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PATHOLOGIC EXAMINATION OF OCULAR SPECIMENS

This chapter describes the most common approaches for the handling of ophthalmic pathology specimens. It is not intended to be a comprehensive document for grossing, but rather to highlight the essential techniques and features of each type of ophthalmic specimen removed for the diagnosis and treatment of neoplasia.

GLOBE

Enucleation

In an enucleation procedure, the eye (globe) is usually removed intact, with an attached segment of an optic nerve and small amounts of periocular soft tissue (fig. 1-1). In a procedure known as evisceration, the surgeon cures the intraocular contents and removes corneoscleral tissue leaving the shell of the sclera behind. The evisceration technique is not appropriate in the context of a suspected neoplasm. Evisceration carries the risk of tumor spread into the orbital tissues when an intraocular neoplasm is present.

Enucleation is the preferred approach to remove an eye with a tumor. Fresh tumor retrieval techniques for genetics and molecular testing are described in other chapters. Most eyes come to the pathology laboratory fixed in 10 percent formalin. Fixation is recommended for at least 48 hours to obtain complete penetration by the fixative of the sclera and intraocular tissues, including the tumor. Injection of formalin or opening of the anterior chamber is discouraged because these procedures may alter the interpretation of the relationship between the tumor and the intraocular tissues and ocular coats. The recommended approach is to place the eye in at least five times its volume of formalin for adequate fixation.

The specimen is first identified as a right or left eye. The extraocular muscles include the four recti and the two oblique muscles. The recti muscles insert a few millimeters behind

the limbus. The distance from the limbus to the muscle insertion progressively increases from the medial rectus muscle, to the inferior rectus muscle, to the lateral rectus muscle, to the superior rectus muscle (farthest from the limbus), forming an opening spiral (fig. 1-2). The differences between muscles are minute, however, making this approach impractical for confirming laterality of the eye (right or left eye). Also, various ocular pathologic conditions may distort the anatomic placement of rectus muscles relative to the corneoscleral limbus.

The most reliable way to confirm the laterality of the eye is to identify the oblique muscle insertions. The oblique muscles insert in precise locations, with the inferior oblique inserting directly into the temporal side of the optic nerve behind the location of the macula in the retina. The superior oblique inserts slightly temporal (lateral) to the center of the eye (12:00

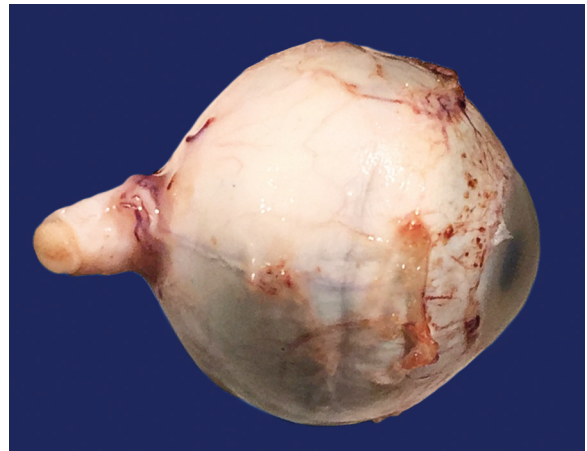


Figure 1-1

ENUCLEATED EYE

An enucleated eye with an attached segment of the optic nerve (to the left). No significant amount of orbital tissue is present.

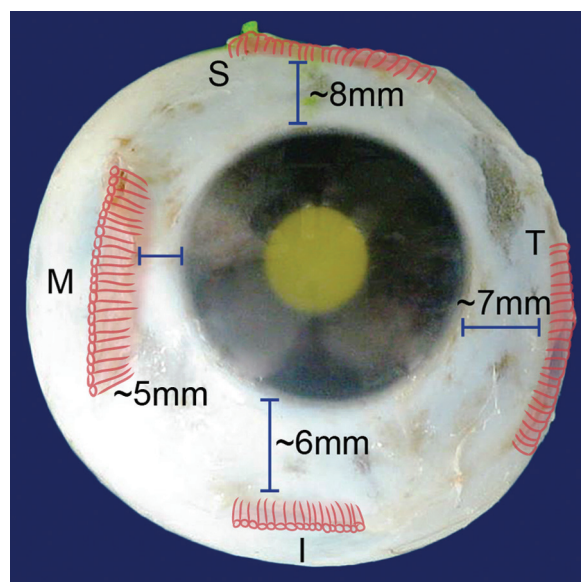


Figure 1-2

ENUCLEATED EYE

Frontal view of an enucleated eye with the distance of the superior (S), temporal/lateral (T), inferior (I), and medial/nasal (M) recti extraocular muscle insertions to the limbus. The insertion openings from medial to superior rectus muscle insertions are spiral shaped.

o'clock position), anterior to the equator, and the insertion is through a long tendon.

In many eyes, the track of the long ciliary nerves/vessels is seen as a light blue/gray line that runs horizontally, a few millimeters from the optic nerve to the equator. This feature is helpful in orienting the eye for the localization of the horizontal plane and finding the insertion of the inferior oblique muscle (fig. 1-3).

After orientation, it is recommended to place a mark on the sclera at the 12:00 o'clock position to maintain orientation during grossing. The eye is then measured: first, anterior-posterior (from the center of the cornea to the edge of the sclera meeting the optic nerve); then horizontal (at equator); and finally, vertical (at equator) preferably using measuring calipers. Also recorded are the horizontal and vertical measurements of the cornea from one edge of the limbus to the other, iris color, iris appearance, and status of the anterior chamber and pupil (fig. 1-4).

The length of the optic nerve is measured from the edge of the sclera to the tip of the cut margin while extending the nerve to correct

the natural fixation shrinkage of the tissue (this is particularly useful in eyes harboring retinoblastoma). In some laboratories, the maximum width of the nerve near the scleral exit of the nerve is measured.

There are two approaches to taking cross sections of the optic nerve. In some laboratories, a cross-section is obtained 2 to 3 mm posterior to the insertion of the nerve into the sclera because tissue at the actual surgical margin may be crushed. In cases of retinoblastoma, a cross section at the 2- to 3-mm point that is free of a tumor means that the resection margin is also free.

Many laboratories take a cross section of the nerve near or at the surgical resection margin. Ink is applied to the optic nerve margin, and a transection perpendicular to the long axis is performed to obtain the margin for histopathologic examination.

If a laboratory receives the optic nerve as a separate piece of tissue, the length and width should be measured. The surgical resection margin is then inked (fig. 1-5).

For eyes suspected of harboring uveal melanoma, some pathologists sample the vortex veins before opening the eye. These veins are believed to be a pathway for metastasis. The vortex veins exit the sclera posteriorly, and these structures can be cut with scissors and submitted individually in separate cassettes (fig. 1-6). Other laboratories do not submit this tissue for histologic sectioning because there is no evidence that involvement of the veins by uveal melanoma is associated independently with metastasis. Practices even vary across the laboratories of the contributors to this text.

Transmission of light through the eye may be helpful in locating an intraocular tumor before sectioning. In a darkened room, an intensive light source is focused onto the center of the cornea, and the eye is slowly rotated to find the edges of the shadow if present. A marker is used to trace the edge of the shadow on the sclera. The orientation of the sectioning of the eye is centered on the tumor shadow to obtain the central anterior-posterior section, the pupil-optic nerve (PO) section, through the tumor (fig. 1-7).

