

Anatomical Pathology Patient Interest Association (APPIA) TOPS Tissue Handling Guidelines illustrate an ongoing education initiative to improve the understanding of pre-analytical factors and promote standardization in tissue workflows.

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Introduction:

According to the World Health Organization, cancer accounted for nearly 1 in 6 deaths globally in 2020, however, as deadly as cancer is, many cancers can also be cured if detected early and treated effectively¹. Receiving the right diagnosis and the right treatment at the right time is vitally important to a patient's probability of a successful fight against cancer. Thanks to advancing technologies and an increasing emphasis on precision medicine related to biomarker testing, the processes for appropriately handling and preparing clinical tissue samples have only grown in importance. Determining cancer in a patient often begins with acquiring a tissue sample. The journey of a patient's tissue sample from acquisition to diagnosis, however, is a long, multi-step process. All the steps of collection, handling, and processing of clinical tissue samples make up what is referred to as the pre-analytical portion of the process. Unfortunately, pre-analytics in anatomic pathology often involve multiple manual processes that are wrought with the potential for error. In fact, an estimated 60-70% of laboratory-associated errors involve pre-analytical factors². Examples include errors like insufficient or prolonged fixation, incorrect patient identification, improper grossing thickness, inadequate tissue processing, etc. Inconsistencies and errors during pre-analytical processes have the potential to negatively impact the quality of the tissue and the downstream analytical testing, ultimately jeopardizing the patient's diagnosis.

Those involved in the process of delivering a final patient result, whether they are laboratory professionals or pathologists, hold the responsibility for ensuring the quality of specimens for testing. Proper education and standardization of best practices in pre-analytics are keys to empowering pathology professionals to optimize tissue preservation and quality that enables the delivery of an accurate diagnosis in an efficient fashion.

The Tissue Optimization and Pre-Analytical Standardization (TOPS) Tissue Handling Guidelines developed by the Anatomical Pathology Patient Interest Association (APPIA) is part of an ongoing education initiative to improve the understanding of pre-analytical factors and promote standardization in tissue workflows.

Materials & Methods:

The TOPS guidelines are separated into the ten (10) main sequential steps of the pre-analytical process and list the industry best practices related to each step. The ten primary steps include tissue specimen labeling, tissue specimen handling, time to fixation, tissue specimen storage and transportation, tissue grossing and in-lab fixation, duration of fixation, tissue processing, tissue embedding, microtomy, and slide and block storage.

Content for the best practices was aggregated from known and respected industry sources, including the American Society of Clinical Oncology (ASCO), College of American Pathologists (CAP), and Clinical and Laboratory Standards Institute (CLSI) guidelines.

Once the information was collected, the APPIA board, which consists of industry professionals throughout anatomical pathology, organized and reviewed the content to ensure proper alignment within the ten sequential steps. A 2-page infographic was then created from the resulting content and best practices to be distributed to laboratories as an educational aid.

In addition, the material has been presented via webinar and on the APPIA webpage

Conclusions:

Optimization of patient outcomes in tissue-based testing can benefit from improving pre-analytical factors, beginning with specimen procurement and collection, and including the important steps of specimen fixation, transportation, and all in-laboratory processes for the eventual histopathologic evaluation by a pathologist. APPIA's TOPS Tissue Handling Guidelines promote and reinforce best practices and support the goals of laboratories to provide the best patient care.

Results:

The TOPS Guidelines are presented in an illustrated, 10-step infographic that outlines best practices for handling specimens from tissue procurement through laboratory diagnostics, including the pre-analytical steps of acquisition, fixation, and transportation of the specimen before it arrives in the laboratory for analysis. TOPS is APPIA's first education program for the entire health care delivery team emphasizing the importance of pre-analytics while serving the interests of patients.

