Original Article

Bone and Soft Tissue Extirpations: Whole-Specimen Freezing Delivers Superior Pathological Evalulation

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ABSTRACT

Background: Bone resections involved by either benign or malignant disease are complex specimens requiring special processing. Irrespective of experience, many pathologists, residents, and pathologists' assistants (PAs) are apprehensive of these resections due to their infrequency. For these extirpations, serial slab-sections are ideal for identifying margin status, size of tumor, documentation of diagnosis, tumor classification, imaging correlation and presence of discontinuous lesions. However, the variable density of bone and soft tissues creates a challenge for prosectors to reliably yield multiple intact thin slabs. Standardized protocols provide both reassurance and a systematic approach and herein we describe a method as implemented at the sister institutions of Mayo Clinic Hospital in Phoenix and Phoenix Children's Hospital.

Methods: Utilizing the approach of whole-specimen freezing and slab-sectioning, we prospectively processed 41 cases of bone and soft tissue resections between 2011 and 2014 and histologic sections were retrospectively evaluated for freeze artifact, bone dust, thermal injury and immunoreactivity. Slab-sectioning following whole-specimen freezing resulted in crisply visible anatomic relationships across multiple planes allowing for superior gross inspection, easy correlation with prior imaging, photographic documentation and ease in the selection of histologic sections.

Result: Microscopically, freeze artifact was present in 6 of 39 (15%) cases available for review, but was insignificant to interpretation and was not affected by freeze duration (up to 72 hours). No loss of immunoreactivity was present (0 of 5 cases) and neither bone dust nor thermal injury were significant findings in any of the cases.

Conclusion: The protocol is easy to follow, yields reproducible results and induces no significant freeze artifact, providing excellent histomorphology regardless of tumor type involving bone. We recommend slab-sectioning following whole-specimen freezing and we offer our procedure in detail.

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Introduction

Specimens submitted for pathological examination composed of bone range from core biopsies to curetted lesions to large-scale resections which often require specialized handling, additional processing steps and unique tools for analysis. As these tissues are procured from many different surgical procedures, bone tumors may be received in myriad states. Specialized equipment, such as diamond or band saws, is usually required as is the necessity for additional processing steps such as decalcification, radiography and photography. The goals of specimen evaluation are multiple, including documentation of diagnosis, margin status, disease extent, tumor classification, imaging correlation, and response to neoadjuvant treatment, if applicable.[1] To this end, standard protocols for processing bone extirpations are desirable because they yield reproducible results. The following procedure is easy to follow and affords the pathologist a precise gross examination and exceptional histology, thereby yielding all of the information necessary to render a clear diagnostic report.

Materials and Methods

We detail here our standard method of whole-specimen freezing and slab-sectioning process; standard grossing elements are beyond the scope of this discussion and have been previously described.[2-5] Upon receipt of the intact specimen, gross photographs are obtained and archived in the institutional lab information system (LIS). The specimen is radiographed as roentgenograms provide diagnostic information and delineate tumor burden for the prosector, thus determining how the specimen should be sectioned.[1-2,4,6-8] Radiographs may be obtained with a Faxitron x-ray cabinet or if the specimen is too large it may be transported in a safe, mindful manner to radiology for imaging. If radiographing is not a feasible option, the patient's previous imaging is reviewed in conjunction with the clinical and radiologic impressions which can also assist in proper orientation for subsequent sectioning and analysis.[2,4-5,7-9] The specimen is oriented and measurements are taken, making note of vessels, soft tissue, skin, etc. as described.[2-5] Prior to sectioning, communication with the submitting surgeon ensures all margins or areas of concern are addressed. The margins of interest are sampled before inking the specimen, i.e. vascular or neurovascular bundles, as these may be difficult to find after inking. These samples are placed in a duly labeled cassette for future processing. The specimen is inked in one or more colors depending on preference or the number of margins to be assessed. All areas of previous sampling, such as a vascular margin, may be inked with a different color for re-identification. Metal hardware, if either adherent to or embedded within the specimen, is extricated. Radiographing the specimen allows the prosector to visualize the extent of implanted hardware that cannot be removed and helps guide sectioning. The specimen is placed in a freezer (-70° C to -140° C) for a minimum of four hours before sectioning. Ensure the specimen is completely frozen to prevent thawing during sectioning; it may be left in the freezer overnight or over the weekend without risk of introducing artifact.

