CHAPTER

1

Basics of Grossing Techniques

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The macroscopic examination of surgical specimens is called 'grossing' in surgical pathology. Grossing or "cut-in," or "histological dissection" is an art that requires skill and knowledge. It involves the accurate macroscopic description of an intact specimen, sectioning specimens in the correct fashion, examination of the cut surface, and selection of the most appropriate tissue blocks. This requires the correlation of clinical details, imaging information, knowledge of surgical anatomy of the organ/tissue, normal macroscopy, distinct gross features of the disease being suspected, and the related prognostic factors.

ROLE OF THE SURGEON

The surgeon has a vital role in helping a pathologist to generate a quality report. It is the operating surgeon who knows the most accurate whereabouts of the specimen. He knows the clinical details and the relevant laboratory investigations. He has already made up a provisional diagnosis or has narrowed it down to at least a set of differential diagnoses before operating the case. He knows where he placed his knife and what he had dissected out. The attending surgeon is the best person to orient any surgical resection specimen and identify the resection margins. The surgeon should endorse the warm and cold ischemia time on the requisition form.

Warm Ischemia Time

The warm ischemia time (WIT) is a non-modifiable pre-analytical factor. It is defined as the time from the surgical interruption of the blood supply to the lesion of interest to the excision of the tissue specimen.

Cold Ischemia Time

The standard cold ischemia time (CIT) is the time between tissue removal and the initiation of formalin permeation into the tissue. The exact time of tissue removal and placement of the specimen in formalin/transport medium should be documented. Delayed formalin fixation or prolonged CIT may impact the optimal expression of biomarkers or molecular analysis, especially if the specimen is kept at room temperature. Although the tissue specimens should be placed in formalin for fixation as soon as possible after resection, the permissible CIT is 30–60 minutes, particularly in breast specimens.

The Requisition Form

A requisition form contains the following set of information:

a. Patient particulars:
   - Patient full name
   - Age and gender
   - Unique patient identifiers–at least two assigned identifiers such as patient ID, hospital accession number assigned laboratory number, or barcode number

b. Date and time of collection/procedure
c. Referring clinician
d. WIT and CIT
e. Transport medium
f. Clinical diagnosis
g. Site, procedure, and type of specimen
h. Number of containers (if multiple) with designated labels for different containers
i. Relevant details—clinical/examination findings, radiological investigations, previous cytology/biopsy reports, and per-operative findings are to be mentioned as indicated.
j. Prior treatment history, if any
k. Specimen orientation specifics like sutures/clips/ink/diagrammatic representation
l. Tissue preservation for additional investigations/biobanking
m. Instructions related to the disposition of specimens like mounting for academic purposes/request for burial as for products of conception/medical devices like prostheses be returned to the manufacturer/remnants to be returned to the patient.

WORKFLOW IN A HISTOPATHOLOGY LABORATORY

The workflow in a histopathology laboratory is shown in Fig. 1.1.

Accession

Proper labeling of specimen container(s) and filling of requisition form with at least two unique patient identifiers/barcodes must be used for specimen identification to avoid serious errors. In case of any discrepancy or inappropriate identification of specimen or unattached requisition form or specimen without fixative or tissue autolysis, the concerned clinician must be immediately informed. The date and time of the accession and the acknowledgment of the specimen being received by the laboratory staff should be endorsed in the records. This is especially important for evaluating turn-around time (TAT). Specific histopathology laboratory/accession numbers should be assigned to the specimen and mentioned in the requisition form and on the specimen containers to prevent mix-up at any step in subsequent processing. Any identification number or details related to the specimen should not be mentioned over the container lid. Containers should be checked for any leakage and the presence of an adequate amount of fixative. Additional fixative should be added if the quantity is inadequate. Ideally, the fixative should be about 15–20 times the specimen volume. Data related to specimens should be recorded in a register or laboratory information system-related software.

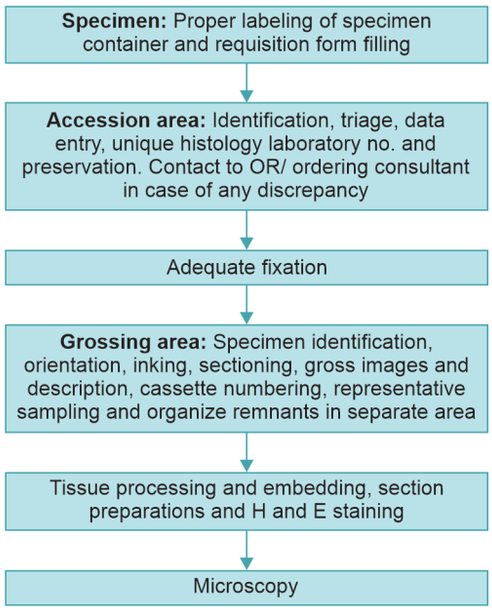

Fig. 1.1: Diagrammatic representation of histology laboratory workflow components in the pre-analytical phase