Using a sharp blade, the eye is opened starting from the back at less than 1 mm from the edge of the optic nerve into the sclera while firmly holding the eye with the fingers on top of

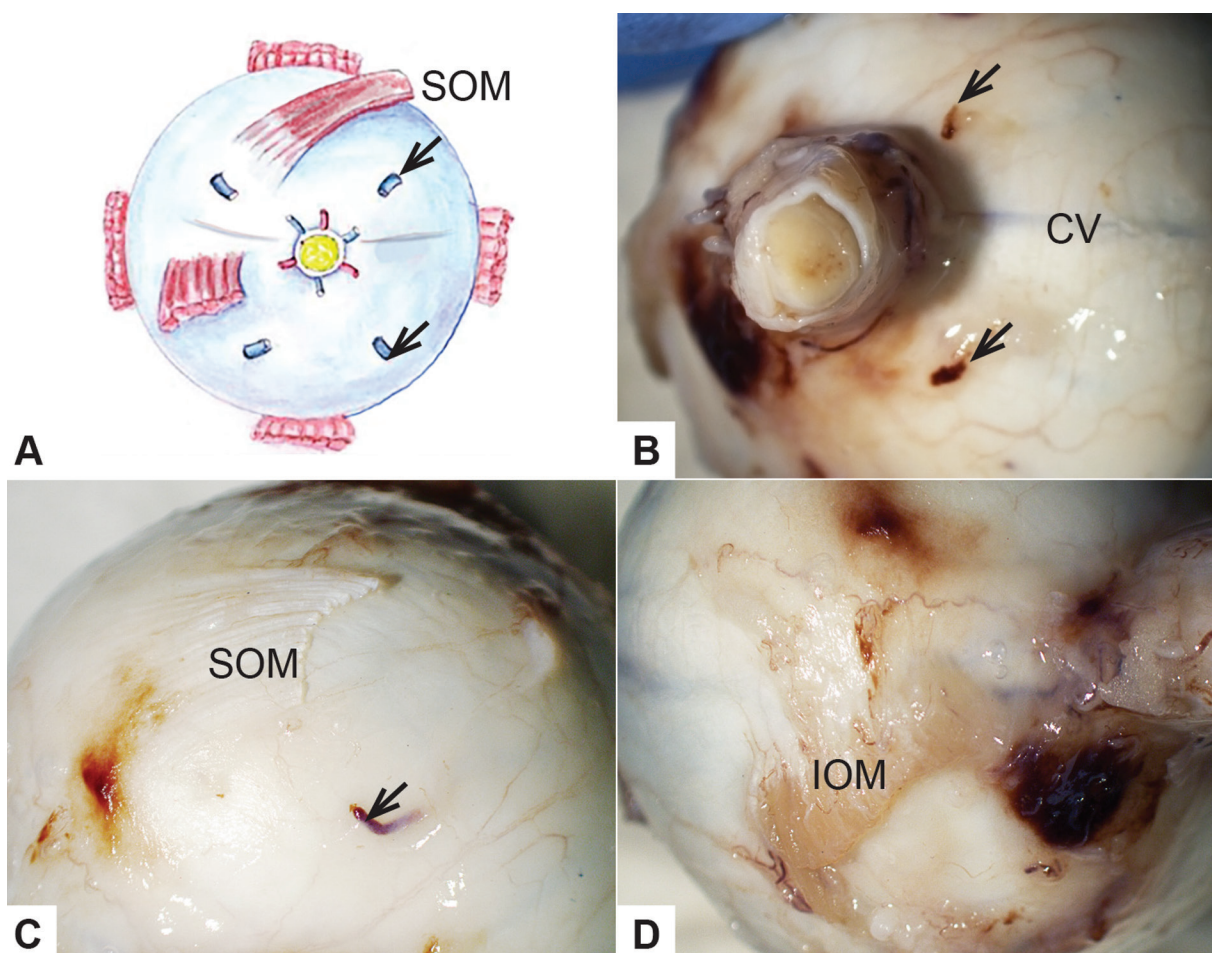


Figure 1-3

POSTERIOR VIEW OF THE EYE

A: Schematic representation of the back of a left eye. The tendinous insertion of the superior oblique muscle (SOM) superior and temporal to the optic nerve (in yellow). The four vortex veins (arrows) exit at the center of the quadrants and posterior to the equator. Slightly superior to the optic nerve in the sclera is the long ciliary vessels in the horizontal plane, seen as gray lines. The insertion of the inferior oblique muscle is seen. This muscle has a direct insertion into the sclera without a long tendon (seen in this illustration just left of the optic nerve).

B: Arrows point to the superior-nasal and inferior-nasal vortex veins in this enucleated eye.

C: The shiny white tendinous insertion of the SOM and the superior-nasal vortex vein (arrow) are seen.

D: In contrast with the SOM, the inferior oblique muscle (IOM) inserts directly into the sclera, without a tendon.

the equator on a nonslippery surface. The blade is advanced with a lateral zigzag motion toward the anterior segment, aiming to enter the anterior chamber at about 2-mm internal to the limbus.

When the blade reaches the anterior portion of the globe, after having crossed the equator, the blade is kept in place while the eye is rotated onto the corneal surface. Then, using both hands to hold the blade, it is possible to cut through the remainder of the eye with a swift guillotine-like movement toward the cornea,

passing through the lens and cornea without dragging intraocular tissues (fig. 1-8).

The interior of the eye is examined after removing the first calotte (cap), and the tumor is described (size at the base in contact with the choroid or sclera and maximum height on the cut surface), noting any morphologic effects on the intraocular tissues. The eye is then turned down on the cut surface, and the same cutting method is used to remove the opposite calotte. The central PO nerve section is about 7.0 mm

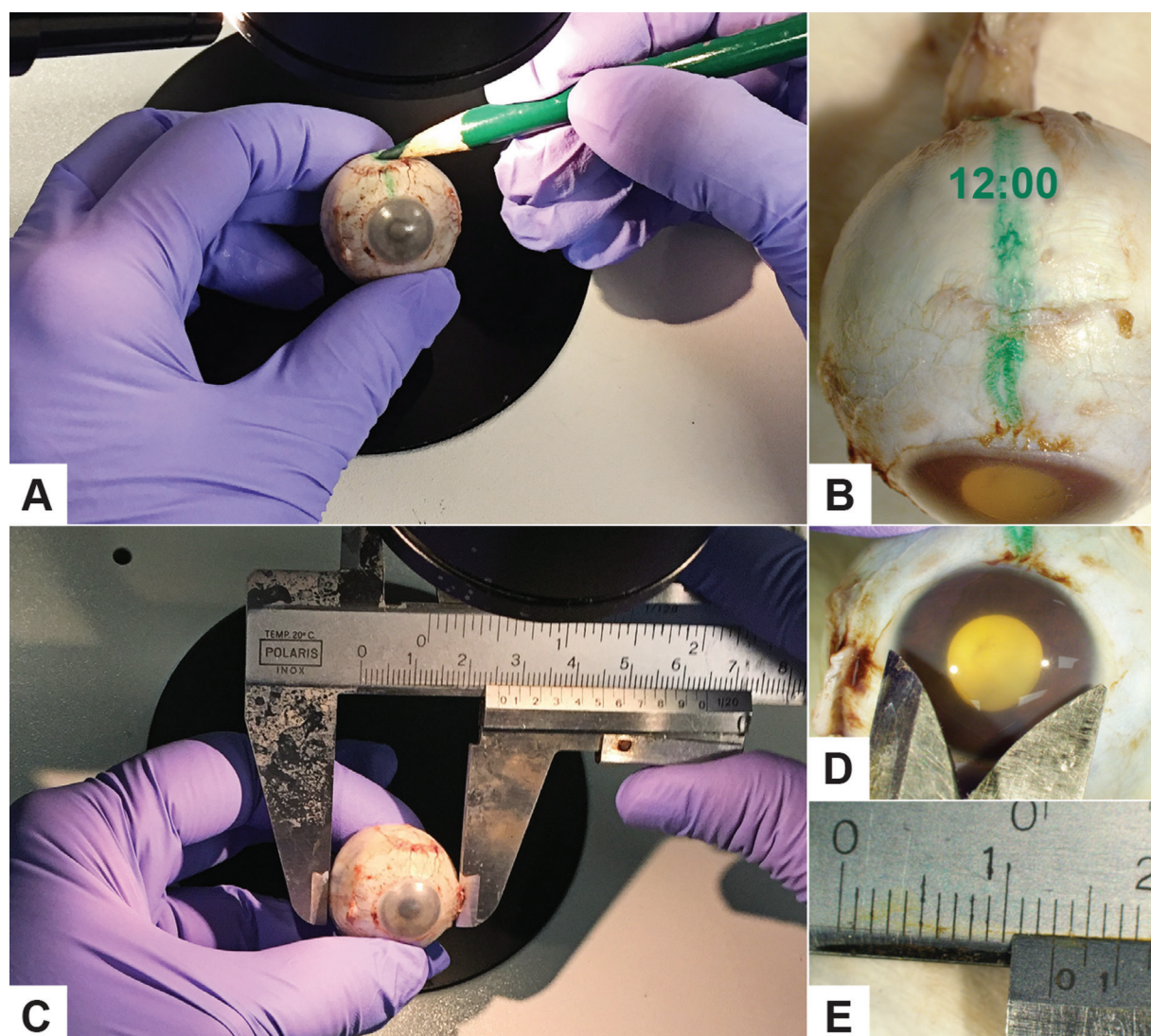


Figure 1-4

ORIENTATION OF THE EYE DURING GROSSING

A: After conferring laterality to the eye, the 12:00 o'clock position starting at the limbus is marked with a colored pencil or a similar implement.

