, R. LM., Hanson, I., Katzmarzyk, C., Parson, T.J. 2014. Prioritized sampling of bone and teeth for DNA analysis in commingled cases. In: Adams, B. and Byrd, J.E. (eds.). *Commingled Human Remains: Methods in Recovery Analysis and Interpretation*. Pp: 275-305. London: Elsevier.

### **Mandibular Body**

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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)*

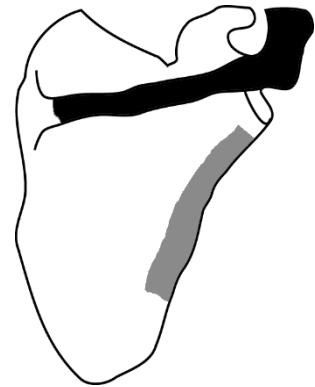


### **Scapula**

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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**

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

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



### **Manubrium and Sternum**

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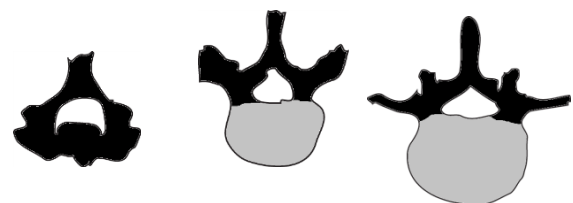
A complete or mostly complete manubrium or sternum may be sampled. Select the area(s) best preserved with the most cortical bone.

### **Vertebrae (Cervical, Thoracic, and Lumbar)**

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The transverse and spinous processes (black) have first priority for the thoracic and lumbar vertebrae. The vertebral body (gray) are second priority. A complete cervical vertebra may be submitted, if possible.

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



**Cervical**

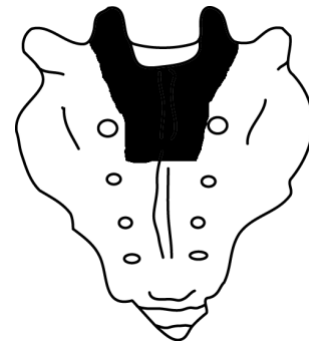
**Thoracic**

**Lumbar**

## Sacrum

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

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

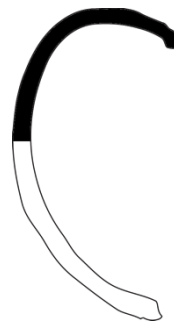


## Ribs

The vertebral half (black) of one of the middle ribs is a priority. A complete or mostly complete upper or lower rib (gray) have second priority.



**Upper (1<sup>st</sup> to 3<sup>rd</sup>)**



**Middle (4<sup>th</sup> to 9<sup>th</sup>)**

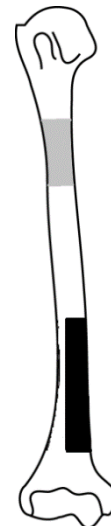


**Lower (10<sup>th</sup> to 12<sup>th</sup>)**

## Humerus

A window of bone from the distal medial shaft approximately 2 cm above the plane of the trochlea (black) has first priority. A window section at or near the proximal mid-shaft (gray) has second priority.

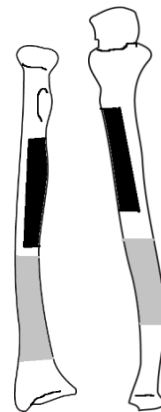
*Location and relative size of a humerus sample  
(A right humerus is illustrated)*



## Ulna and Radius

A window of bone removed from the proximal shaft along the interosseous crest (black) has first priority. A cross-section of the distal mid-shaft (gray) has second priority.

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



## Hand Bones

The metacarpal and capitate are the preferred bones when sampling a complete hand.

### Hand Phalanges

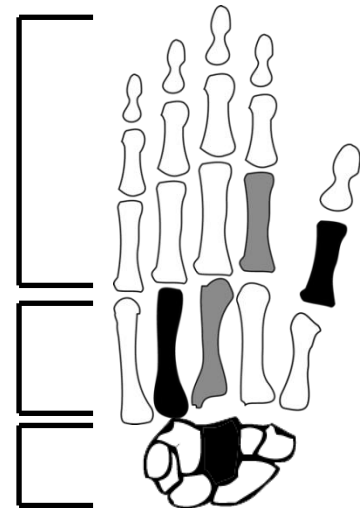
The 1<sup>st</sup> proximal phalanx (black) has first priority.  
The 2<sup>nd</sup> proximal phalanx (gray) or another large hand phalanx have second priority.

### Metacarpals

The 4<sup>th</sup> metacarpal (black) has first priority.  
The 3<sup>rd</sup> metacarpal (gray) has second priority.

### Carpals

The complete capitate (black) has first priority.  
Any available larger carpal bones have second priority.

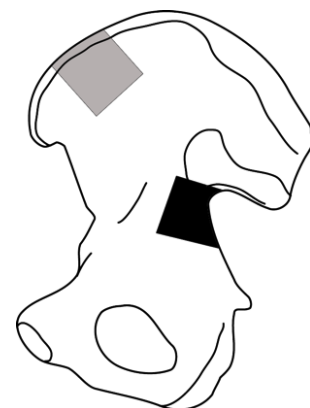


## Innominate Bone

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

Use a fantail blade to extract the sample from the innominate bone.

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

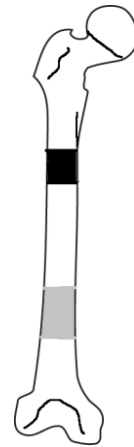


## **Femur**

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A window of bone removed from the proximal anterior shaft below the plane of the lesser trochanter (black) has first priority. A window of bone removed from the distal anterior shaft (gray) has second priority.

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



## **Patella**

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A complete or mostly complete patella may be sampled.

## **Tibia**

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A window of bone removed from the anterior proximal shaft (black) has first priority. A window of bone is removed from the distal anterior shaft (gray) has second priority.

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



## **Fibula**

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A window of bone is removed from the distal shaft. In some cases, a cross-section of the shaft is necessary. If so, the remaining 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.

### **Foot Phalanges**

The 1<sup>st</sup> proximal phalanx (black) has first priority.  
The 2<sup>nd</sup> proximal phalanx (gray) or another large foot phalanx have second priority.

### **Metatarsals**

The 5<sup>th</sup> metatarsal (black) has first priority.  
The 1<sup>st</sup> metatarsal (gray) has second priority.

### **Smaller Tarsals**

The complete navicular (black) has first priority.  
The cuboid (gray) has second priority.

### **Talus**

A complete or mostly complete talus may be sampled.

### **Calcaneus**

The posterior area or the body of the calcaneus (black) has first priority.