Triaging the Specimen

After accession, the specimen may need triaging, according to the laboratory policies. Specimens may be prioritized based on the clinician's request, clinical diagnoses of critical alert, labeled as 'Rush' or 'Urgent' specimens. Small and large specimens can be processed according to the laboratory workflow management for achievable TAT. Due to obvious reasons, specimens sent for frozen sections must be dealt with urgently to provide a timely report.

Fixation

Fixation prevents cellular changes due to biochemical and proteolytic changes secondary to denaturation and autolysis. Fixation aims to prevent autolysis and denaturation, fix the tissue in its near-alive state, inactivate infectious agents, and stabilize or harden the tissue for further processing steps. Most importantly, the motto of fixation is to render the best possible tissue morphology on routine histology and immunohistochemical evaluation. Apart from physical factors like temperature and pH variation, prior tissue hypoxia (due to increased warm ischemia time) or dehydration, mishandling of the specimen, and addition of phosphatases, RNAases, or proteinases from the environment or dying cells affect the integrity of the tissue molecules.

An **ideal fixative** should be able to meet these aims of fixation. In addition, it should be fast in action, cheap, easily disposable, compatible with a wide variety of tissues, non-inflammable, and non-toxic. It should have a long shelf life and good tissue penetration, prevent tissue breakdown, minimize enzymatic degradation, and support long-term tissue storage. To date, there is no "ideal" fixative, but of all those options available, 10% neutral buffered formalin (NBF) is the most widely used and accepted fixative for histopathology.

Multiple factors affect the process of fixation: The factors affecting the fixation process are discussed below:

1. **Temperature**: An increase in temperature increases a fixative's penetration rate. This principle reduces the fixation time to about 20 min by microwave-induced heating. A rise in temperature to approximately 45°C does not alter tissue morphology significantly.
2. **The rate of fixative penetration and tissue thickness affect the fixation duration:** The time required for tissue fixation is directly proportional to the square of the depth of tissue to be penetrated.

   $$d = k\sqrt{t}$$

   d = depth of penetration, t = time duration, and k = coefficient of diffusion of fixation, (e.g. for formalin, $k = 0.79$)

   The rate of penetration of formalin is 1 mm/hour. The greater the thickness of the tissue to be fixed, the greater the time required for fixation. Hence, serial cuts at an interval of 3–5 mm in specimens like breast, spleen, etc. aid in increasing the surface area exposed to the fixative while reducing the tissue's thickness to be penetrated. A duration of 24 hours is recommended for fixation by formalin. The presence of blood also impedes the penetration of fixatives. Thus, washing the tissue before fixation can be helpful. If the fixation duration is required to last for >24 hours, the fixative can be changed daily.
3. **Specimen to fixative ratio**: The fixative should ideally be 15–20 times the volume of the specimen. The ratio may be optimally compromised to 10: 1.
4. **pH and buffer:** The effectiveness of fixation by formaldehyde is reduced in acidic pH as the H^+ reduces the cross-linking reaction. This also means that acidic or unbuffered formalin is better for immunochemical recognition of antigens. However, the drawback of acid formalin is that at pH <5.7, formalin pigment is formed, which gets deposited in the tissues.
5. **Concentration and osmolality of fixative:** Use of higher concentrations of formalin (>10%) results in increased hardening and shrinkage of the tissue. A higher concentration of formalin can result in white precipitate formation. On the other hand, a lower concentration of a fixative will result in poor fixation. The hyperosmolality of a fixative leads to shrinkage of the cells, while hypo-osmolality results in cell swelling. The slight hypertonicity of a fixative is expected to result in the best tissue morphology. However, the osmolality of the 10% formalin is relatively high, and we are used to viewing the resultant tissue morphology.

TYPE OF FIXATION METHODS

Based on the mechanism of action, the methods of fixation can be classified as Fig. 1.2 and **commonly used fixatives** are tabulated in Table 1.1.