B: The line extends to the back of the sclera until meeting the optic nerve.

C: Using a vernier, the eye is measured in the horizontal dimension (seen here) and also anterior posteriorly and vertically.

D: The diameter of the cornea is measured with the vernier from one aspect of the limbus to the other.

E: The measuring scale shows 13 mm for the measurement of this cornea in the horizontal plane.

in thickness at the center, wider in the cornea (7.5 to 8.0 mm) and narrower by the optic nerve (about 5.5 mm). Findings on the two calottes are reported after separating them.

The eye can be submitted entirely in three cassettes. The calottes may be further sectioned in anterior-posterior segments to increase the surface of tumor and intraocular structures stud-

ied in the histologic sections (see figs. 1-8 and 9-20). It may be helpful to obtain photographs of the cut surface of the eye (1,2).

Alternatively, for suspected uveal melanomas, the eye may be opened in a plane that permits the pathologist to visualize the tumor from the same perspective as the ophthalmologist. In the case of uveal melanoma in the posterior pole,

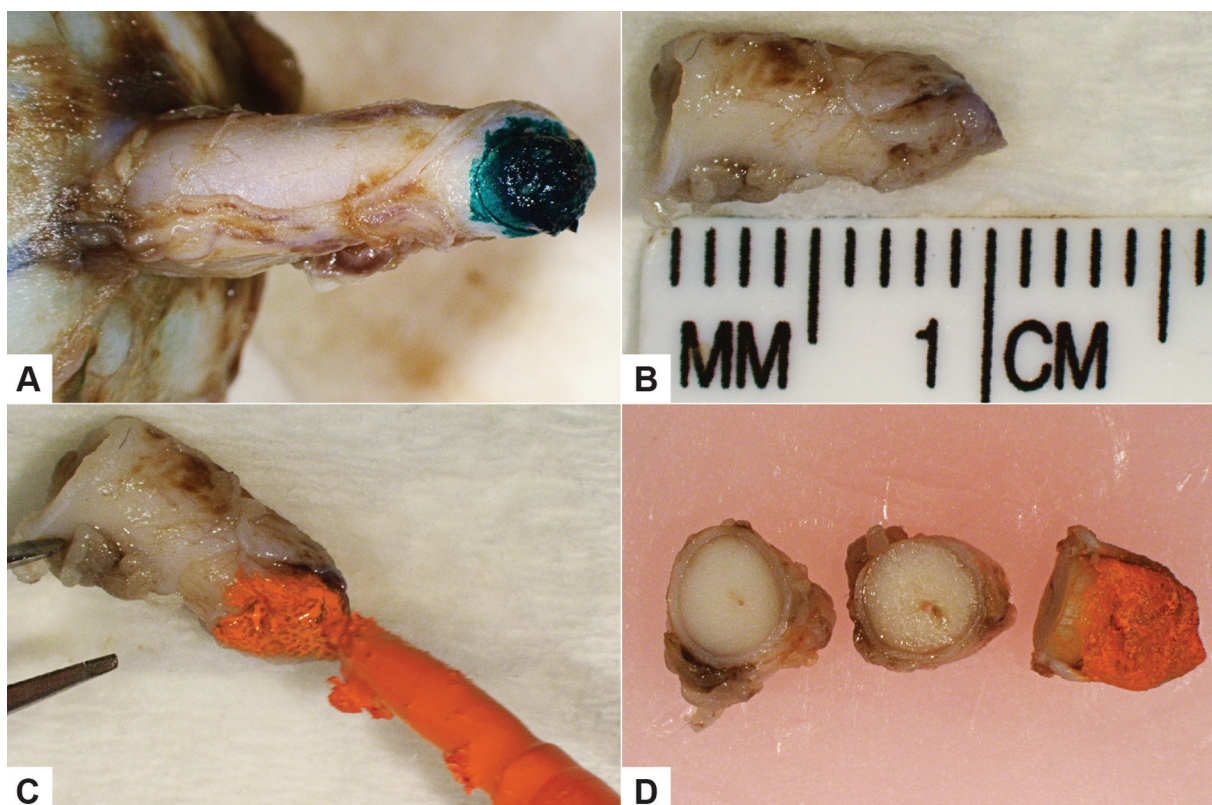


Figure 1-5

OPTIC NERVE MARGIN

- A: The optic nerve margin is inked and sectioned to be submitted separately.
- B: A separately received optic nerve in an enucleation for retinoblastoma is measured to the tip of the margin.
- C: Ink is applied to the real margin (note the slant rough cut surface).
- D: The nerve is serially sectioned and submitted for examination in one cassette.

it is possible to take a coronal section (parallel to the surface of the cornea) through the pars plana, revealing the tumor in the posterior pole, just as the ophthalmologist visualized the tumor. This maneuver permits the pathologist to take sections through the tumor to address questions that the ophthalmologist may have about the clinical appearance (for example, to sample a particular margin precisely). If an alternative protocol is used for tumors anterior to the equator of the eye, then the eye is opened in a plane that is parallel to the apex of the tumor, allowing for the generation of precise section planes through areas of interest by direct tumor visualization (3).

Each paraffin block is sectioned to obtain representative sections of all the intraocular structures of importance for providing prognostic

information from the histopathologic review. For example, in retinoblastoma, it is crucial that the PO nerve section demonstrates the optic nerve in its entirety, with prelaminar, laminar, post-laminar components. For retinoblastoma, experts recommend trisecting the calottes (caps) and submitting them for histopathologic review (see chapter 9).

Although the hematoxylin-eosin (H&E) stain is used conventionally for uveal melanoma and retinoblastoma, most laboratories also generate slides stained by periodic acid-Schiff (PAS) because this stain reveals pathology in the various basement membranes of the ocular interior. Most experts also favor the bleaching of melanin with H&E counterstaining when studying uveal melanomas and other highly pigmented intraocular tumors.

