Bone & Soft Tissue Grossing Practices & Techniques

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Bone & Soft Tissue

Bone & Soft Tissue Pathologists are:

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Remember: For any tumor adjacent to / involving bone, if any part of the tumor is SOFT and does not NEED decalcification, please isolate 1-2 sections and submit as non-decalcified tumor (important for possible molecular testing). Then proceed with decal as necessary for the remainder of the case. Alternatively, if molecular testing is anticipated, fix and decalcify a portion of tumor in EDTA.
Challenge: Closely associated bone, soft tissue, and tumor

Such as this hand amputation:
Challenge: Closely associated bone, soft tissue, and tumor

- With large specimens such as this, ideally they should be triaged **same-day** as receipt
  - Prefer to “open up” the tumor to start fixation
  - Do not immerse entire specimen in formalin and expect tissues to fix properly

- If very late in the day, can place in fridge over night and address first thing in morning
# OPTIONS for grossing:

<table>
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<th>How</th>
<th>Pros</th>
<th>Cons</th>
<th>When to consider</th>
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</thead>
<tbody>
<tr>
<td>Score</td>
<td>Score the soft tissue and cut through bone with saw</td>
<td>• Can be done fresh or fixed</td>
<td>• May result in uneven sections depending on skill of grosser / complexity of specimen</td>
<td>• When most pathology is in the soft tissue and there is a single / simple core of bone (mandible)</td>
</tr>
<tr>
<td>Shave</td>
<td>Shave off as much soft tissue as you can and gross soft tissue and bone separately</td>
<td>• Can be done fresh or fixed</td>
<td>• Does NOT preserve relationships of bony / soft structures</td>
<td>• When most pathology is in the bone (osteosarcoma)</td>
</tr>
<tr>
<td>Freeze</td>
<td>Freeze the FRESH specimen in liquid nitrogen (until hard) or deep freezer (overnight) then cut entire specimen with saw</td>
<td>• Preserves relationships of bony / soft structures</td>
<td>• Can only be done FRESH – time sensitive</td>
<td>• When soft and hard parts and tumor are intimately admixed (soft tissue tumor surrounding and invading bone OR bone tumor invading soft tissue)</td>
</tr>
<tr>
<td>Fix &amp; Decal</td>
<td>Fix the specimen fully, decalcify it entirely, and cut with regular long blade</td>
<td>• Preserves relationships of bony / soft structures</td>
<td>• Requires decalcification of entire specimen and eliminates the possibility of molecular testing</td>
<td>• When bone is sparse, thin, delicate, and would suffer fragmentation from the above (maxilla, alveolar ridge)</td>
</tr>
</tbody>
</table>
Challenge: Closely associated bone, soft tissue, and tumor

Such as this hand amputation:

The sarcoma surrounds small bones
Example of *Freeze*:

Good when soft and hard parts and tumor are *intimately associated*

Bones surrounded by tumor / soft tissue
Maintains relationships in specimens with complex anatomy:

Tumor
These slices were ~1 cm in thickness. Too thick for submitting in cassettes. What are our options? (after fixation)

Isolate select areas of bone OR tumor interface with bone. Decalcify ONLY those sections. Thin down with knife after decal.

Isolate areas of soft tumor without bone. Do NOT decalcify. Thin down with knife.
Challenge: Closely associated bone, soft tissue, and tumor

Such as this arm amputation:

The sarcoma is in the humerus
Example of *Shave*:
Good when most pathology is in the *bone*

Bone was isolated and then cut with saw:
Challenge: Closely associated bone, soft tissue, and tumor

The carcinoma is in the mucosa / soft tissue.

Such as this mandibulectomy:
Example of Score:
Good when most pathology is in the *soft tissue* and there is a single / simple core of bone

Soft tissue/tumor was scored with knife:

Bone and teeth were cut with bone saw in the same plane
If these slices were ~1 cm in thickness...
Too thick for submitting in cassettes.
What are our options? (after fixation)

Isolate select areas of bone OR tumor interface with bone.
Decalcify ONLY those sections.
Thin down with knife after decal.