Utilizing proper protective equipment (gown/apron, gloves, Kevlar gloves, impact-resistant face shield and respirator mask), the specimen is retrieved from the freezer and is sectioned. A saw suitable for bone and soft tissue is employed, such as a Torrey or comparable butcher saw. The first cut is a transverse section to include the bone and soft tissue margins--an en face section--which is subsequently fixed, decalcified and submitted. Positive surgical margins are correlated with local recurrence[7-8,10] and a predictor of poor prognosis.[11] If the extirpation is large, such as a leg disarticulated at the hip, the specimen may be sectioned into smaller components—tumor versus non-tumor—for ease of subsequent cutting. Utilize imaging studies for reference to divide the resection. The specimen should then be cut along its long axis in 4-6 mm serial slices, either coronal or sagittal, in the plane demonstrating maximum tumor burden and using one continuous cut.[1-2,4-6,8-9] The opposing end/side slabs may be further sectioned in a perpendicular fashion to demonstrate tumor relationship to these margins. The resulting slabs are gently cleaned of bone dust under cool, running water with the aid of a sponge, scour pad or surgical brush. Save tissue for ancillary studies if warranted. Slabs are wrapped in moist paper towels to prevent sections from adhering to one another taking care to maintain orientation. The specimen slabs are then photographed (**Fig.1**) and the images stored in the LIS. Reconstruct the specimen and record tumor measurements and margin relationships. Consult with the case pathologist and review the specimen to determine which slab should be submitted. Once selected, this slab should be photographed separately and stored as described. Keep in mind the overall aims of histologic sampling when determining areas for submission: tumor classification/subclassification, presence or absence of lesional tissue, anatomic distribution, if tumor is present how much is viable versus necrotic and documentation of any reactive processes.[1] One of the best prognostic factors following neoadjuvant chemotherapy is tumor necrosis greater than 90%.[1,3,6-7,9-11] To this end, one entire slab must be submitted for histologic evaluation. Please note some primary bone malignancies other than osteosarcoma and the Ewing's family of tumors may not require extensive evaluation. In these cases, refer to your institution's standard of practice regarding submission guidelines.

