APPIA

Anatomical Pathology Patient Interest Association

General Tissue Handling Guidelines

Best practices for handling specimens from tissue procurement through laboratory diagnostics

Tissue Optimization and Pre-analytic Standardization (TOPS)

Before laboratory:

1

Tissue specimen labeling

- Specimen label must contain two unique patient identifiers, and the source of specimen.
- Container should be labeled with the type of fixative used.
- Ensure label matches patient requisition identification.

2

Tissue specimen handling

- Use 10% aqueous Neutral Buffered Formalin (NBF) only.
- The fixative volume to tissue volume ratio should be 10:1 minimum.[3]
- If needed, bisect or open the specimen to ensure complete penetration of the fixative solution or as instructed by the Pathology laboratory.
- Ensure that the entire specimen is immersed in the fixative.

3

Time to fixation

- Limit cold ischemia time to <5 minutes, but never exceed 1 hour.[1]
- Specimen should be immersed in fixative immediately at time of collection if possible, or immediately upon receipt at the laboratory if transported fresh.
- Document and record time of collection and start time of fixation (time 0).
- Proper fixation preserves specimen integrity and enables optimal tissue preservation and quality.
- Formalin penetrates tissues quickly (approx. 1mm per hour) but fixes slowly. If needed, specimens need to be opened, incised or sliced in the laboratory and left to fix for an adequate period of time prior to processing.

4

Tissue specimen storage and transportation

- Do not store specimens overnight at room temperature or at 4°C without fixative solution.[2]
- Fresh specimens should be transported to the lab immediately.
- Ensure that the specimen is transported via courier at ambient temperature (18°- 25°C).

(continued on next page)

1. Hammond M, Hayes D, Dowsett M, et al. American Society of Clinical Oncology/ College of American Pathologists guideline recommendations for immunohisto- chemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). Arch Pathol Lab Med 2010. 34 (7):e48–e72.
2. Khoury T, Sai S, Hwang H, et al. Delay to formalin fixation effect on breast biomarkers. Mod Pathol. 2009;22:1457-1467.
3. CLSI. Quality Assurance for Design Control and Implementation of Immunohisto- chemistry Assays; Approved Guideline – Second Edition. CLSI document I/LA28-A2. Wayne, PA: Clinical and Laboratory Standards Institute (CLSI). 2011.
4. Westra WH, Hruban RH, Phelps TH, Issacson D. eds. Surgical Pathology Dissection: An Illustrated Guide. 2nd ed. New York, NY: Springer-Verlag New York, Inc; 2003.
5. CLSI MM13 guidelines.

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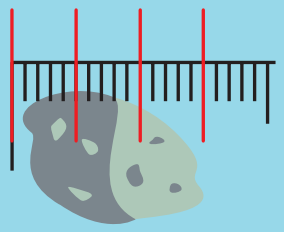
In laboratory:

5 Tissue grossing and in-lab fixation

- Depending on tissue type/size, slice at 5-10 mm intervals (red bars) after inspection/margin designation and immerse in fixative.[4]
- Specimens placed into cassettes must be “nickel thick” (2-3 mm max.).
- Verify that labeling of the tissue cassette matches the identifier(s) on the specimen container.
- Verify the number of specimens vs. the requisition, document any discrepancies.



6 Duration of fixation

- Total fixation time in 10% NBF at room temperature is no less than 6 hours, and no greater than 72 hours for most tissues, including fixation time on the tissue processor.[1,5]
- Fatty tissue may be fixed up to 48 hours or, as noted for some breast markers, up to 72 hours.[1,5]
- Under-fixation is a greater concern than over-fixation for all routine and IHC testing.[3]

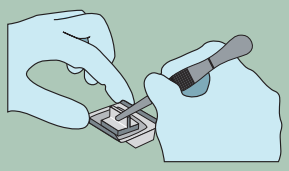
10% neutral buffered formalin

7 Tissue processing

- Use an optimized, laboratory-validated processing protocol that is specific for that tissue type/size.
- Gradual dehydration through graded alcohols yields best results.
- Monitor reagent usage and follow the lab’s validated reagent exchange protocol to ensure proper reagent efficacy.
- All processing protocol and instrument temperatures should be monitored and recorded daily.

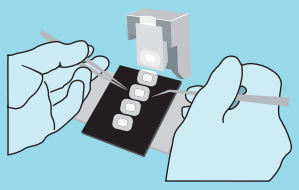
8 Tissue embedding

- Ensure proper tissue orientation following in-lab protocols.
- Ensure only a single patient cassette is embedded at a time to reduce errors.
- Confirm tissue specimen counts and other special instructions from grosser, document any discrepancies.
- Properly clean forceps, embedding molds and embedding system between cassettes to avoid tissue cross contamination.
- All embedding protocols and instrument temperatures should be monitored and recorded daily.



9 Microtomy

- Microtome must be cleaned between each block to reduce tissue cross-contamination.
- Use a clean, sharp blade for best sections or “ribbons”.
- Cut sections at 4-5µm for routine staining.
- Use charged slides for special stains and IHC/ISH.
- Verify that labeling of the slide matches the identifier(s) on the tissue cassette.
- Flotation water bath must be cleaned between each block to reduce tissue cross-contamination.
- Oven dry at 56°- 60°C for no more than 1 hour[3] or air dry at ambient temperature of 18°- 25°C overnight.
- Ensure section is a full face of the tissue block and free of holes, folds, tears, wrinkles, other artifacts.



10 Slide and block storage

- Paraffin blocks and slides should be stored in a temperature and humidity-controlled environment.
- Store slides and blocks for a minimum of 10 years per local and national guidelines.