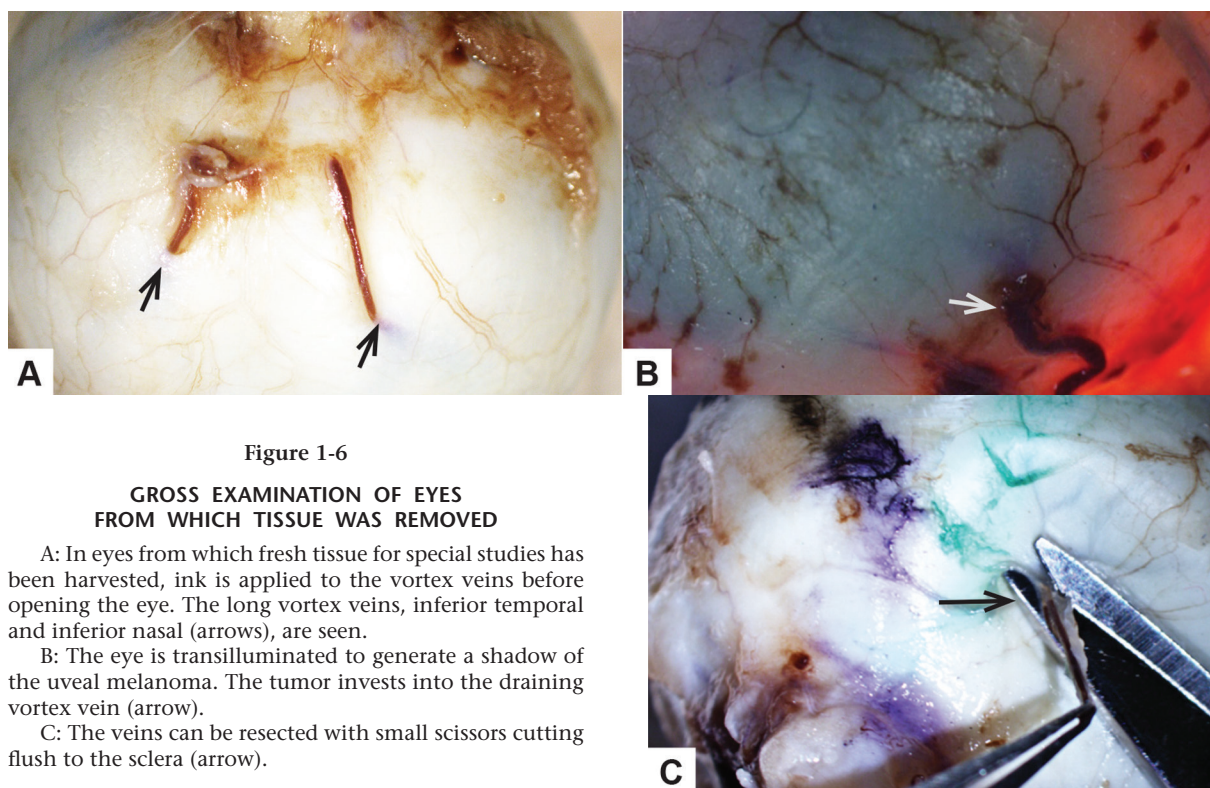


Figure 1-6

GROSS EXAMINATION OF EYES FROM WHICH TISSUE WAS REMOVED

A: In eyes from which fresh tissue for special studies has been harvested, ink is applied to the vortex veins before opening the eye. The long vortex veins, inferior temporal and inferior nasal (arrows), are seen.

B: The eye is transilluminated to generate a shadow of the uveal melanoma. The tumor invests into the draining vortex vein (arrow).

C: The veins can be resected with small scissors cutting flush to the sclera (arrow).

Segmental Excision

Segmental excisions of iris or iridociliary tumors are seldom performed. Orientation by the surgeon for laterality and identification of the clock position occupied by the lesion contribute significantly to a thorough evaluation of margins.

It is helpful for surgeons to submit iridectomy specimens on a piece of thick paper or other flat surface material (a piece of wooden tongue depressor facilitates the adhesion of the fresh tissue to the wood before placing into formalin) that preserves the shape of the specimen during fixation for adequate orientation and sectioning. The use of a stereoscopic microscope is encouraged when grossing these specimens, since the lesions may be less than 5 mm in maximum diameter and the localization and orientation of the margins may be challenging without the magnification. Handling of these tiny and flimsy specimens can benefit from the use of gel-tissue embedding before processing at the time of the grossing. This protocol may facilitate the maintenance of specimen orientation

(see Pretissue Processing Embedding Technique below; fig. 1-15).

Iridectomy Specimen. Excision of iris tumors usually is accompanied by a small amount of iris surrounding the lesion and the pupil margin. The lesion may be inked for the peripheral margins (toward the sclera, and either inferior and superior, temporal and nasal, or preferably called by the clock hour to identify the margin). The pupillary margin and the deep and superficial margins are not attached to any structure. Thus, these are not margins unless there are synechias to the lens or the cornea. In these cases, the surface may be inked and referred to as cornea or lens surface.

After inking, the specimen is serially sectioned using a sharp thin blade (razor blades are better for small specimens). They are then embedded on edge (in pretissue processing gel if available), preferably no more than two pieces in each cassette.

Iridocyclectomy Specimens. Tumors in the ciliary body or iris and ciliary body are sometimes excised “en block” with small amounts of adjacent iris, ciliary body pars plana, and sclera. In these excisions, similar to the iridectomies, it is

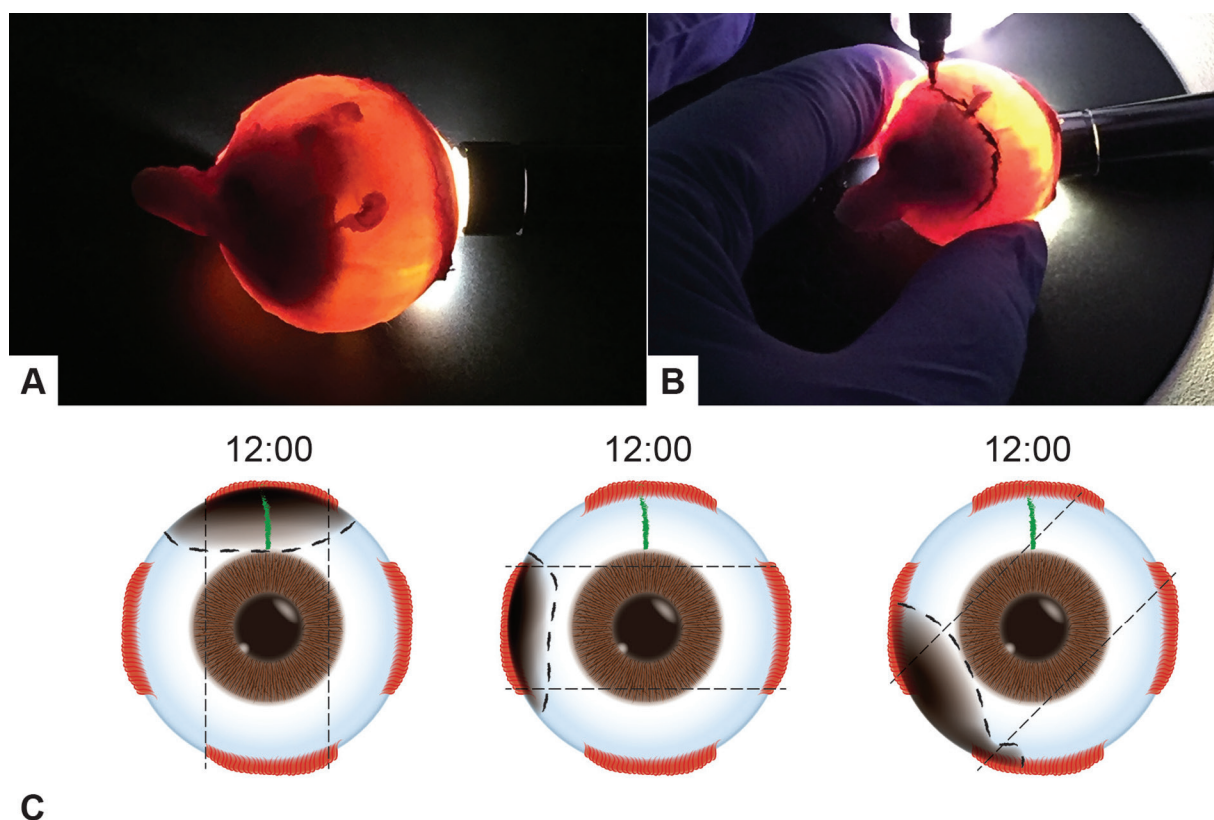


Figure 1-7

TRANSILLUMINATION OF THE EYE

A: In a darkened room, an intense light is focused onto the cornea, rotating the globe to show the shadow and its limits.

B: A marker is used to delineate the shadow.

C: After the shadow is delineated, a decision of how to cut open the eye is made based on the location of the tumor. The goal is to create a central pupil-optic nerve section to have all essential structures in one section, including the tumor. The diagram shows the possibilities depending on the location of the tumor. The globe on the left has a tumor in the superior quadrant, and a vertical section is selected. The globe in the middle has tumor in the medial or nasal quadrant, and the best section is horizontal. If the tumor is in the middle of the quadrants, then the section should be oblique, as seen in the globe on the right.

helpful to know the clock position of the excision to orient the margins. The posterior margin in this excision is the margin with the remainder of the ciliary body, usually the pars plana. The pathologist should ink the scleral margin in an iridocyclectomy specimen because the scleral margin is a critical landmark. After the specimen is inked, it is serially sectioned into pieces (as in a pie) with each section taken with a pupillary margin and a scleral margin (fig. 1-9).