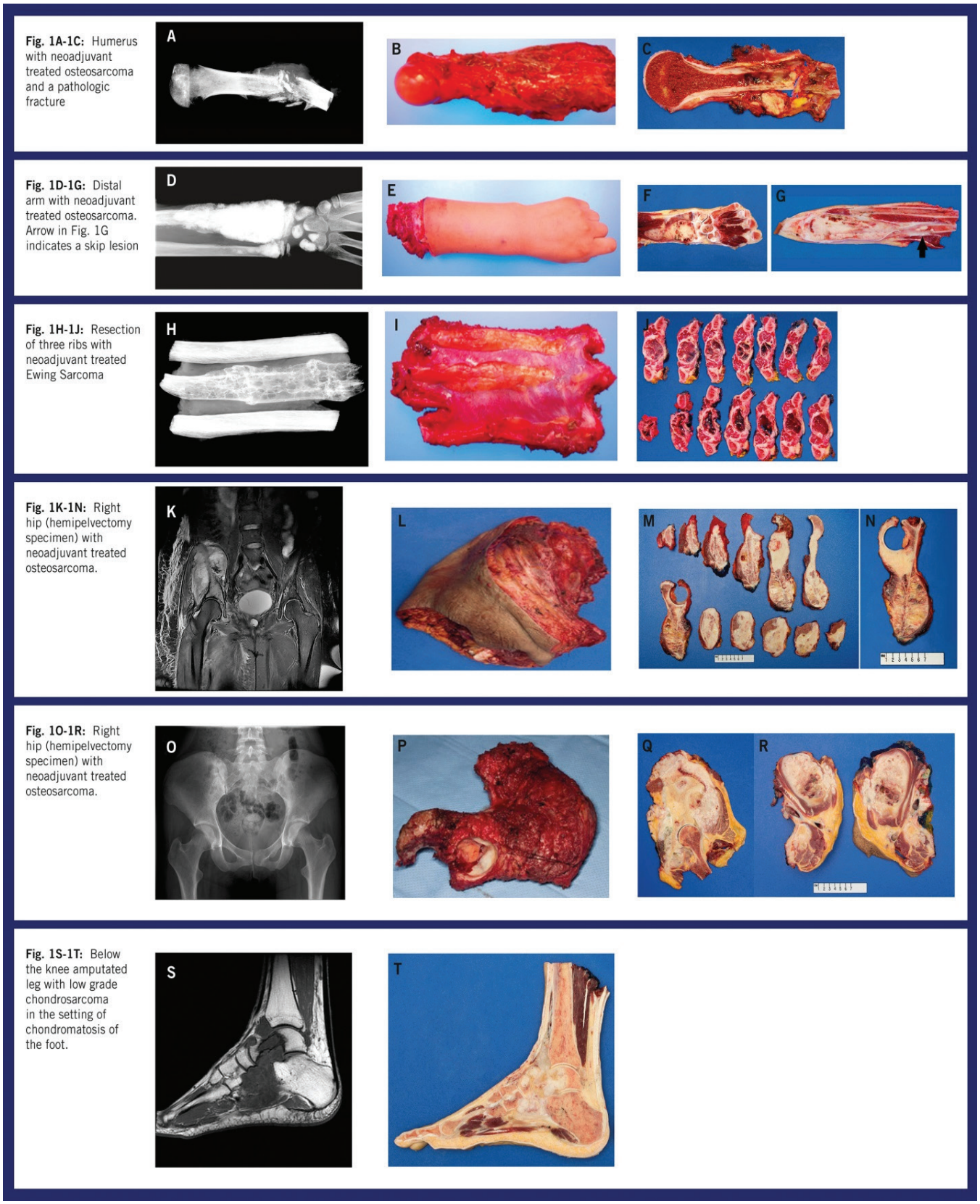
Fig. 1A-1C: Humerus with neoadjuvant treated osteosarcoma and a pathologic fracture
A
B
C
Fig. 1D-1G: Distal arm with neoadjuvant treated osteosarcoma. Arrow in Fig. 1G indicates a skip lesion
D
E
F
G
Fig. 1H-1J: Resection of three ribs with neoadjuvant treated Ewing Sarcoma
H
I
J
Fig. 1K-1N: Right hip (hemipelvectomy specimen) with neoadjuvant treated osteosarcoma.
K
L
M
N
Fig. 1O-1R: Right hip (hemipelvectomy specimen) with neoadjuvant treated osteosarcoma.
O
P
Q
R
Fig. 1S-1T: Below the knee amputated leg with low grade chondrosarcoma in the setting of chondromatosis of the foot.
S
T

Fix the entire specimen for a minimum of 24 hours. Proper fixation ensures excellent morphology even with subsequent decalcification. Once the specimen is fixed, a lymph node search may be performed and all soft tissue margins and malleable areas of noncalcified tumor are sampled prior to placing the specimen into decalcification fluid. Decalcify the specimen in its entirety in the event additional sections are required. Check the specimen on a routine basis to prevent over decalcification which, depending on the agent employed, may hinder routine histochemical or immunohistochemical staining. At the termination of decalcification, rinse the specimen in running water to ensure removal of all decalcifying agent and sample. The sections submitted should be annotated on an accompanying map diagram. The corresponding archived images may be printed off and the map sections carefully drawn onto the picture which will go with the report for the pathologist to reference during interpretation. Further, the image may be manipulated in a picture editing program, such as Power Point, and the map diagram printed off and delivered to the pathologist for reference at microscopic review. All map diagrams should be archived and saved in the patient's permanent record.