Formaldehyde

In anatomic pathology, formaldehyde is the most commonly used fixative. Formaldehyde combines with water and gets converted to methylene hydrate. This methylene hydrate binds with the side chains of various proteins to form reactive hydroxymethyl side chains. This is the primary and characteristic reaction that occurs early in the fixation process. A similar reaction occurs with nuclear proteins and nucleic acids later in time. Still later and at higher temperatures, cross-linking reactions commence in proteins, DNA, and

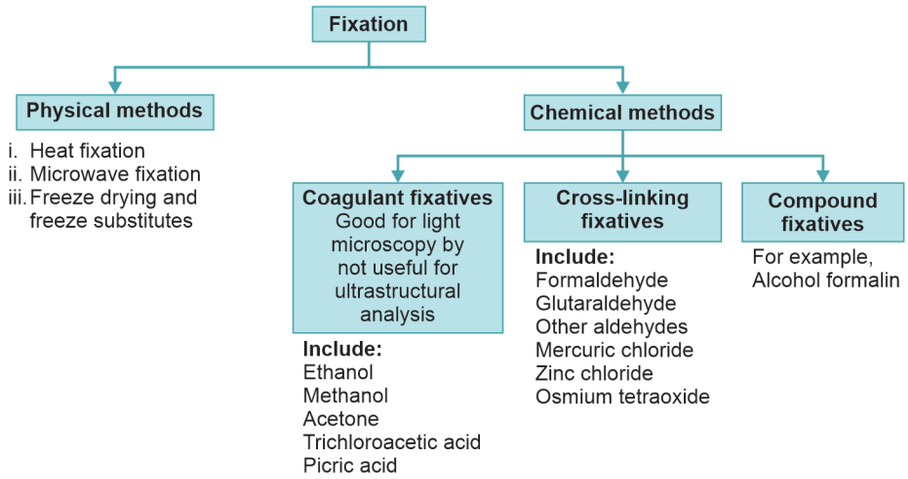

Fig. 1.2: Different methods of fixation

Table 1.1: Commonly used fixatives in a histopathology laboratory

Fixative	*Composition*	*Quality of H&E staining*	*Ultra-structure analysis*	*Applications and advantages*	*Disadvantage*
Formaldehyde	10% buffered at pH 7.2-7.4	Good	Good	General all-round fixative in anatomic pathology	Skin and eye irritant, toxic on inhalation and ingestion, corrosive; Formalin pigment on H&E, esp in blood rich biopsies
Glutaraldehyde	2% buffered at pH 7.4	Good	Excellent	Good for electron microscopy if used with Osmium tetroxide post-fixation	Expensive, slow penetration, not suitable for IHC
Zenker's fixative	Mercuric chloride + potassium dichromate sodium sulfate + acetic acid	Good	Poor	Excellent for hematopoietic tissues and lymphoid tissues like lymph nodes, spleen, thymus and bone marrow	Carcinogenic, irritant, corrosive, toxic Mercury pigment artefact on H&E
B-5	Mercuric chloride + sodium acetate + formaldehyde	Good	Poor		
Osmium tetroxide	1% solution buffered at pH 7.4	Poor	Good (better visualization of membranes)	Used as secondary fixative for electron microscopy	Toxic

Contd.

Table 1.1: Commonly used fixatives in a histopathology laboratory

Fixative	*Composition*	*Quality of H&E staining*	*Ultra-structure analysis*	*Applications and advantages*	*Disadvantage*
Bouin's fixative	Aqueous picric acid + formalin+ Glacial acetic acid	Good	Poor	Good for connective tissue stains, e.g. Trichome; Useful for gastro-intestinal biopsy, testicular biopsy, endocrine tissue, liver and muscle	The fixation process results in yellowish discoloration of the tissue. Destroys nuclear membrane and mitochondria; carcinogenic
Alcohol formalin	10% formaldehyde + 90% ethanol		Poor	Good general fixative; better visualization of lymph nodes on gross evaluation (Removes fat from tissues); suitable for immuno-fluorescence studies	Not to be used for renal specimens

unsaturated lipids. Formaldehyde does not interact with carbohydrates. The cross-linking can be reversed by washing in water for a certain period.

Various preparations of formaldehyde are available commercially. A few are given below:

1. **10% formalin**: Formaldehyde, in its pure form, exists as vapor. When dissolved in water, the concentration of formaldehyde in the so-called 'formalin' is 37–40%. Hence, the 10% formalin contains only 4% of formaldehyde.
2. **10% formal saline**: A 100 ml of this solution consists of formalin (10 ml), sodium chloride (0.9 g), and distilled water (90 ml).
3. **Neutral buffered formalin (NBF):** One liter of NBF contains formalin (100 ml), distilled water (900 ml), sodium phosphate monohydrate (4 g), and sodium phosphate dibasic anhydrous (6.5 g). It is the most frequently and widely used preparation for fixation of histopathology specimens. The pH should be 7.2–7.4. The pH must be checked daily. It is suitable for immunohistochemical analysis also.
4. **Formol calcium acetate**: It consists of formalin (100 ml), distilled water (900 ml), and calcium acetate (20 g). It is suitable for enzyme histochemistry.