Isolate areas of soft tumor without bone.
Do NOT decalcify.
Thin down with knife.
Example of *Fix & Decal*:
Good when bone is *sparse, thin, delicate,* and would otherwise suffer fragmentation

- If you want to decalcify a **whole** specimen
  - (after formalin fixation of course)
- **DISCUSS WITH AN ATTENDING FIRST**
- **DETERMINE IF THERE IS A PRIOR NON-DECALCIFIED BIOPSY**
- Consider all options before deciding on this “last ditch” approach
Example of *Fix & Decal*:

Good when bone is *sparse, thin, delicate,* and would otherwise suffer fragmentation

Palate/maxillary sinus or Alveolar ridge:

The carcinoma is in the mucosa covering bone

May destroy lesion by shaving or using harsh bone saw
Example of **Fix & Decal**:

Good when bone is *sparse, thin, delicate,* and would otherwise suffer fragmentation

Palate/maxillary sinus or Alveolar ridge:

- Tissue covering bone is scant and delicate
- Bone has been sectioned intraoperatively such that marrow space is opened (not a full circumference of dense cortex)
Example of *Fix & Decal*:

Good when bone is *sparse, thin, delicate,* and would otherwise suffer fragmentation

- In these circumstances, you may want to consider full fixation and full decalcification
- Teeth and bone fix/decalcify/cut similarly
- Fillings and cement do NOT fix/decalcify/cut
  - These need to be removed
- Sometimes decal “thins out” the tooth / bone so that fillings can be popped out
Example of *Fix & Decal*:

Good when bone is *sparse, thin, delicate,* and would otherwise suffer fragmentation

Now you have full sections of all important components:

Tumor with interface to tooth and bone.
Score, Shave, Freeze, Fix & Decal

• Questions, comments?
Challenge: Need for molecular testing

• Not a problem with soft tissues
• Becomes a potential problem with bony / calcified tissues in which molecular testing is needed for
  – Neoplasms (to detect mutations)
  – Infections (to detect organisms)
    • Valves, synovium, bone
    • Molecular testing is less utilized here so we won’t focus on it
  – You name it
• Need decalcifier that preserves nucleic acids
  – NGS, PCR, FISH
Decal

• Decalcification can be performed for
  – Bone, teeth, otherwise calcified tissues
  – That cannot cut with your knife (and therefore also cannot cut with a microtome in histology)
  – Don’t forget about Decal in:
    • Atherosclerotic arteries in limbs/hearts
    • Calcified heart valves
    • Partially calcified tumors

• **FULL FIXATION IS ALWAYS PERFORMED BEFORE DECALCIFICATION**
How to Decalcify

• **Never** decalcify an **entire** specimen before consulting with an attending first
  – Check to see if there is a pre-existing recent non-decalcified biopsy
  – Determine extent of HCL vs EDTA (will discuss)

• **Always** try to **isolate** soft lesional tissue to submit WITHOUT decalcification:
  – Tumor next to bone
  – Soft tumor within bone
  – Noncalcified tumor
How to Decalcify

• If there is **no way** to submit tumor without decalcification:
  – Bone core for metastasis
  – Predominantly ossified tumor (osteosarc)
  – Tumor diffusely infiltrating bone with no soft component

• If it is a biopsy:
  – Decalcify entirely in **EDTA after fixation**

• If it is a medium-large specimen:
  – Decalcify at least 1 (if not more) sections in **EDTA after fixation**
  – **WHY?**
Decal Solutions & Limitations

• **Strong acids**
  - Hydrochloric acid*, nitric acid
  - Fastest (hours to days) but destroys nucleic acids
  - **ALL BONY/CALCIFIED TISSUES HAVE ROUTINELY BEEN DECALCIFIED IN HCL at UChicago**

• **Weaker organic acids**
  - Formic acid
  - Slower and somewhat more gentle but not great at nucleic acid preservation

• **Chelating agents**
  - Ethylenediaminetetraacetic acid (EDTA)
  - Slowest (hours to days to weeks) but the most gentle and best for preserving nucleic acids
How to evaluate nucleic acids?