Result

Using this method, we prospectively processed 41 cases of bone and soft tissue resections between 2011 and 2014. All slides available for review were analyzed by the Pathologists' Assistants with the Pathologists. Parameters examined included presence or absence of freeze artifact, bone dust and thermal injury and their significance to interpretation and also effects of this method on immunoreactivity and molecular testing. Our cohort of 39 tumors available for review included osteogenic sarcoma (26), Ewing sarcoma (4), chondrosarcoma (2), synovial sarcoma (2), metastatic renal cell carcinoma (1), neuroendocrine carcinoma (1), chordoma (1), enchondroma (1) and ghost cell odontogenic carcinoma (1). Histologically, each case demonstrated a minor degree of freeze artifact in the attached soft tissues. In relation to the lesional components, freeze artifact was identified in 6 of 39 (15%) cases available for review, but was insignificant to interpretation and was not affected by freeze duration (up to 72 hours). In fact, lack of nuclear detail and cytoplasmic shrinkage were attributed more to over-decalcification and inadequate fixation, respectively, than to freeze artifact. No loss of immunoreactivity was seen (0 of 5 cases). Cases requiring molecular analysis were performed on previous biopsy material. Neither bone dust nor thermal injury was significant in any of the cases (0 of 39). Slab-sectioning following whole-specimen freezing resulted in crisply visible anatomic relationships across multiple planes and provided excellent histomorphology regardless of tumor type. Further, pathologic fractures and both small and large joints remained intact with this method. This method was easy to employ and gives the prosector a standardized protocol to follow that yields excellent, reproducible results (**Fig. 2**).

Discussion

Bone tumors are procured from many different surgical procedures and thus may be received in various forms: curetted fragments to whole limb extirpations. The goals of specimen evaluation are multiple, including documentation of diagnosis, margin status, extent of disease, tumor classification, imaging correlation, and treatment efficacy, if applicable. To this end, a systematic approach to prosect these specimens is desirable because it yields easily reproducible results and provides the pathologist with the information necessary to render an accurate diagnosis encompassing all of the required report elements.

As described above, the entire extirpation is frozen for ease of sectioning and excellent retention of margin status. Several sources have proposed dissecting away the soft tissue to expose the normal and lesional bone before sectioning; [1-2,4-9] however, this may prove problematic in accurately submitting and reporting margin relationships. Shaving off soft tissue in the "region" of the tumor fails to accurately access tumor relationships. Freezing allows for all of the soft tissue margins to be definitively described and reported as the attached tissue stays intact versus "shredding" when cut at room temperature. Some extirpations may be large; i.e. an entire leg or arm with attached shoulder. These specimens should be approached in the same manner; however, they may be separated into smaller more manageable segments, such as tumor versus non-tumor. Strict correlation with previous or current imaging is integral if the prosector divides the specimen as to not impair tumor relationships. From our experience, table saws are the most efficient at sectioning the larger resections. Diamond saws, such as the table top Isomet (Lake Bluff, IL) may be used for smaller resections. Devices to assist in specimen stabilization are helpful; most table saws are equipped with guides to aid specimen cutting. Blocks of wood may be employed as wedges if proper guides are not available. As with all procedures in the gross room, safety is paramount. All personnel should be properly trained in the safety and use of saws and don appropriate protective equipment prior to their operation.

Using the protocol described above, we prospectively processed 41 bone and soft tissue tumors encompassing a wide variety of malignancies and retrospectively analyzed these cases for freeze artifact, thermal injury, bone dust, immunoreactivity and molecular testing results. Overall,

Nuclear Detail (H&E)