Common health hazards related to formalin include toxicity and allergic reactions to the respiratory system, eyes, and skin. Formaldehyde has been categorized as a class 1 carcinogen. In the last decade, the concept of a formalin-free laboratory system has emerged, which intends to make the histopathology laboratory eco-friendly and integrate routine cellular pathology with molecular pathology to understand the dynamic molecular events in health and disease. A few alternatives to formalin have been suggested (Table 1.2).

Table 1.2: Alternative methods for a formalin free histopathology laboratory

Non-formalin based fixatives	*Tissue transfer under vacuum*	*Tissue preservation for biobanking*
▪ Non-toxic ▪ Maintain the integrity of nucleic acids, proteins and histology ▪ Variable shelf-life ▪ Few can be home made; cost effective Examples: ▪ Carnoy's fixative ▪ Modified methacarn ▪ Zinc based fixatives ▪ Ethanol based fixatives ▪ HOPE (Hepes-glutamic acid buffer mediated organic solvent protection effect) ▪ Universal molecular fixative (UMFIX) ▪ Acetone-methylebenzoate-xylene (AMeX)	▪ Vacuum sealed device ▪ Transport from OT to lab; also, for tissue transplantation and tissue banking ▪ No chance of drying ▪ Slow autolysis (no air) ▪ Faster cooling of tissues ▪ Preservation of specimen or morphology ▪ Excellent histology, IHC and molecular results ▪ Tissue banking ▪ Eco-friendly and totally formalin free ▪ Large specimens easily sent (Light container)	▪ Transport on ice, snap freezing by liquid nitrogen ▪ Storage in low temperature (–800°C) ▪ RNAlater solution; prevention of degradation in tissues and stabilizes expression levels of genes

Some recommendations for laboratory personnel in the anatomic pathology:

1. Avoid scrupulously sniffing the chemicals in the histology laboratory.
2. Use prefilled containers to transport biopsies so the lids are opened quickly to remove the specimens.
3. Use latex or nitrile gloves and wear a disposable gown and goggles to minimize contact with skin and eyes.
4. Enhance ventilation at the grossing station and increase humidity in the room by running a tap to diminish formaldehyde evaporation.
5. Acute exposure to formaldehyde vapors causes throat irritation. Drinking milk or an alkaline drink is helpful in this condition. Otherwise, the affected individual can keep these fluids in the mouth or gargle in an emergency to minimize mucosal damage.
6. Washing the eyes with cold water after formalin splash is essential (at least for two minutes)
7. Used formalin should be discharged in small volumes into the wastewater system.

Grossing Area

It is good to have a separate designated room for grossing. The grossing room should be well-lit lighted ventilated. It must essentially have an exhaust system to allow formalin fumes to exit and a formaldehyde meter to measure its concentration in the air. Indoor formaldehyde level should be below than 0.03 ppm and critical level for symptomatic presentation ranges between 0.10 and 1.1 ppm.

Grossing Station (Fig. 1.3)

A basic grossing workstation or gross laboratory model includes:

- Extracted working area with two perforated plates (holes of variable size 9–23 mm)
- Basin (sound attenuating insulated, free from sharp edges), tap for water

Fig. 1.3: Gross station

- Drainage system
- Formalin waste drain
- Glove dispenser
- Electric lights, power supply, magnified lens, lamp LED, exhaust fan
- Some additional features may be provided in advanced models like the *Optical system* include camera, attached barcode reader, computer system with relevant software, USB port, etc.

All the necessary equipment for grossing and measuring the specimens should be available. These may include a smooth, surfaced, light-colored board, scalpel, blades, scissors, knives, forceps, a kidney tray, scale, and a sieve. The blotting paper, cotton rolls, ink brush, inks of four colors, and fixative for ink should be readily available. In addition, there should be access to a weighing scale to weigh the surgical specimens. A provision for taking photographs should also be included.

The grossing personnel should attend to the following points:

1. While handling the tissue specimens, **universal safety precautions** should be followed, and personal protective equipment (e.g., gloves, mask, lab coat, or apron) should be used. Check the formaldehyde meter to record and monitor the levels of formalin vapor before grossing. All required equipment and reagents should be in good working condition. The grossing station should be cleaned appropriately. Containers filled with formalin should not be kept open for extended periods.