The TOPS Program was introduced to audiences on November 29, 2020, by APPIA and Dr. David G. Hicks, Director of Surgical Pathology, Department of Pathology and Laboratory Medicine at the University of Rochester Medical Center, Rochester, New York in a webinar entitled: "[Optimize Patient Outcomes by Improving Pre-Analytics](#)". The TOPS program is endorsed by the National Society of Histotechnology (NSH). *Click on the infographic below for an expanded view:*

General Tissue Handling Guidelines
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.¹
 - If needed, incise 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 immunohistochemical testing of estrogen and progesterone receptors in breast cancer [unpublished]. Arch Pathol Lab Med 2010; 34 (7):e48-e72.
2. Khoury T, Se S, Heang H, et al. Delay to formalin fixation effect on breast biomarkers. Mod Pathol. 2009;22:1467-1467.
3. CLSI. Quality Assessment for Design and Implementation of Immunohistochemistry Assays: Approved Guideline – Second Edition. CLSI document H43-A2. Wayne, PA: Clinical and Laboratory Standards Institute (CLSI); 2011.
4. Steyer WA, Hohenfeldt P, Pugh TH, Johnson D, eds. Surgical Pathology Dictionary: An Illustrated Guide, 2nd ed. New York, NY: Springer-Verlag New York, Inc; 2003.
5. CLSI M43-G 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.⁴
 - Fatty tissue may be fixed up to 48 hours or, as noted for some breast markers, up to 72 hours.^{1,3}
 - Under-fixation is a greater concern than over-fixation for all routine and IHC testing.¹
- 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.
 - Fixation water bath must be cleaned between each block to reduce tissue cross-contamination.
 - Oven dry at 50°-60°C for no more than 1 hour⁴ 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.

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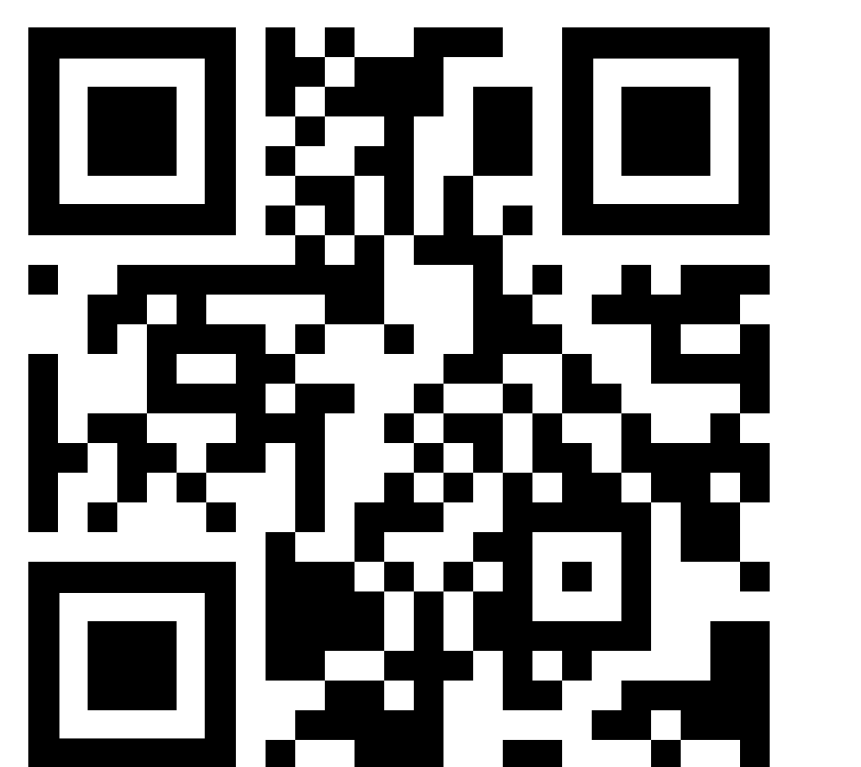
Citations:

1 "Cancer." World Health Organization, 03 Feb. 2022, <https://www.who.int/news-room/fact-sheets/detail/cancer>.