Biopsy

Rarely, ophthalmologists perform biopsies of the interior of the eye: intraocular biopsies. Retina and chorioretinal biopsies are useful in

rare tumors that do not shed cells to the vitreous. A transvitreal or transscleral approach may be used to obtain the biopsies. The transvitreal approach is preferred because the surgeon can visualize the lesion. This technique requires specialized instruments (laser, special scissors and forceps, and a vitrectomy instrument) which are used to minimize trauma to the retina and vitreous, and avoid bleeding. The resulting biopsy is delicate and small. Ideally, it is placed on filter paper to maintain orientation and flat architecture. Some of these biopsies, however, are received in fluid because they are suctioned through the vitrectomy instrument, to avoid further retinal damage or bleeding.

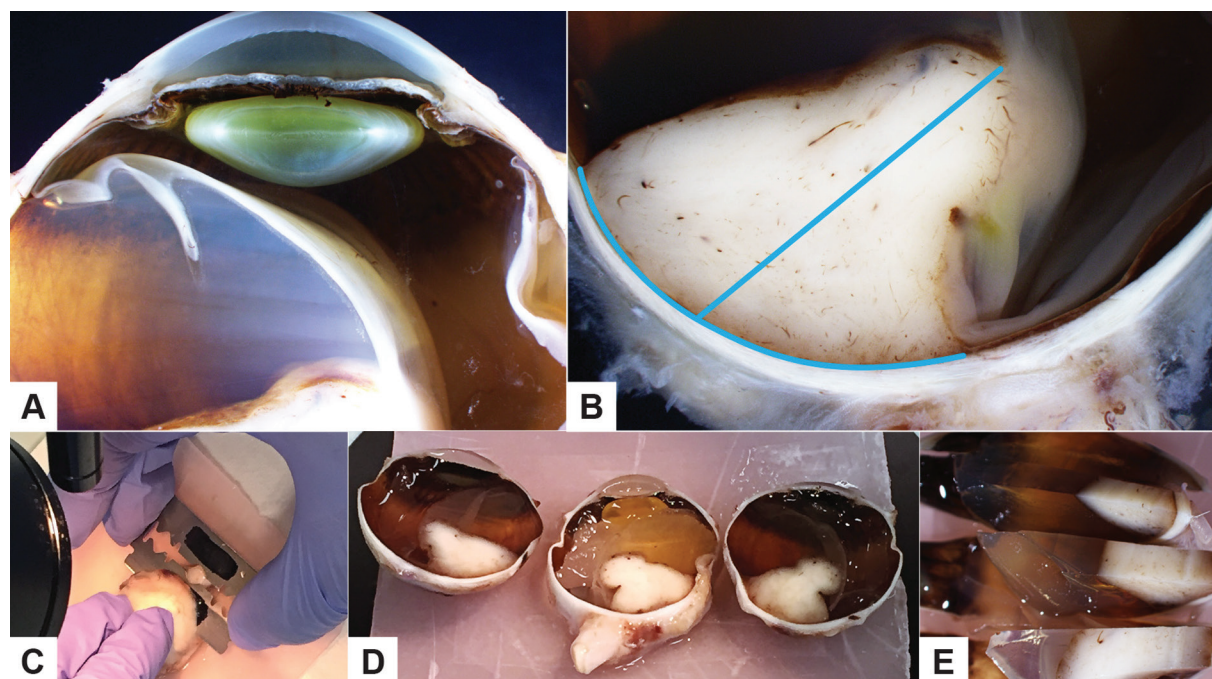


Figure 1-8

INTERIOR OF THE EYE

A: Cutting the first calotte allows the pathologist to visualize the interior of the eye. At the top, the cornea, anterior chamber, and lens are without pathologic alteration. The pathology appears in the posterior portion of the eye with a detached retina (white dome-shaped membrane).

B: The detached retina overlies the amelanotic mushroom-shaped uveal melanoma. The tumor is measured at the base in contact with the sclera as a tangent to the scleral curve and the tumor's maximum height at the cut surface is also recorded.

C: The second calotte is obtained by a section parallel to the first section, starting in the sclera adjacent to the optic nerve. The two fingers of the hand not holding the blade (inferior left) are firmly securing the eye on the equator and away from the track of the blade.

D: After obtaining the two calottes (left and right), a central pupil optic nerve section is obtained (at center) containing the tumor.

E: The calottes are further sectioned in a bread-loaf manner to achieve larger surface of tumor and ocular tissue for histopathologic evaluation. Here, segments were generated from one calotte.

Ophthalmologists who typically obtain biopsies from the choroid through the sclera commonly place tissue in formalin. If the clinical diagnosis points to intraocular lymphoma, then the tissue sample may be submitted in RPMI medium.

In any of these scenarios, the small tissue sample may be grossed under a dissecting microscope. It may be beneficial to consider using gel embedding to facilitate orientation (choroid or retina).

Fine-Needle Aspiration Biopsy. Most diagnoses of intraocular tumors depend on clinical and imaging findings. The reasons to avoid biopsies include the risk of complications of the biopsy techniques that may compromise visual acuity when the naturally clear media of the eye

(cornea, anterior chamber fluid, lens, and vitreous) become cloudy with hemorrhage, scarring of the intraocular tissues, or retinal detachment.

In retinoblastoma, the possibility of seeding tumor during the biopsy procedure and converting an intraocular tumor into an extraocular and possibly metastatic tumor is one of the main reasons to avoid taking biopsies. In uveal melanoma, the most common indication for intraocular fine-needle aspiration biopsy (FNAB) is to obtain tumor cells for molecular prognostic testing. Some ocular oncologists only obtain the sample and send the entire specimen for molecular testing; others submit some sample to cytology to confirm the diagnosis, and most importantly, adequacy of the sample. Few practices

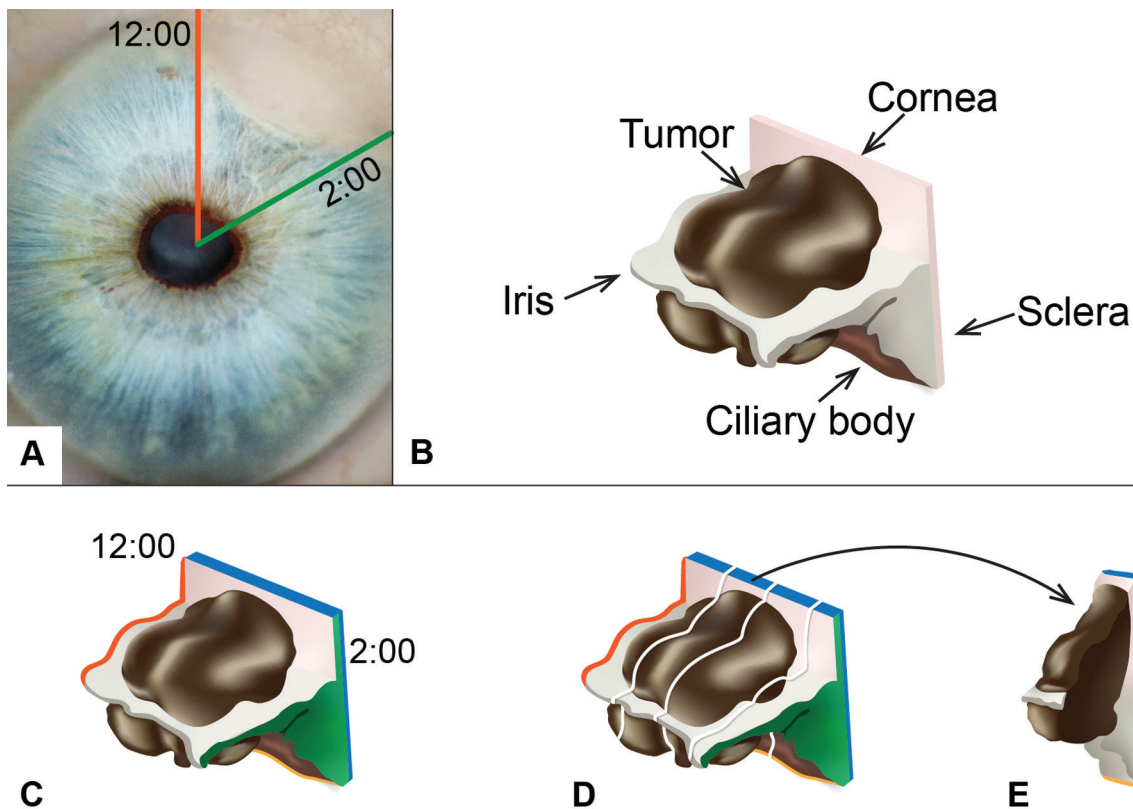


Figure 1-9

IRIDOCYCLECTOMY SPECIMENS

- A: The tumor at the root of the iris extends into the iris at the 12:00 to 2:00 o'clock position.
- B: Schematic representation of a segmental excision (iridocyclectomy) of this lesion includes a small amount of cornea, sclera, ciliary body, iris, and tumor.
- C: The margins are inked with different colors. Apart from the corneoscleral margin and the posterior pars plana margin (yellow), the lateral margins are inked and represent the hour of the clock that limits the lesion (12:00 in red, 2:00 in green).
- D: The specimen is then sectioned anterior posteriorly to obtain the margins and the center of the specimen. The sections contain all the essential anatomic structures and the margins.
- E: The section generated by the step illustrated in "D".

include an on-site cytologic evaluation of the specimen at the time of the biopsy for adequacy of tumor sampling for molecular testing.