2. **Organization of specimens according to triage:** Effective triaging mainly depends on the specimen's category, priority for processing (urgent/rush/stat), and status of specimen preservation. Grossing personnel should be involved in the biopsy triaging and know the basis of the process for effective workflow control. Laboratories may set their standard protocol or policy for significant workloads or workflows.
3. **Specimen identification:** At least two unique patient identifiers on containers and the requisition form should be matched. Specific accession numbers assigned by the laboratory should be used for further patient or case identifier numbering of cassettes, tissue blocks, and glass slides.
4. Clinico-imaging details, suspected diagnoses, purpose of the procedure or any specific instruction in requisition form, should be assessed by the grossing personnel to proceed with the processing accordingly.
5. **Type of specimen/procedure:** The grossing personnel should know the type of specimen received, e.g. incisional/punch/endoscopic/vacuum assisted/excisional biopsies/resections. The steps of grossing and selection of blocks may differ with the type of specimen.
6. **Gross description:** The laboratory assistant should record at least the following information and endorse it on the laboratory report form.
   - Type of specimen
   - Orientation and measurements of the specimen
   - Photograph the specimen
   - Ink or not to ink? If inked, the ink coding should be recorded.
   - External examination
   - Open the specimen and describe the cut surface
   - Capture the close-up images of distinct gross features of cut surface
   - Adequate fixation- whether to fix further or proceed for taking sections?
7. **Sampling of surgical margins:** Surgical margins are the borders of a tissue specimen when it was removed from body during the surgery. The distance between the tumor and margin is referred as **surgical clearance.** A positive surgical margin is defined as presence of residual tumor at the surgical margin. The tumor can be seen macroscopically or only microscopically at the margin. A positive margin with disease present or very close margin state increases the risk of recurrence. These are the candidates for neoadjuvant therapy or a revision surgery.

   The purpose of the surgical procedure suggests a lot about whether the surgical margins need to be sampled or not. Specific margins to be sampled depend upon the type of procedure, tumor site, and tumor extent. Figure 1.4 shows the descriptors commonly used in medical practice. For example, most of the resection specimens should be considered as a cubical box, and various margins (anterior, posterior, superior, inferior, medial, lateral, etc.) should be sampled; bone resection specimens may be taken as cylinder and both resected margins (proximal, distal, etc.) should be sampled. The skin surface may be considered a sheet, and margins (superior, inferior, medial, lateral, etc.) should be submitted accordingly.

   Margins are commonly of two types:

   - **Shave or en-face type** particularly when the margin is widely free from a tumor and the complete surface is sampled. The distance between the tumor and the margin cannot be determined microscopically.

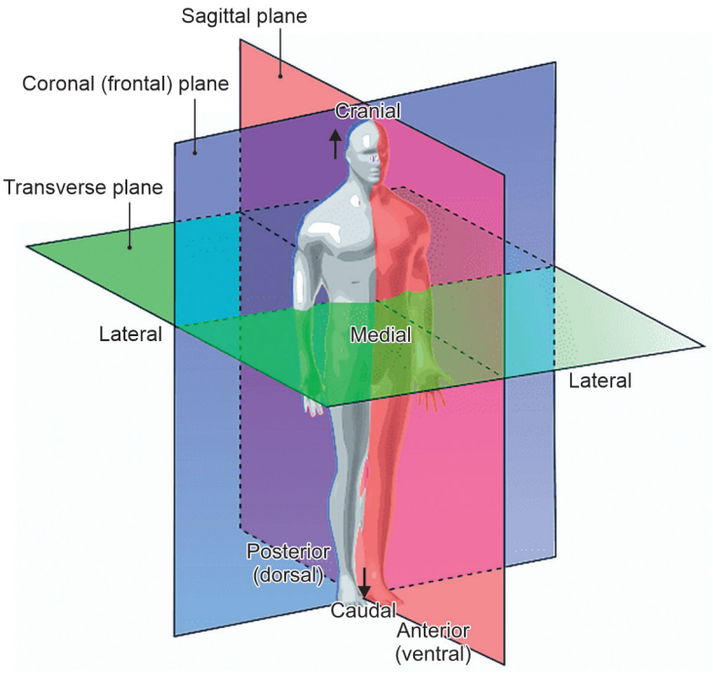

Fig. 1.4: Anatomical planes of body

- **Radial or perpendicular type** when the tumor is close to the margin or a minimum distance of the tumor from the margin need to be reported. The representative sections including tumor tissue and the surgical plane of cut with closest distance must be sampled and this distance can be measured by microscopic examination. (Fig. 1.5)

Always ink the margin ***before*** *cutting the specimen,* to avoid any stray ink in the microscopic section or seepage of the ink inside the specimen. The method of inking of the specimen is discussed later. If the received specimen is already incised or fragmented or a sheared margin is present, always document the limitation for evaluation of margin status.