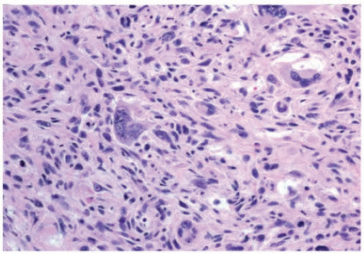

Fig. 2A: Neoadjuvant treated osteosarcoma with viable tumor – 20x

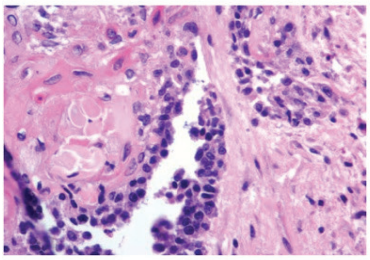

Fig. 2B: Odontogenic ghost cell tumor – 40x

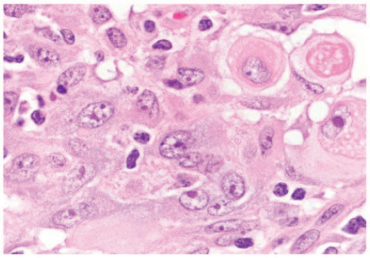

Fig. 2C: Chondrosarcoma – 100x

Immunohistochemistry

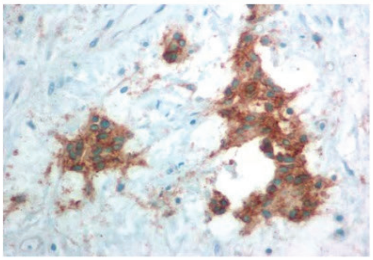

Fig. 2D: Ewing sarcoma – CD99 40x

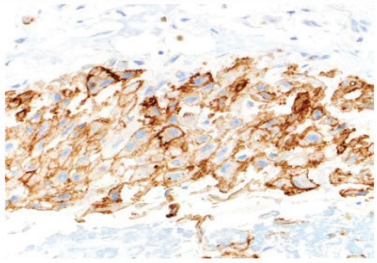

Fig. 2E: Chordoma – EMA 40x

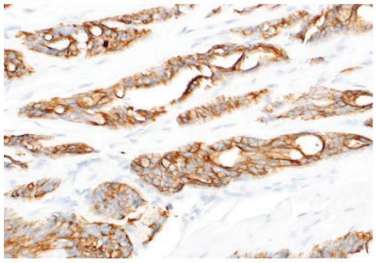

Fig. 2F: Synovial sarcoma – PanCK 40x

Freeze Artifact

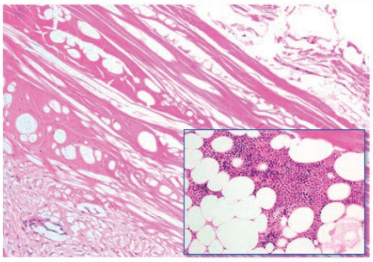

Fig. 2G: Artifact in soft tissue away from Osteosarcoma – 10x . Inset - Marrow with excellent nuclear detail from same specimen – 20x.