There are two approaches to FNAB in uveal melanomas: transvitreal or transscleral. Transvitreal biopsies require a surgical microscope and vitreoretinal instrument to visualize the tumor and minimize complications. Although some surgeons use a 25-gauge vitrectomy instrument to obtain a core of tissue, this technique increases the likelihood of bleeding.

Most surgeons use a 25- to 30-gauge needle. The needle is connected to long tubing and suction is created by a syringe in a biopsy gun. Usually, one or two passes are done (fig. 1-10).

The transscleral approach requires of transillumination of the eye to localize the tumor, and the needle is introduced through the sclera after the conjunctiva is dissected (fig. 1-10). If adequacy evaluation is required, a small drop of the sample is shared on a slide to create a smear for Diff-Quick staining and on-site cytologic evaluation. The needle is rinsed after collecting samples for molecular testing and fixed for cytologic evaluation, which may include a cell block (fig. 1-10). Samples are usually scant but diagnostic, since the normal melanocytes lack atypical prominent nucleoli, abundant cytoplasm, epithelioid appearance, and discohesion, features seen in melanoma (fig. 1-10)

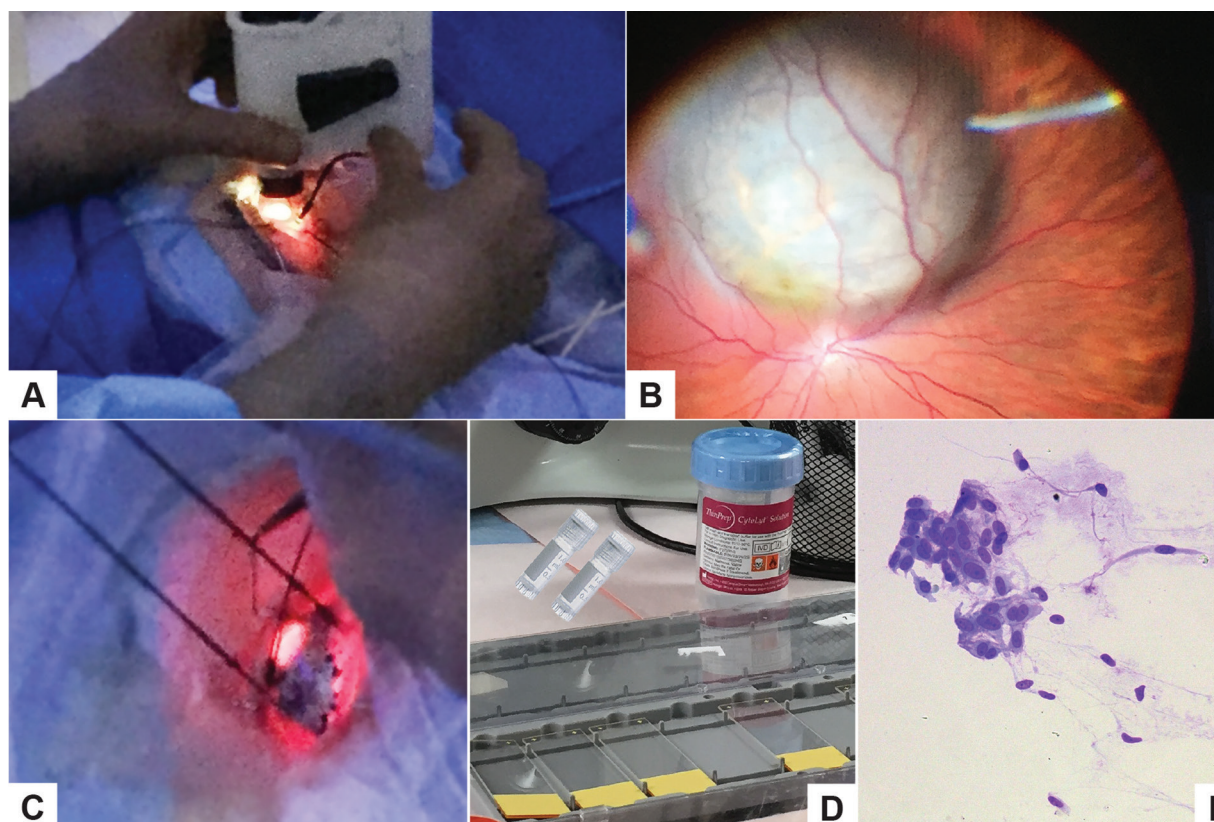


Figure 1-10

FINE-NEEDLE ASPIRATION BIOPSY (FNAB) OF INTRAOCULAR TUMORS

A: Transvitreal biopsy performed using an operating microscope allows visualization of the intraocular structures.

B: A 25-gauge needle (left) is entering the partially amelanotic choroidal tumor and seen through the operating microscope.

C: In a transscleral approach to the choroidal tumors, transillumination is used to localize the tumor, as seen here where the tumor cast a shadow. Traction sutures (to the muscles) are used to position and handle the eye during the procedure.

D: In the rapid on-site evaluation of the FNAB of uveal melanoma, the biopsy is evaluated by creating a slide with one drop of the needle and allocating the rest of the specimen in cryovials for molecular analysis and in cytology fixative for possible cell block and liquid-based cytology from the needle rinses.

E: Groups of spindle melanoma cells are seen in this Diff-Quick-stained smear prepared during an adequacy check to confirm the presence of tumor in the sample.

(4,5). Vitreous biopsies or vitrectomy specimens are used to diagnose intraocular vitreoretinal lymphomas (see chapter 11).

EYELIDS

Full-Thickness Wedge Resection

Segmental resections of eyelids for tumor are usually full-thickness wedge (pentagonal) resections that include all layers. Knowing the precise origin of a biopsy sample assists the pathologist in orienting the tissue to evaluate the margins. For example, the location of the eyelashes (eyelid margin-conjunctival/epidermal junction)

assists in orienting the tissue because, in lower eyelids, the eyelid margin is on the superior area of the specimen while in the upper eyelids it is located inferiorly.

After the pathologist has determined the laterality (right or left) and the origin of the specimen (upper or lower eyelid), the temporal (lateral) and nasal (medial) margins are assessed. In lower eyelid wedge biopsies, the inferior margin is assessed, and in upper eyelid biopsies, the superior margin is assessed. There is no “deep margin,” because in a full-thickness eyelid resection, the surface of the specimen opposite the cutaneous surface of the eyelid is conjunctival mucosa. The