Comment in the gross description whether the margin is grossly involved or not. Endorse which all margins are grossly involved. If margin(s) are not involved, specify and record the closest macroscopic distance from the tumor as well as the type of

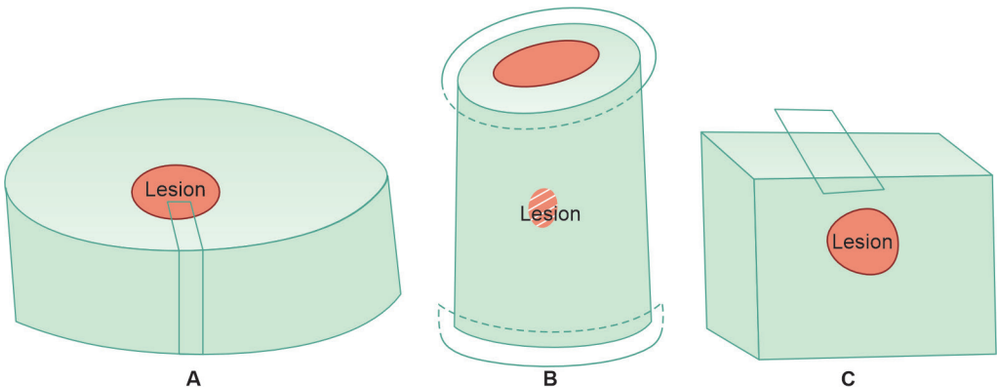

Fig. 1.5: Types of margins—Radial (A) and Shave (B and C)

section of margin taken (shave versus radial). Adequate surgical clearance depends on the tumor site and type (Table 1.3).

8. **Sampling of the tumor or abnormal area as indicated**: The number of sections from the tumor or abnormal area depends on the tumor site, size, type, tumor extent, pathological staging criteria specific to the site, preoperative therapy, and differential diagnoses. Representative or minimum number of sections will be discussed in respective sections.

   A grid diagram may be drawn to demonstrate which section is derived from which area of the tumor tissue, particularly in post-therapy cases where residual tumor size needs to be measured or in case more sections are needed later for extensive grossing.
9. **Sampling of grossly normal appearing tissue**: It should be made a thumb rule that one or two sections should be sampled from the tumor/lesion and the peritumoral/perilesional interface. Also, a few sections should be sampled from the macroscopically normal tissue to identify associated diseases or any predisposing condition.
10. **Sampling of lymph nodes as indicated (discussed in respective sections)**
11. **Type of submission–complete, representative, informative:** Always document whether the entire tissue has been submitted or remnants of tissue are kept.
12. **Handling of the cassettes and tissues in the cassettes:** Endorse the number of tissue sections or fragments in each cassette. Check whether the number labels have been placed correctly in the cassettes. Check that all the cassettes are tightly closed. A loosened cassette may result in losing the tissue during processing.
13. There should be clear-cut instructions to the histo-technician on which surface of the section will be cut so that embedding can be done accordingly. As a thumb rule, it should be practiced that the surface of the tissue facing downwards should be the one that is cut. This is important because sometimes the lesions are too small that the reversal of the surface being cut may lead to missing lesions.
14. At the end of grossing of a specimen, the number of cassettes should be counted in the gross description as well as on the working station and an immediate check should be carried out in case of any discrepancy. Remove used swabs and dispose in the trash. The grossing slab and the instruments should be cleaned after the grossing of every sample to eliminate floaters in the resulting slides.

Table 1.3: Examples for adequate pathological margins according to common tumor site and type

System	*Tumor*	*Adequate pathological margins (Fresh)*
Head and neck	Oral squamous cell carcinoma	≥5 mm
Respiratory system	Lung carcinoma	≥10 mm
Breast	Invasive carcinoma	≥1 mm
Gastrointestinal system	Colorectal carcinoma	Radial or circumferential margin >1 mm Resected margins >1 cm
Female genital system	Cervical carcinoma	>5 mm
	Vulvar carcinoma	8 mm
Genitourinary system	Prostate carcinoma	>2 mm
	Urothelial carcinoma	≥1 mm
	Renal cell carcinoma	≥1 mm
Skin	Squamous cell carcinoma	>5 mm
Soft tissue	Sarcoma	≥10 mm

Decalcification

Bone specimens and calcified tissues need to be decalcified to make the bony tissue soft enough to render the microtomy possible. The 'incomplete decalcification' can damage the microtome blade and can also damage the adjacent soft tissue in the block during microtomy. On the other hand, 'over decalcification' affects tissue morphology with poor nuclear and cytoplasmic details. There is increased eosinophilia and loss of nuclear basophilia on routine microscopy. The specimen should be adequately fixed before decalcification to reduce the damaging effect of acid-decalcifying fluid on the tissue.

