

## CHAPTER 2

# Quick-Start Guide: Specimen Handling and Special Tests and Procedures in Pathology



*This chapter includes related videos.*



*This chapter includes a related activity.*



*Indicates selected key points within the chapter.*

### Highlights

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- Clear communication between the ophthalmologist and pathologist via the pathology request form and pathology report is essential for obtaining an appropriate specimen, ensuring proper handling, and facilitating an accurate diagnosis.
- In special circumstances, such as suspicion of malignancy or determination of a critical diagnosis, direct discussion with the pathologist during preoperative planning is vital.
- Although some histopathologic tests and procedures require fresh tissue, tissue fixation is typically required to arrest decomposition and preserve cellular morphology.
- Histochemical stains allow contrasting color staining of various cellular and extracellular elements and/or identification of specific material in tissue sections.
- Special procedures, such as immunohistochemistry and molecular genetic techniques, have led to improvements in the diagnosis of eye diseases.

### Glossary of Select Pathology Terminology

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**Circulating tumor cells (CTCs)** Cells shed from a primary tumor that may be detected in the blood of patients with early or advanced cancers using antibody-based assays or molecular methods. The presence of CTCs has been linked to unfavorable prognosis, and detection offers opportunities for individualized risk assessment.

**Cytology fixatives** Examples include ethanol, methanol, and Saccomanno fixatives, which are used to fix and preserve cells in smears and liquid-based cytology specimens (eg, corneal

scrapes, aqueous and vitreous fluid, and fine-needle aspiration biopsies) (see Table 2-1). Direct smears are air-dried and stained without fixatives.

**Direct immunofluorescence** Similar to indirect immunohistochemistry, except that this method uses a single antibody directly conjugated to a fluorophore that binds to the selected antigen(s). Fluorophores emit a signal that can be visualized using a fluorescence microscope. This technique requires fresh tissue samples (see Table 2-1).

**Flow cytometry** A method used to analyze the physical and chemical properties of cells moving in single file in a fluid stream. This is the most used method for immunophenotyping hematopoietic proliferations and requires fresh tissue (see Table 2-1).

**Formalin fixative** The most used fixative, 10% neutral-buffered formalin, is a 40% formaldehyde solution that stabilizes proteins, lipids, and carbohydrates and crosslinks proteins to prevent the destruction of tissue by enzymes (autolysis) (see Table 2-1).

**Glutaraldehyde fixative** Fixative used for electron microscopy that quickly penetrates tissues, preserving proteins, glycogen, and structures such as microtubules and smooth endoplasmic reticulum (see Table 2-1).

**Histochemical stains** Tissue dyes, principally hematoxylin and eosin (H&E) and periodic acid–Schiff (PAS), used to stain tissue sections for microscopy (see Table 2-2).

**In situ hybridization** Localization of a specific DNA or RNA sequence in a tissue section using a labeled complementary DNA, RNA, or modified nucleic acid strand (or probe). This method can be used on paraffin-embedded formalin-fixed tissue.

**Indirect immunohistochemistry** A method that detects the presence of 1 or more selected antigen(s) in cells using a primary antibody that can bind specifically to the queried antigen(s). A secondary antibody is then applied to bind with the primary antibody, producing a colored compound (chromogen) that can be detected with a light microscope. This method typically utilizes paraffin-embedded formalin-fixed tissue sections or cytology specimens (see Table 2-1).

**Microarray** A molecular biology technique used to survey the expression of thousands of genes in a single assay, the output of which is called a gene expression profile (GEP).

**Polymerase chain reaction (PCR)** A molecular biology technique used to amplify a single strand of nucleic acid by several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

**Roswell Park Memorial Institute (RPMI) medium** Cell culture medium that supports cell viability in biological samples (ie, “fresh tissue media”). RPMI is used when laboratory testing requires fresh, unfixed specimens (eg, flow cytometry to rule out lymphoma) (see Table 2-1).

**Tissue processing** An automated process involving the dehydration of tissues to allow infiltration with paraffin to make a tissue block, which can then be sectioned onto glass slides.

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## Introduction

This chapter covers the approaches that pathology laboratories commonly use to process and analyze tissue specimens, such as globes, intraocular contents, and ocular adnexal tissues. Glass slide preparations are typically used for histopathologic diagnosis and additional tests. In addition to diagnosis, tissues are evaluated for prognosis and therapeutic suitability. The main steps involved are

- communication with the pathologist
- appropriate handling and transfer of tissue, including submission of a requisition form containing relevant clinical information
- gross dissection to prepare the specimen for histologic sectioning
- tissue processing, slide preparation, and histochemical tissue staining
- consideration of special histological techniques and molecular clinical diagnostics

### TAKE NOTE

*Prior to biopsy or surgery, it is critical to communicate with the attending pathologist to plan for appropriate specimen collection and tissue fixation.*

## Communication With the Pathologist

Communication with the pathologist before, during, and after surgical procedures is an essential component of quality patient care. The ophthalmologist is responsible for providing relevant details regarding the clinical history when submitting the specimen to the laboratory. This history facilitates clinicopathologic correlation and enables the pathologist to provide the most accurate interpretation of the specimen.

