

Wills Eye Hospital

Instructions for Submission of Specimens to the Ophthalmic Pathology Laboratory

Fixation and Specimen Containers

With the exception of lymphoid tissue for flow cytometry and vitrectomy specimens, tissue should be fixed by immersion in a sufficient volume of **neutral buffered formaldehyde** prior to submission to the laboratory for histopathologic evaluation. The size of the container should be appropriate for the specimen.

Specimen Containers and Path Forms are Available on Request

The laboratory will provide contributors with specimen bottles filled with fixative and pathology submission forms on request. Four sizes of specimen bottles are available: very small (20 ml with 10 ml of fixative), small (30 ml with 20 ml of fixative), medium (120 ml with 80 ml of fixative) and large (480 ml with 200 ml of fixative). A copy of our **pathology submission form** can be downloaded as a pdf on this website.

The Container Size Should Be Appropriate For The Specimen

Small containers are suitable for corneas, and small biopsies of conjunctiva or eyelid tissue. **They are not suitable for enucleated eyes.** Enucleated eyes need to be immersed in a relatively large volume of fixative and must be submitted in the larger, medium size 120 ml container available in the Wills Surgery Center OR. The latter contain approximately 80 ml of fluid when completely full. The very large containers are designed to hold orbital exenteration specimens.

Saline Is Not A Fixative!!

Specimens should never be submitted in saline. Saline is not a fixative! Specimens of fresh tissue (e.g. lymphoma for flow cytometry) should be forwarded in a closed, moist chamber (specimen bottle with lid and piece of moistened gauze) If a specimen is inadvertently submitted in saline (and laboratory personnel are not notified) it is possible that they will assume that it is in fixative – with potentially disastrous consequences.

Always Notify The Lab When Submitting Fresh Unfixed Tissue

The laboratory should always be notified by phone whenever fresh unfixed tissue is submitted. This assures that it will be processed properly and expeditiously.

Make Sure Trephined Eyes Are Submersed in Fixative!

Care should be taken when submitting enucleated eyes that have been trephined in the OR to allow harvesting of fresh tissue. They may contain entrapped air introduced by the trephination. If the bubbles are not expelled, the eyes will float on the surface of the fixative and will not be properly fixed.

Specimen Containers Must Be Properly Labeled

All primary specimen containers must be appropriately labeled. The label on each primary specimen container must contain at least **2 unique patient identifiers**. In addition to the patient's name, these identifiers may include date of birth, patient age, patient's home address, social security number hospital or surgery center account number or social security number. Printed patient labels generated in the Wills Surgery Centers are appropriate. Containers filled with formalin fixative also must bear a standard **formaldehyde warning label**. The labels should be placed on the side of the container. They should never be affixed to the lid.

Sending Specimens To The Laboratory

FedEx is an appropriate method of forwarding specimens to the laboratory. It is fast, reliable and specimens can be tracked. Specimens must be properly packaged!! (see below)

Specimens Must Be Packaged Properly To Leakage and Loss

Specimens must be properly packed for transit to avoid leakage and potential specimen loss. Commercial carriers like FedEx can. **and will** discard packages wetted by leaking containers. Specimens always should be submitted jars or plastic specimen containers that have tight fitting, leak-proof screw caps. (Urinalysis containers leak and are unsuitable.)

Placing A Container Within A Container Prevents Leakage

The first specimen container should be placed within a second leak-proof container such as sealable plastic bag that is designed to contain spills if a lid accidentally comes off, or the container is broken. FedEx has special packaging designed for use with specimens.

Further Comments About Specific Types of Specimens

Conjunctival Biopsies

Biopsies of conjunctival tissue tend to "ball-up" when they are immersed directly in fixative. The latter makes assessment of surgical margins, which is always a challenge with conjunctival specimens, nearly impossible. For this reason, fresh conjunctival specimens should be carefully spread on a thin piece of cardboard or heavy paper prior to fixation. The piece of paper bearing the specimen is then immersed in fixative and the specimen is fixed as a sheet. The cards packaged with surgical sutures are excellent for this purpose. Even if tissue is handled in this manner, it is often difficult to assess the margins of conjunctival specimens. If surgeons are concerned about margins, they are encouraged to submit separate specimens of marginal tissue.

Lymphoid Lesions

If possible, lesions known or suspected to be lymphoid in nature should be submitted for flow cytometric analysis. This requires the expedient submission of a sufficient quantity of fresh unfixed tissue. The fresh tissue should be placed in a moist container, e.g, an empty specimen container with a moistened piece of gauze. It should not be immersed in saline. An alternative media for specimens submitted from surgical centers outside of Wills Eye Hospital is RPMI (pink media), which can be provided by Pathology Department at the request of the surgeon. A sufficient quantity of tissue generally is generally stated to be greater than the size of a "pea". It often is impossible to perform flow cytometric analysis on small quantities of suspected lymphoid tissue excised from the conjunctiva. In such instances, immunohistochemical analysis can be performed on paraffin sections. However, the latter technique does not allow quantification or evaluation of clonality by assessing immunoglobulin light chains.