Commonly used decalcifying agents include neutral ethylene diamine tetra-acetic acid (EDTA) solution, 5–10% nitric acid, formalin-nitric acid, 5–10% formic acid, and 5% trichloro-acetic acid.

- Bone/cartilage/hard tissues/tooth should be adequately fixed and may require slicing into thinner slices.
- Specimen should be kept in the adequate amount (10–20 times of volume of the specimen volume) of decalcifying solution at least for 24–48 hours.
- The end-point of decalcification should be checked twice in 24 hours. This can be done by adding NH_4OH or liquid ammonia dropwise to 5 cm^3 of the used decalcifying agent until it turns alkaline to litmus. Now, add 5 cm^3 of saturated ammonium oxalate solution. A resultant cloudy solution indicates incomplete decalcification, while a clear solution indicates the end-point of decalcification. Other methods of decalcification include performing an X-ray (especially useful in large bone resection specimens) and physical methods, e.g. needling the bone.
- Specimen should be washed thoroughly for 1–2 hours after decalcification and then proceed as routine tissue processing.
- Solution to be changed after every 5–7 days or more often if indicated until the bone is decalcified.
- Avoid weekends or holidays for keeping the specimen in a decalcifying solution.

Some specimens require only gross examination and sections cannot or are not required to be sampled. Some examples of these specimens are prosthetic valves, accessory digits, toenails, traumatic amputations, foreign bodies or calculi. In some circumstances, if the clinician requests, further pathological examination can be performed in these cases.

Inking

As discussed in earlier sections, the surgical margins are the surfaces of a surgical specimen where the specimen was cut away from the surgical site. The resection margins are often demarcated by the operating surgeon by application of clips, sutures or nicks. But, when the specimen is received in the histopathology laboratory, the histopathologist usually uses ink(s) to identify the different resection margins. For inking a surgical specimen, ink, it's applicators and appropriate mordant are required.

Ink to be used: India ink and acrylic colors are commonly used in histopathology laboratories for inking specimens. Both types of inks have their advantages. A comparison between the two types of ink is shown in Table 1.4. Commercially available tissue marking dyes (e.g. by Thermo Shandon, SkyTek, etc.) can also be used, however, they are costlier, hazardous, have longer drying time, and affect immunohistochemistry results. It is a good

Table 1.4: Comparison of two most widely used types of ink in a histopathology laboratory	
Acrylic colors	***India Ink***
Available in a greater number of color options	Available in fewer color options
Take less time to dry	Takes more time to dry
Good visibility on naked eye examination	Good visibility on naked eye examination
Good visibility under microscope	Better visibility under microscope than acrylic colors
Contamination of tissue processing fluid is more	Lesser contamination of tissue processing fluid by the ink
Interferes with cellular and nuclear details	Does not interfere with cellular and nuclear details
Penetrates into deeper tissues	Does not penetrate into the tissues
Cheaper	Costlier
Non-toxic	Toxic

practice to keep 6 or more different colors available at the grossing station. The acrylic colors–crimson (shade 04), turquoise blue (22), dark green (06), burnt sienna (01), black (02), orange (17), mauve (15), and even, white color- can be used for inking. The use of colors like deep brilliant purple (64), pink (18), golden yellow (09), and ultramarine blue (23) should be avoided. The Alcian blue, colored gelatin and starch have also been tried as surgical inks.

Applicator: The cotton-tipped applicator or the fine painting brushes are good enough for inking. The cotton tipped applicators can be prepared in-house by using wooden sticks and cotton. The reverse end of the stick can be used to apply ink on small resection margins, e.g. polyp base.

Mordant: Acetone, acetic acid, formaldehyde-acetic acid, alcohol, or Bouin solution can be used for fixing the ink to the tissue.

Method: First, dry blot any excess formalin on the surface to be inked. This is going to prevent any dilution and carryover of ink. In specimens like mastectomy, the fat on the surface can be removed by dipping the specimen in alcohol. Remove any access alcohol. Apply ink sufficient to cover the required surface only. Blot and dry any excess ink. Allow it to dry for 1–2 minutes. Apply any of the mordants for 5–10 seconds to fix the stain. Do not apply these mordants in excess. The tissue can now be used for further processing.