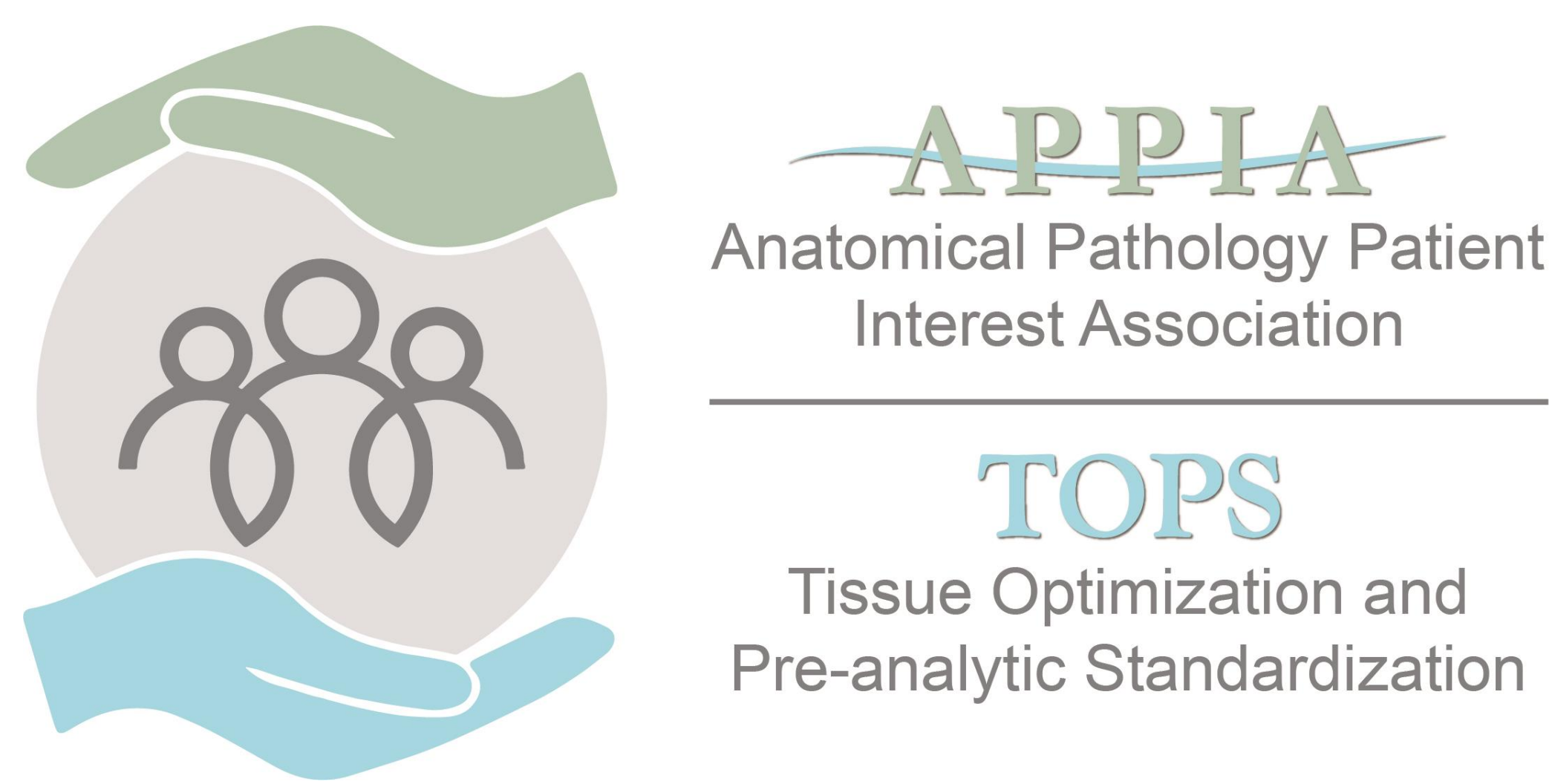
2 Compton, C. et al. Preanalytics and Precision Pathology: Pathology Practices to Ensure Molecular Integrity of Cancer Patient Biospecimens for Precision Medicine. Arch Pathol Lab Med 1 November 2019; 143 (11): 1346–1363. doi: <https://doi.org/10.5858/arpa.2019-0009-SA>

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<http://appiagroup.org/tops>



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Results:

The TOPS Guidelines are presented in an illustrated, 10-step infographic that outlines best practices for handling specimens from tissue procurement through laboratory diagnostics, including the pre-analytical steps of acquisition, fixation, and transportation of the specimen before it arrives in the laboratory for analysis. TOPS is APPIA's first education program for the entire health care delivery team emphasizing the importance of pre-analytics while serving the interests of patients.

