# Q&A column

## Editor: Frederick L. Kiechle, MD, PhD

Submit your pathology-related question for reply by appropriate medical consultants. CAP TODAY will make every effort to answer all relevant questions. However, those questions that are not of general interest may not receive a reply. For your question to be considered, you must include your name and address; this information will be omitted if your question is published in CAP TODAY.

---

**Q. I am part of a two-pathologist practice in a rural community hospital of 110 beds. We have been asked more frequently lately to evaluate liver and kidney biopsies for organ transplantation. We are hesitant to evaluate these biopsies for transplantation purposes due to frozen section artifacts and because we send all of our kidney biopsies performed by local nephrologists to a reference laboratory and do not evaluate kidney biopsies. It seems that regardless of what we say about the biopsies, the surgeons transplant the organs. We believe it is out of our scope of practice to evaluate liver and kidney biopsies for organ transplantation. What do you think?**

A.Evaluating these organs is not out of our scope of work as pathologists, and there are standards for accepting or rejecting organs that the surgeons have to follow. It is not entirely correct that no matter what pathologists say, the surgeons transplant these organs. Organs have been rejected, transplanted, and transferred on the basis of frozen section results, and UNOS (United Network for Organ Sharing) data will show this for anyone who may be interested. If a surgeon does decide to transplant even if the frozen section read shows it is “a bad organ,” that is the surgeon’s decision and liability. But without the clinical details pertaining to the recipients (which pathologists usually do not have), it is not always possible to judge the surgeon’s decision.

That said, pathologists should not be made to practice in an area in which they are not comfortable or do not feel competent. Each group has to develop a policy for automatically diverting such biopsies to a center with resources to evaluate them. In Minnesota, for example, several hospitals do not look at these biopsies, and the University of Minnesota Medical Center has an understanding with the transplant groups in each region to have these biopsies sent to the medical center, even when the donor is located at another hospital. At the University of Minnesota, we are also in the process of introducing whole slide imaging and leveraging this technology in the transplant donor assessment workflow.

The organ procurement agency typically provides a worksheet that walks the pathologist step by step through what to evaluate, which makes it straightforward. For liver, this would include portal inflammation, fibrosis, fat content (macrosteatosis), and any obvious malignancy. Assessment of microsteatosis on frozen section should be discouraged as it has little influence on transplant decision and is prone to overcall or undercall. For kidney, it is mainly a percentage of sclerosed glomeruli and inflammation and any obvious malignancy.

A final note: If the pathologists who submitted the question are credentialled to perform this evaluation, they should be able to perform it. If they are not credentialled, they should not be required to do so.

*Oyedele A. Adeyi, MBBS*
*Staff Pathologist*
*University of Minnesota Physicians*
*Professor of Pathology*
*Department of Laboratory Medicine and Pathology*
*University of Minnesota Medical School*
*Minneapolis, Minn.*
*Member, CAP Surgical Pathology Committee*

*Sabrina C. Sopha, MD*
*Chairman and Medical Laboratory Director*
*Department of Pathology*
*University of Maryland*
*Baltimore Washington Medical Center*
*Glen Burnie, Md.*
*Member, CAP Surgical Pathology Committee*

**Q. What is the minimum and maximum formalin fixation time for cytology specimens for optimal immunohistochemical and nucleic-acid–based molecular testing?**

A. For most IHC stains performed on surgical pathology specimens, the American Society of Clinical Oncology/College of American Pathologists breast biomarker recommendation of six to 72 hours of formalin fixation is appropriate.[1,2] For cytologic samples, there is insufficient data to make an alternative recommendation. Available studies used cell blocks fixed within that range (for example, Sauter, et al., used six hours in their validation study of cell blocks[3]) or did not list the duration of formalin fixation.

Should cytologic samples be held to the same standard as surgical pathology specimens? Most cytologic preparations contain only single cells or small clusters of cells suspended in fluid, or small clotted tissue fragments aggregating to a few cubic millimeters. Thus, if needles were rinsed immediately with formalin or if fluid samples were pelleted and resuspended in formalin, it is reasonable to hypothesize that the minimum fixation time could be considerably shorter for many preparation types. Despite careful review of published literature on the influence of preanalytic factors on cytology specimens, we did not identify time-course studies on cytologic preparations that would answer this question.