If the mordant is not available, then, the specimen can be kept for overnight fixation after the steps of inking and drying. Then, on the next day, the ink can be re-applied and further processing can be carried out. This technique also gives good results.

Maintenance of Equipment

Grossing tools should be scrubbed with cotton and dried after use. Tools should not be soaked. Make sure that there should not be any remnants of tissue over scalpel/cutting blades/scissors/paint brushes/working slab.

Grossing station should be cleaned on daily basis after the completion of grossing and no body fluid/tissue/ink should be left on the station or tables. Sink or basin should be free of debris with clear drain. All containers of reagents should be kept closed and wiped clean.

Discarding the Specimens

Different organizations/laboratories may exercise individual protocols for retention of grossing specimens, paraffin blocks and slides. The Royal College of Pathologists, UK recommends preservation of blocks permanently and histology slides for 10 years and specimen remnants/wet tissue for at least 4 weeks after dispatch of the report. However, it is a good practice to preserve slides and blocks for 10 years and for 25 years in cancer referral centers.

Radioactive substances are used for identification of the sentinel lymph nodes. Although these substances carry little risk of radiation exposure, the laboratory should develop mechanisms for their storage and disposal.

SPECIAL REQUESTS

Intra-operative Consultation

Because the turn-around time in intra-operative consultation is up to 30 minutes, the rapid processing of the tissue is of paramount importance. The frozen sections generated with the help of a cryostat machine are used in this case. The tissue is hardened by freezing it at a temperature of –30 to –500°C by using a coolant in the cryostat. The tissue sent is placed onto the chuck of a cryostat in an optimum cutting medium. The metal weight in the cryostat is placed on it for a few minutes. The chuck is then transferred to the cryotome for serial sections.

Immunohistochemistry

Additional 2–3 μ thick sections over poly-L-lysine coated slides may be taken without wasting the tissue particularly in small or core biopsies, if immunostaining is needed. Optimal antigen retrieval depends on pre-analytic variables like fixation time (delayed fixation >30 minutes, over fixation >48 hours or uneven/under fixation), tissue processing (conditions of hydration, clearing or paraffin impregnation) and optimal temperature maintenance.

Molecular Studies

Although the ability to perform diagnostic molecular studies in formalin fixed paraffin embedded tissue has substantially diminished the need to collect fresh tissue on every case, however, frozen tissue may be needed to enter patients into treatment protocols. Nevertheless, discretion should be used in triaging tissue from sarcomas. Adequate tissue should be submitted for conventional light microscopy before setting aside samples for cytogenetics, electron microscopy, or molecular analysis. Fresh tissue for special studies should be collected at the time the specimen is received.

It may be important to snap freeze a small portion of tissue and in general, approximately 1 cm^3 of fresh tissue (less is acceptable for small specimens, including core biopsies) should be cut into small, 0.2–0.3 cm. fragments, reserving sufficient tissue for histologic examination. This frozen tissue should ideally be stored at –70°C and can be shipped on dry ice to facilities that perform ancillary testing.

Molecular/cytogenetic preparation steps (curetting, biopsy specimens or untreated resection specimens): Before you begin the macroscopic examination, gather the materials needed for special studies.

- Vial of minimum essential medium (MEM) or other suitable transport media for cytogenetics.
- At least 4 empty small plastic vials or pieces of aluminum foil to snap freeze tissue.
- Dewar containing liquid nitrogen.
- Extra-long forceps for immersion of plastic vials or aluminum foil containing tissue.
- Glass slides for touch preparations.

Molecular/Cytogenetic Handling Guidelines

Once sufficient tissue is reserved for histologic examination, a 1 cm^3 piece of fresh tumor should be cut into 0.2 cm fragments and snap frozen. The frozen tissue can be stored at –700ºC and shipped on dry ice, if necessary, for molecular analysis to the appropriate reference laboratory. Analysis may include a variety of molecular assays for tumor-specific molecular translocations that help molecular classification of the tumors.

For small specimens, such as core biopsies, less tissue is acceptable. A frozen section residuum, previously used for evaluation of specimen adequacy and viability, can be saved, frozen at –700ºC, and used for this purpose.

FISH and RT-PCR studies are used to evaluate tumor-defining translocations like for the Ewing sarcoma family of tumors. Due to increased sensitivity, snap frozen tissue is preferred, and every effort should be made to procure a sample.

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