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General Tissue Handling Guidelines

Best practices for handling specimens from tissue procurement through laboratory diagnostics

Tissue Optimization and Pre-analytic Standardization (TOPS)

Before laboratory:

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 - 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
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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.

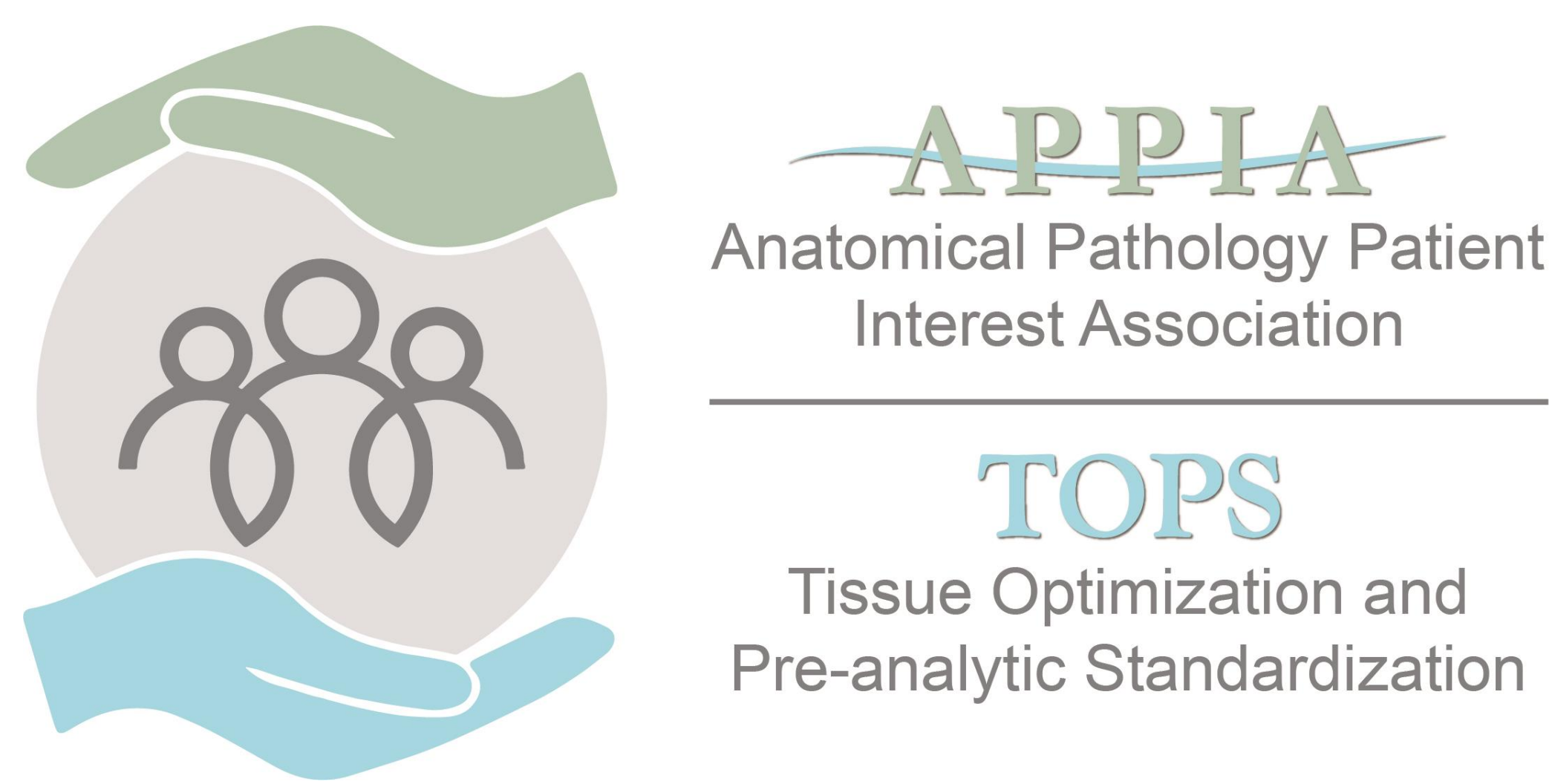
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Conclusions:

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 - 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 hour³ 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.
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