Information is usually sufficiently conveyed by the pathology request form and pathology report. However, if there are any special circumstances, such as suspicion of malignancy or identification of a critical diagnosis, direct and personal communication between the ophthalmologist and the pathologist is essential. Discussion prior to surgery allows all involved physicians to consider the best way to collect a specimen and submit it to the laboratory. Checklist 2-1 is a preoperative checklist for handling routine specimens.

### CHECKLIST 2-1

#### Ophthalmic Pathology Consultation Checklist for Routine Specimens

1. Fill out requisition form or electronic order, providing
  - a. two patient identifiers
  - b. sex and age of patient
  - c. location of lesion (laterality and exact location)
  - d. previous biopsies of the site and diagnosis

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- e. pertinent clinical history
- f. clinical differential diagnosis
- g. ophthalmologist phone, pager, and/or fax number
2. Submit specimen in adequately sealed container with
  - a. ample amount of 10% formalin (at least 10 times the volume of the biopsy specimen)
  - b. label with 2 patient identifiers and location of biopsy site on the container itself, not the cap
3. If relevant, include diagram indicating the biopsy site and landmarks for orientation of resection margins (eg, complete resection of eyelid or conjunctival malignancies, ciliary body/iris tumors)

If a previous biopsy has been performed, the clinician should request a review of the prior slides, especially if there is a history of malignancy. The pathologist can compare the current and prior morphological features and diagnoses, and the subsequent treatment plan can be tailored based on the findings.

### TAKE NOTE

*If there is a significant discrepancy between the clinical diagnosis and the histologic diagnosis or between the initial and subsequent histologic diagnoses, the ophthalmologist should promptly contact the pathologist to resolve the discrepancy.*

### EXAMPLE 2-1

If the initial diagnosis was squamous cell carcinoma in situ but the repeat biopsy reveals a poorly differentiated, invasive squamous cell carcinoma, a more aggressive resection with lymph node biopsy may be more appropriate.

College of American Pathologists. Cancer protocol templates. Accessed June 10, 2023. [www.cap.org/protocols-and-guidelines/cancer-reporting-tools/cancer-protocol-templates](http://www.cap.org/protocols-and-guidelines/cancer-reporting-tools/cancer-protocol-templates)  
 Couce M, Dharamraj AM, Saad EAH, et al. Eye. PathologyOutlines.com. Accessed June 10, 2023. [www.pathologyoutlines.com/eye.html](http://www.pathologyoutlines.com/eye.html)

## Handling and Transfer of Tissue

### Fixatives

Table 2-1 lists many of the fixatives and transport media and their indications. Formalin is the most used fixative and diffuses quickly through tissues. In general, a specimen should be submerged in a volume of fixative that is at least 10 times the volume of the tissue. Tissue fixation time varies depending on the size and composition of the specimen. When a globe is sent for pathologic evaluation, for example, it should be suspended in formalin for at least 24–48 hours before processing. However, it is not necessary or desirable to open



**Table 2-1 Fixatives Used in Ophthalmic Pathology**

Fixative	Color	Examples of Use
10% neutral-buffered formalin (NBF)	Clear	Routine fixation of all tissues
Bouin solution	Yellow	Small biopsy specimens (eg, conjunctiva)
Absolute ethanol or methanol	Clear	Crystals (eg, corneal urate crystals)
Cytology fixatives (ethanol, methanol, or Saccomanno fixative)	Variety of colors	Liquid specimens or smears (eg, vitreous, aqueous humor, fine-needle aspirates, corneal smears)
Glutaraldehyde (2.5%)	Clear	Electron microscopy (eg, corneal microsporidia)
Michel or Zeus transport medium <sup>a</sup>	Clear	Immunofluorescence (eg, conjunctival biopsy for mucous membrane pemphigoid)
Roswell Park Memorial Institute (RPMI) tissue culture medium <sup>a</sup>	Pink, salmon	Tissue culture and fresh media (eg, orbital tumor for cytogenetics or flow cytometry) or transport medium for molecular studies

<sup>a</sup> Not a true fixative but prolongs tissue decomposition.

the eye, inject fixative, or create a scleral window to ensure adequate fixation despite the relatively large size and volume of the globe. Opening an eye before fixation may damage or distort sites of pathology, making histologic interpretation difficult or impossible.

While most pathology tests can be performed on fixed tissue, some situations require a fresh specimen. For example, biopsies to rule out lymphoproliferative disorders or conjunctival biopsies for suspected ocular cicatricial pemphigoid typically require ancillary diagnostic testing that can only be performed on fresh tissue. In these cases, tissue should be either submitted in a small volume of saline, wrapped in saline-soaked gauze, or submerged in tissue culture media (see Table 2-1). Because different institutions may use different protocols, preoperative consultation with the pathologist is critical to determine the appropriate approach.

### TAKE NOTE

*Direct immunofluorescence studies for the evaluation of ocular cicatricial pemphigoid and other blistering disorders are usually performed in specialized pathology laboratories. Preoperative communication with the pathology laboratory is essential to ensure appropriate tissue submission and handling.*

Ocular cytology specimens, such as corneal or conjunctival scrapings, aqueous fluid samples, vitreous fluid samples, and fine-needle aspiration biopsies (see further discussion under Special Techniques and Procedures), may require specific fixatives or special tissue handling (Activity 2-1). For example, conjunctival and corneal scrapings are usually submitted as direct smears or may be alcohol-fixed like cytology specimens.