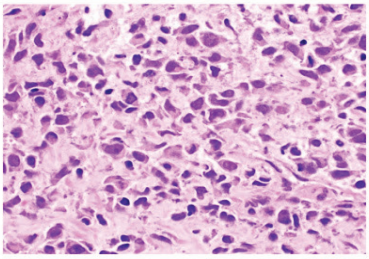

Fig. 2H: Neoadjuvant treated Osteosarcoma – 60x

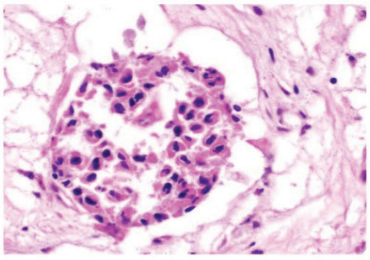

Fig. 2I: Neoadjuvant treated Ewing sarcoma – 60x

Bone Dust

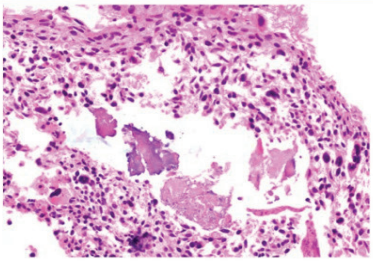

Fig. 2J: Osteosarcoma with no deleterious effect on diagnostic content – 20x

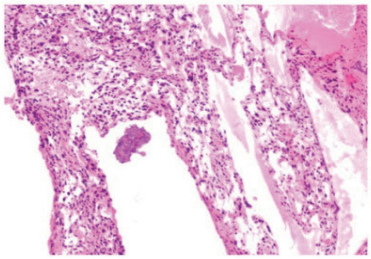

Fig. 2K: Osteosarcoma with no deleterious effect on diagnostic content – 10x

Fig. 2L: Chondrosarcoma case with minimal bone dust at resection margin – 10x

the method provided not only allows for excellent gross inspection but also yielded superb histoarchitecture. Of the 39 cases available for review, each case revealed minor freeze artifact in the attached soft tissue. In regards to the lesional/treated components, 6 of 39 (15%) cases harbored some degree of freeze artifact but was insignificant to interpretation and was not affected by freeze duration up to 72 hours. The artifact may have resulted from large, bulkier resections freezing more slowly than smaller resections. Further, lack of nuclear detail and cytoplasmic shrinkage were attributed more to over decalcification and inadequate fixation, respectively, than freeze artifact. Indeed, fixation is critical and the slabs should be separated by paper towels/gauze to ensure adequate exposure to the fixative of choice. Further, frequent monitoring for the end-point during decalcification is also integral for subsequent histologic preparation. Decalcifying specimens over the course of the weekend without proper monitoring should be avoided. Immunoreactivity was retained in 100% of the cases (5 of 5). Thermal injury inflicted by the cutting implement was not identified; however, cautery artifact from the surgical procedure employed was present in virtually every case. Bone dust was present in both resection margins and routine sections but was insignificant to interpretation. Care should be taken to thoroughly scrub the slabs to remove all macroscopic evidence of bone dust as to not impair histologic interpretation. Unfortunately, we could not gauge molecular test results as these studies were carried out on previous specimens.

Slab-sectioning following whole-specimen freezing resulted in crisply visible anatomic relationships across multiple planes examined and provided excellent histomorphology regardless of tumor type. We offer our standardized protocol in detail (**Table 1**).

Table 1: Whole-Specimen Freezing and Slab-Sectioning Protocol

Take gross photographs and store as either a hard copy or in the lab information system (LIS)
Evaluate the specimen as described. Sample margins of interest and ink the specimen.
Radiograph the specimen as x-rays provide diagnostic information, delineate tumor burden and drive specimen section or review the patient's previous imaging and/or radiological impressions which provide similar information.
Remove any metal hardware, if possible, following radiographic imaging.
Place the specimen in a freezer (-70 to -140°C) for a minimum of four hours - longer duration will not induce detrimental freeze artifact.
Use a saw suitable for cutting bone and soft tissue, such as a band saw or comparable butcher saw.
The first cut should be transverse to include the entire bone and soft tissue margin which is subsequently fixed, decalcified and submitted. Positive surgical margins correlate with local recurrence and are a predictor of poor prognosis.
Following cuts should be made along the long axis in 4-6 mm serial slices, either coronal or sagittal, in the plane demonstrating maximum tumor burden, utilizing one continuous cut. The opposing end/side slabs may be further sectioned in a perpendicular fashion to demonstrate tumor relationship to these margins.
Gently clean slabs from bone dust under cool running water with the aid of a sponge or surgical brush and then photograph and save the images as a hard copy or archive in the LIS.
Review slabs with the Pathologist to determine the section most representative of the tumor. One of the best prognostic factors following neoadjuvant chemotherapy is tumor necrosis greater than 90%. To this end, one entire slab must be submitted for histologic evaluation.
Fix the selected slabs for analysis in 10% neutral buffered formalin for a minimum of 24 hours.
Following fixation, perform lymph node search and sample all soft tissue margins and areas of noncalcified tumor prior to decalcification. Submit remaining sections following decal.
Map the sections as they are taken on the corresponding image.
Sectioning maps are utilized by the Pathologist during review and then stored either in the LIS or with the patient's permanent record.

Conclusion

Due to their infrequency in standard practice, resections harboring bone tumors may intimidate even the most experienced prosector. Whole-specimen freezing followed by slab-sectioning keeps the specimen strikingly intact. These multiple thin slabs allow for exceptional gross evaluation providing a vivid overall assessment of the tumor in relation to the soft tissue and bone margins, correlation with radiologic studies, photographic documentation and determining tissue for histologic sampling. The protocol is easy to follow, yields reproducible results and induces no detrimental freeze artifact, providing superb histomorphology regardless of the tumor type involving bone. We recommend slab-sectioning following whole-specimen freezing for all bone tumor resections.

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Competing Interests

None

Reference:

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