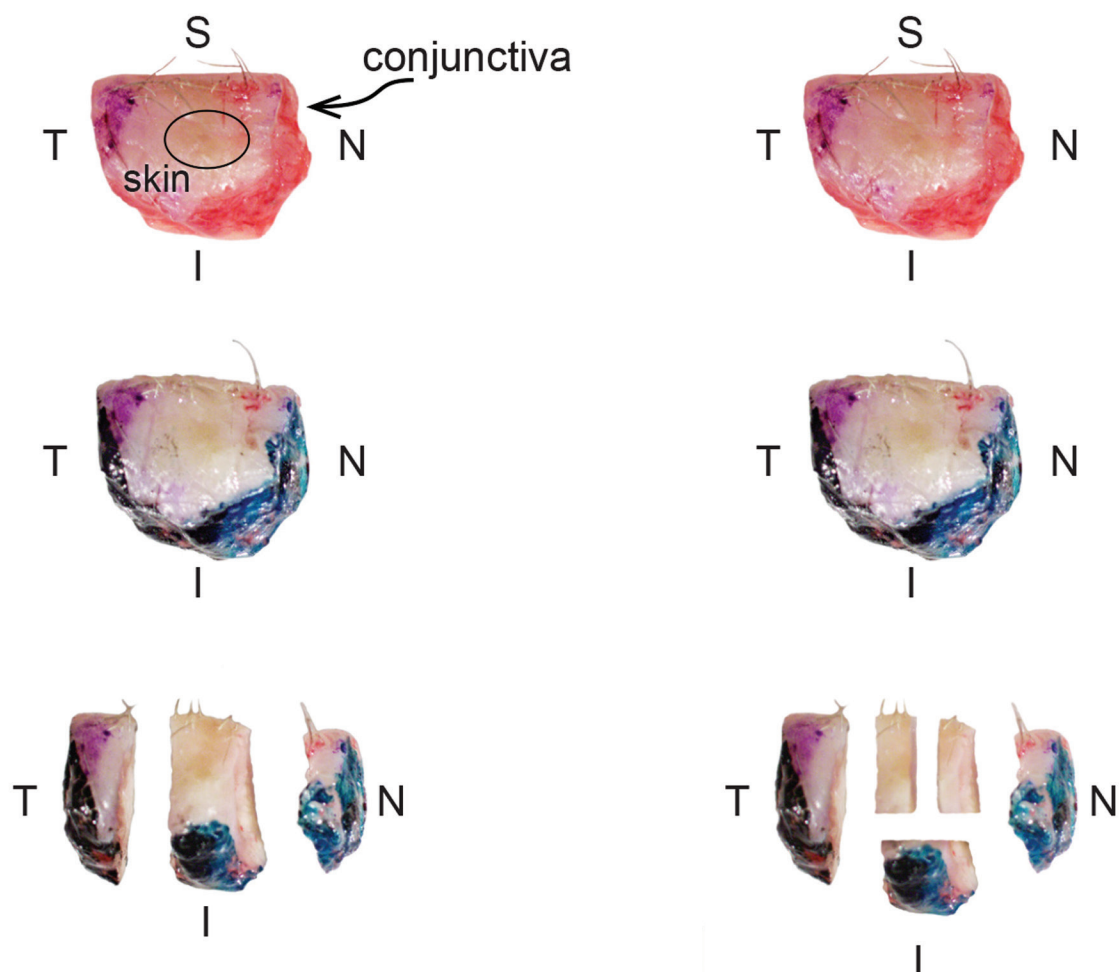


Figure 1-11

WEDGE EXCISION OF AN EYELID

Left: It is crucial for the surgeon to provide clinical information about laterality (right eye or left eye) and whether the specimen originates from a superior or inferior eyelid. The eyelashes are at the free border of the eyelid, and in this right lower eyelid, the temporal/lateral margin is on the left side. The anterior surface is the skin and the posterior surface the tarsal conjunctiva. The margins are inked as temporal and nasal (or medial), extending the color to the center of the inferior margin. The specimen is then trisected to obtain a temporal (T) section, including the temporal margin, a central section, including the inferior margin (I), and the nasal/medial margin (M), including the medial margin.

Right: Margins are taken separately from the central portion of the resected tissue that contains the tumor.

conjunctiva lining the undersurface of the eyelid is known as the palpebral conjunctiva.

There are two main dissecting approaches for full-thickness wedge resections (fig. 1-11) to examine the three margins (temporal, nasal, and either superior or inferior) while preserving the anatomic structures in the sections. In both approaches, the surgical margins are inked, temporal half one color and nasal half a different color, extending the colors into the center of

the superior or inferior margin. If the specimen is large enough, ink is applied to the inferior or superior margin with a third color.

In small specimens, the eyelid is sectioned in three segments: nasal, central, temporal (fig. 1-11 left). In larger specimens, there is the option of obtaining the nasal and temporal margins and then obtaining the inferior margin separate from the central area where the tumor is usually present. The central portion may be

divided into two pieces (fig. 1-11 right). This approach is usually preferred for frozen sections to freeze only the three margins. It is customary to submit the central portion with the tumor for permanent sections, embedding all sections on edge (1,6). Some ophthalmic surgeons take sentinel node biopsies, and the technique is similar to that used in other sites (7).

Partial-Thickness (Lamellar) Wedge Resection

Partial-thickness (lamellar) wedge resection includes the tarsus with conjunctiva, or rarely, the skin with muscle. In these cases, it is essential to ink the margin either anterior/superficial (surface of the tarsus/muscle) or deep (muscle in contact with tarsus) in addition to the superior or inferior margin and the temporal and nasal margins. These types of excisions are performed to preserve the function of the eyelid or for well-localized lesions in the conjunctiva or skin. They usually include the eyelid margin.

Biopsy

An eyelid biopsy is obtained from the palpebral (also known as the tarsal) conjunctiva or the skin, and sometimes from the eyelid margin. Skin lesions may be biopsied similarly to skin biopsies elsewhere, through punch biopsy, shave biopsy, or excisional biopsy. These are oriented and handled in the same manner as those elsewhere. Biopsies from the eyelid margin should be embedded on edge to obtain sections demonstrating the skin, tarsus, and conjunctiva. Biopsies from the tarsal conjunctiva also should be included on one edge for orientation of the epithelium and the tarsus.

CONJUNCTIVA AND CARUNCLE

Conventional Biopsies

An excellent gross evaluation of a conjunctival biopsy begins with the surgeon. Conjunctival tissue is very floppy, somewhat like plastic wrap used in a kitchen. Unless precautions are taken, conjunctival tissue will fold during fixation. Folds in the tissue can distort margins and generate tangential sections through lesions that may require precise measurement of thickness, such as conjunctival melanomas. Therefore, pathologists must play an active role in educating the surgeon on how to handle conjunctival biopsies.

The surgeon must take steps to ensure that the conjunctival mucosal biopsy remains flat before and during fixation. Frequently, ophthalmologists take these biopsies in outpatient settings, even in their offices. Placing the conjunctival biopsy on filter paper for about 10 seconds before immersion into formalin is an effective method. If filter paper is not available, any absorbable surface will suffice, and we have seen ophthalmologists use fragments of their business cards effectively for this purpose.

Pathologists should understand that the treatment of conjunctival malignancies requires a minimal loss of tissue. Even in cases of conjunctival squamous cell carcinoma and melanoma, ophthalmic surgeons do not take wide margins of the type used for cutaneous malignancies. It is essential for surgeons to conserve conjunctival tissue in order to maintain the integrity of the ocular surface. This is an essential objective for minimizing the likelihood of dry eye (xerosis) after the removal of a malignancy. Xerosis can be painful and lead to visual loss. Ophthalmic surgeons understand that surgical margins are not extensive in conjunctival biopsies, and cryotherapy or topical chemotherapy may be delivered to the affected site to augment surgical extirpation.

Some ophthalmologists mark the surgical margins with dye to provide orientation (for example, the margin closest to the limbus). They may use markers that are water soluble, such as methylene blue, and when they place the tiny fragment into formalin, the blue dye spreads across the entire specimen, and the ability to identify a critical margin is lost.

Some ophthalmologists mark margins with sutures. The suture must be thin, because the passage of a thick suture through the flimsy mucosal tissue disrupts the microanatomy and pathology. Drawings of the biopsy sample in the context of the ocular surface may be an effective, nondisruptive method of indicating margins.

Many lesions of the conjunctiva arise at the limbus. The sections should be taken perpendicular to the limbus to obtain histologic sections with one end at the cornea and the opposite end the conjunctiva toward the fornix or caruncle to evaluate invasion into the episclera/sclera, cornea, or conjunctival margins (fig. 1-12).