For laboratories that employ formalin fixation only after creating a cell block, it is advisable to consider the thickness of the cell block in relation to a formalin permeation rate of 1 mm per hour. For very large cell blocks, it is advisable to have an adequate volume of formalin and space within the cassette to mix the formalin freely. In some cases, it may be necessary to serially section large cell blocks and, if needed, submit the blocks in more than one tissue cassette.

It is important to note that some IHC stains may be more sensitive to pre-fixation in alcohol-based fixatives commonly used in cytology, such as CytoLyt, leading to potential false-negative or false-positive results. Please refer to the CAP Pathology and Laboratory Quality Center guideline for more information on analytic validation of immunohistochemical assays.[4] Notably, the size of validation cohorts varies depending on whether an IHC stain is predictive (used to determine a patient's treatment, such as PD-L1) or nonpredictive (performed in the context of clinical and morphologic findings, such as TTF-1 or PAX8).

Compared to fresh tissue or alcohol fixation, formalin fixation causes nucleic acid degradation.[5] Thus, if a fresh sample is not available, alcohol fixation (methanol, ethanol, or commercially available alcohol-based fixatives) will better preserve the nucleic acid in cytologic samples. Options include using scrapings of a smear previously stained with modified Giemsa, Diff-Quik, or a Papanicolaou stain, or submitting a liquid sample in an alcohol-based fixative. Using supernatant liquid generated during the processing of cell blocks or liquid-based preparations are other options.[6]

Many molecular assays originally developed for surgical pathology applications are optimized for the shorter nucleic acid fragments and lower yield of nucleic acid produced by formalin fixation. Thus, a formalin-fixed, paraffin-embedded cell block may be the most appropriate cytologic analog for these molecular assays since this specimen type would not require additional extensive validation.

With a growing need for biomarker testing using cytology samples, which typically have limited volume, and increasing recognition that higher-quality nucleic acid is obtained from nonformalin-fixed cytologic preparations,

many laboratories are expanding the types of cytologic preparations accepted for molecular testing.


1. Wolff AC, Hammond MEH, Allison KH, et al. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update. *Arch Pathol Lab Med*. 2018;142(11):1364–1382.
2. Allison KH, Hammond MEH, Dowsett M, et al. Estrogen and progesterone receptor testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists guideline update. *Arch Pathol Lab Med*. 2020;144(5):545–563.
3. Sauter JL, Grogg KL, Vrana JA, Law ME, Halvorson JL, Henry MR. Young investigator challenge: validation and optimization of immunohistochemistry protocols for use on cellient cell block specimens. *Cancer Cytopathol*. 2016;124(2):89–100.
4. Fitzgibbons PL, Bradley LA, Fatheree LA, et al. Principles of analytic validation of immunohistochemical assays: guideline from the College of American Pathologists Pathology and Laboratory Quality Center. *Arch Pathol Lab Med*. 2014;138(11):1432–1443.
5. Srinivasan M, Sedmak D, Jewell S. Effect of fixatives and tissue processing on the content and integrity of nucleic acids. *Am J Pathol*. 2002;161(6):1961–1971.
6. Roy-Chowdhuri S, Mehrotra M, Bolivar AM, et al. Salvaging the supernatant: next generation cytopathology for solid tumor mutation profiling. *Mod Pathol*. 2018;31(7):1036–1045.



*Kaitlin Sundling, MD, PhD*
*Associate Director, Cytology Section*
*Wisconsin State Laboratory of Hygiene*
*Faculty Director, University of Wisconsin Cytotechnology Program*
*Assistant Professor, University of Wisconsin School of Medicine and Public Health*

*Madison, Wis.*
*Member, CAP Cytopathology Committee*

*Abiy B. Ambaye, MD*
*Professor*
*Medical Director of Histology and Immunohistochemistry*
*Department of Pathology and Laboratory Medicine*
*University of Vermont*
*Larner College of Medicine*
*Burlington, Vt.*
*Member, CAP Immunohistochemistry Committee*

*Sinchita Roy-Chowdhuri, MD, PhD*
*Associate Professor*
*Departments of Pathology and Translational Molecular Pathology*
*University of Texas*
*MD Anderson Cancer Center*
*Houston, Tex.*
*Member, CAP Cytopathology Committee*