The laboratory should always be notified when fresh tissue is submitted to prevent the unfixed specimen from being overlooked among samples of fixed tissue.

Flow cytometric analysis generally can only be performed on tissue excised at the Wills Eye Hospital because fresh tissue is required and it must be processed expediently. In addition, it usually is impossible to perform "flow" if the specimen is received too late in the day (generally after 2:30 PM). In such case immunophenotypic analysis will be performed on paraffin-embedded tissue.

When fresh tissue is received for flow cytometric analysis, a preliminary touch preparation usually is made. If the touch preparation does not disclose a significant number of lymphoid cells, the specimen will be fixed and processed routinely, and will not be forwarded for flow cytometry.

Vitrectomy Specimens

Vitrectomy specimens are processed in the Wills Eye Hospital Laboratory using the cytopsin technique. If sufficient material is available, a cell block of centrifuged particulates embedded in paraffin and sectioned is made. The cytopsin preparation is stained routinely with H&E supplemented with stains for microorganisms or immunohistochemical preparations as necessary. Surgeons are advised to submit a sufficient volume of fluid for analysis. The entire vitrectomy cassette or fluid collection bag should be submitted. This should be done expeditiously since the contents are not fixed and the cells can degenerate rapidly. The specimens should be refrigerated if submission is delayed briefly.

At Wills, vitrectomy specimens currently are submitted for pathologic examination at the request of the surgeon. Most are submitted to assess vitreous lymphoma or rarely amyloidosis. Adequate clinical information and communication helps to assure that such specimens are processed in the most appropriated fashion. The latter is particularly important if infection is suspected. In such cases, submission of a large specimen that can be used to prepare a cytologic cell block will increase diagnostic yield.

Corneal Smears Or Scrapings - Acanthameoba Keratitis

Smears or scrapings of corneal epithelium generally are forwarded to the laboratory to assess possible acanthamoebic keratitis. In the Wills Lab, such smears are routinely stained with hematoxylin and eosin. Evaluation requires an adequate sample of tissue, typically small sheets of epithelium. These should be readily visible to the naked eye on the unstained slide. Slides can be air-dried or preferably fixed with aerosol “spray-cyte” fixative or immersed in 95% alcohol container after they are obtained. The pathology slips that accompany such specimens should state that the smears are being submitted to rule-out acanthamoeba. Fungi, bacteria and acid-fast organisms will not be detected with the H&E stain. If the differential diagnosis includes other organisms, this must be prominently stated on the pathology slip and a sufficient number of slides submitted. In unusual cases, communication with the pathologist is advised.

Infectious disease processes

When any infectious disease processes are considered, including infectious keratitis and corneal ulcer, infectious conjunctivitis, orbital cellulitis, endophthalmitis and others, submission of a separate representative sample to Jefferson Microbiology Department is recommended.

SUBMISSION RECOMMENDATIONS SUMMARY

Surgical site	Specimen type / Diagnostic considerations	Submission recommendations
Eyelid	Routine biopsy	10% neutral buffered formaldehyde - Orient with sutures if not a full-thickness eyelid tissue and margins are required
Eyelid	Lymphoproliferative	2 tissue samples: #1. Fresh in moistened gauze (on site) or in RPMI (off site) #2. 10% neutral buffered formaldehyde
Orbit	Routine biopsy	10% neutral buffered formaldehyde
Orbit	Lymphoproliferative	2 tissue samples: #1. Fresh in moistened gauze (on site) or in RPMI (off site) #2. 10% neutral buffered formaldehyde
Orbit	Sarcoma	2 tissue samples: #1. Fresh in moistened gauze (on site) or in RPMI (off site) #2. 10% neutral buffered formaldehyde
Conjunctiva	Routine biopsy	- Spread on a cardboard prior to fixation. - Orient with drawing or sutures if margins are required
Conjunctiva	Lymphoproliferative	2 tissue samples: #1. Fresh in moistened gauze (on site) or in RPMI (off site) #2. 10% neutral buffered formaldehyde
Cornea	Scrape biopsy (epithelium)	Spray Fix Cytology Fixative Aerosol or 95%

		alcohol in a plastic slide container Or Air dried (less preferable)
Cornea	Evaluation of crystalline material	100% alcohol
Cornea	All other tissues	10% neutral buffered formaldehyde
Lens		10% neutral buffered formaldehyde
Iris, ciliary body, choroid, retina	Non-lymphoproliferative diseases	10% neutral buffered formaldehyde
Iris, ciliary body, choroid, retina	Lymphoproliferative	Discuss with pathologist prior to submission - RPMI (on site) - 10% neutral buffered formaldehyde (off site)
Vitreous	Lymphoproliferative	Discuss with pathologist prior to submission - Fresh or RPMI (on site) - RPMI or 10% neutral buffered formaldehyde (off site)
Vitreous	Non-lymphoproliferative	- Fresh or 10% neutral buffered formaldehyde (on site) - Fresh or 10% neutral buffered formaldehyde (off site)

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