- **DNA quantity:**
  - Qubit2.0 Fluorometer (ThermoFisher)
  - **100 ng quant** is needed for in-house assays

- **DNA quality:**
  - fragment analysis (TapeStation 2200, Roche)

- **DNA quantity and quality:**
  - qPCR (Kapa hgDNA Quantification and QC kit)
# Prior Internal Study

**HCL vs Formic Acid vs 5% EDTA vs None**

<table>
<thead>
<tr>
<th>Qubit ng/ul</th>
<th>total Qubit ng</th>
<th>hgquant ng/ul</th>
<th>hgquant total ng</th>
<th>Decal</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW</td>
<td>0</td>
<td>0.0055281</td>
<td><strong>0.41460761</strong></td>
<td>HCL</td>
</tr>
<tr>
<td>55.4</td>
<td>4155</td>
<td>0.06351738</td>
<td><strong>4.76380316</strong></td>
<td>Formical-2000 (Formic Acid)</td>
</tr>
<tr>
<td>89.8</td>
<td>6735</td>
<td>22.004029</td>
<td><strong>1650.30217</strong></td>
<td>EDTA (5%)</td>
</tr>
<tr>
<td>106</td>
<td>7950</td>
<td>75.7562345</td>
<td><strong>5681.71759</strong></td>
<td>No Decal</td>
</tr>
</tbody>
</table>

Courtesy Lauren Ritterhouse, MD
Prior Internal Study

100 ng as measured by hgquant (red line) is how much DNA is needed for in-house OncoPlus

Courtesy Lauren Ritterhouse, MD
Recent Study
10% EDTA vs 18% EDTA

- Tonsil and Solid tumor tissue
- 0 days through 21 days
- In 10% and 18% EDTA
- All were >100 ng quant
- 18% had less DNA quantity than 10% but still adequate after 3 STRAIGHT WEEKS

Jeremy Segal, MD PhD / Filippo Galbo
DNA quality of the six 18% EDTA tumor specimens remained adequate for sequencing across time points (through 21 days)
The point is...

• Always critically evaluate specimens that need decalcification

• If it is a biopsy for *possible* tumor and **all** needs to be decalcified -> use EDTA 18%

• If it is a medium-large specimen and you can grossly see tumor:
  – First choice: If there is soft tumor:
    • Submit as much soft tumor as you need that does NOT need decal
    • Submit some following HCl
    • If you have DEFINITE soft tumor, EDTA may not be needed (since you already have non-decalcified tumor)
    • Ask an attending if questions
  – Second choice: If there is NO soft tumor:
    • Submit some cassettes following EDTA 18%
    • Submit some following HCl
How to document Decal in CoPath?

- MUST enter Decalcification charge for each container (not necessarily each block) in which you use decal (either HCl or EDTA)
  - Generates a billing charge

- In Histology Data Entry/Edit
- In Stain/Process
- Search for decal
- Select “Decalcification” (Dcal)
- The block designation does not matter, as you only need one entry per PART, regardless of how many cassettes you Decal
How to document Decal in CoPath?

• MUST dictate in your **cassette summary** which cassettes were decalcified and in **which** solution
  – Must do this for all cassettes
  – Confirms that the charge is legitimate

If you don’t specify EDTA vs HCl, we assume HCl:

- Cassette Summary
  - B1-6: Representative tumor from slice #1, *EDTA-decalcified* (see diagram)
  - B7: Marrow margin, following decalcification (from slice #4)
  - B8-9: Nearest anterior margin, perpendicular, following decalcification
  - B10-12: Nearest posterior margin, perpendicular, following decalcification
  - B13-14: Nearest medial margin, perpendicular, following decalcification
  - B15-16: Nearest lateral margin, perpendicular, following decalcification
  - B17-19: Skin, entirely, with lesion, following decalcification
  - B20-40: Full face of bone with tumor, following decalcification (see diagram, slice #2)
  - B41-59: Full face of bone with tumor, following decalcification (see diagram, slice #4)

• Entering Decal in **Vantage** alerts Histology to a possible delay but does not generate a charge
Options

10% EDTA

18% EDTA

HCl