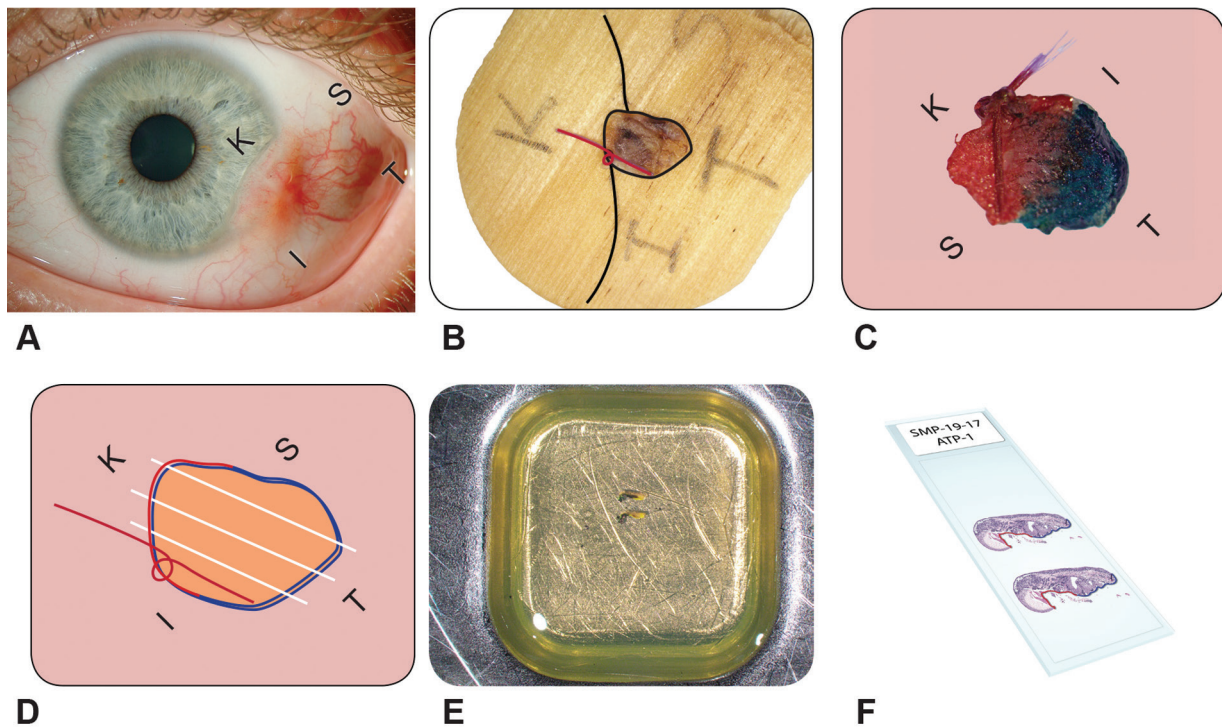


Figure 1-12

CONJUNCTIVAL EXCISIONS

A: The conjunctival tumor involves the cornea (K) at the temporal side of the left eye. Therefore, the superior (S), inferior (I), and temporal (T) margins must be sampled.

B: The excised conjunctival tumor is on a piece of tongue depressor with an orienting drawing and a suture placed in the inferior corneal tip. The specimen remains flat and in position after fixation using this approach.

C: The specimen is turned with the conjunctival surface down to ink the deep and peripheral margins: the temporal half is (T) blue and the corneal half (K) red.

D: After inking, the specimen is turned back to the original orientation with the deep margin down, and serial sections are obtained from the cornea to the temporal margin to obtain a section of the superior margin, a section of the inferior margin, and the central sections, each with corneal and temporal margins.

E: Each of the superior and inferior margins is in a separate cassette, and the central section may be submitted two in one cassette. Pretissue processing gel embedding was performed to orient the two central sections on edge.

F: Diagram of expected sections in the glass slide, with margins inked (temporal, corneal, and deep).

We cannot overemphasize the importance of the pathologist as an educator. Surgeons must be taught in an ongoing bidirectional dialog about the best methods for preparing conjunctival tissue samples for processing and interpretation. Frozen sections of the conjunctival lesion should be discouraged, if possible. Permanent sections are more precise and informative in these limited specimens (6,7).

Conjunctival “Map” Biopsies

The term conjunctival map biopsy describes the taking of multiple small incisional biopsies to determine the extent of ocular surface involvement by the neoplasm. Intraepithelial

squamous neoplasia, conjunctival involvement by sebaceous carcinoma, and what is described clinically as primary acquired melanosis (a conjunctival melanoma precursor) may all affect the conjunctival surface diffusely.

In these clinical settings, the ophthalmologist takes multiple small biopsies, most commonly accompanied by a sketch of the surface of the eye. The site of each biopsy is matched to a number or letter indicating the label on the jar (for example, “1” or “A”). In these cases, it is not necessary for the pathologist to comment on the adequacy of the margins. The pathologist’s goal for each biopsy is to identify the presence or absence of pathology.

Exfoliative Cytology

In cases of squamous dysplasia or carcinoma of the conjunctiva and cornea that has been biopsy proven, the lesion may be too large, multifocal, or recurrent, and treatment with topical chemotherapy is a therapeutic option. Some ophthalmologists choose to follow these patients by the examination of exfoliative cytology.

In general, we discourage ophthalmologists from using exfoliative cytology to follow patients with ocular surface neoplasms by the aphorism, "just because it is possible to do something does not mean that one should do it." Ophthalmologists are not well trained in how to obtain such specimens and how to fix the samples in the office. Furthermore, to obtain exfoliative cytology, topical anesthesia must be applied to the eye, and if this is the case, then more information can be obtained by biopsy than by exfoliative cytology. For example, it is easier to obtain immunohistochemistry on biopsy tissue than on exfoliative cytology specimens. The use of exfoliative cytology in the management of ocular surface neoplasia is yet another area of pathology practice where we believe that pathologists must educate their referring ophthalmologists on the best ways to obtain high-quality material so that the pathologist can render a timely and accurate diagnosis.

ORBIT AND OPTIC NERVE

Exenteration

When malignant orbital tumors infiltrate the soft tissues and possibly orbital bones, the ophthalmologist may elect to remove the contents of the orbital socket. The tissue removed includes the eye and all orbital soft tissue, with resection of adjacent bone if indicated. In many or most cases, the eyelids are removed with the orbital content, although, as described below, the procedure may be modified to spare eyelid tissue.

Exenteration is a surgical procedure for any malignancy that invades the soft tissues of the orbit, including squamous or basal cell carcinoma of the eyelid and malignancies of the conjunctiva. Other tumors of the orbit often necessitating exenteration are those from the lacrimal gland (e.g., adenoid cystic carcinoma), optic nerve (e.g., infiltrating meningiomas), or intraocular tumors that extend through the

sclera and into the orbit (e.g., uveal melanoma, an extraocular extension of retinoblastoma).

The most common indications for radical exenteration (including orbital bones) include invasive basal cell or squamous cell carcinomas of the eyelids or malignancies originating from the sinuses into the orbit. Some cases of adenoid cystic carcinoma of the lacrimal gland require this surgery.

It is common practice for surgeons to submit tissue for a frozen section during an orbital exenteration. The margin that is most likely to be positive (closest to the tumor or in squamous cell carcinoma, the nerve adjacent to the tumor) is submitted separately by the surgeon for frozen sections to evaluate whether there is need to resect bone or adjacent skin. At times, the surgeon may submit the entire exenteration specimen as fresh tissue and request a frozen section examination of areas of concern.

There are several approaches to the gross examination of orbital exenteration specimens, and each approach begins with the proper orientation of the specimen. The orbit has a cuboidal shape with six walls. The eyelids and associated skin represent the anterior surface. The other surfaces include the nasal (the side where the eyelids have the lacrimal puncta, the conjunctiva, and caruncle), superior, temporal (the lacrimal gland is at the junction of the superior and temporal walls), inferior, and posterior (optic nerve cut margin) surfaces (fig. 1-13A).

The specimen should be fixed in formalin for about 3 to 5 days to allow the formalin to penetrate the tumor and intraocular tissues. The specimen is measured from front to back, from side to side, and from top to bottom. Areas of the eye are described and measured (globe, cornea, and segment of the optic nerve) and the dimensions and location of tumor tissue measured and identified in the report.

The soft tissue margins are inked, one color for each (each wall of the cube) margin (fig. 1-13B–D). The ink should extend to the anterior surface for the inferior and superior margins if the skin is absent from the eyelids (fig. 1-13B,D). The posterior margin includes the optic nerve margin, and the same color ink is used to designate the posterior soft tissue (fig. 1-13C).

After the margins are inked, a vertical section is taken through the entire specimen with the

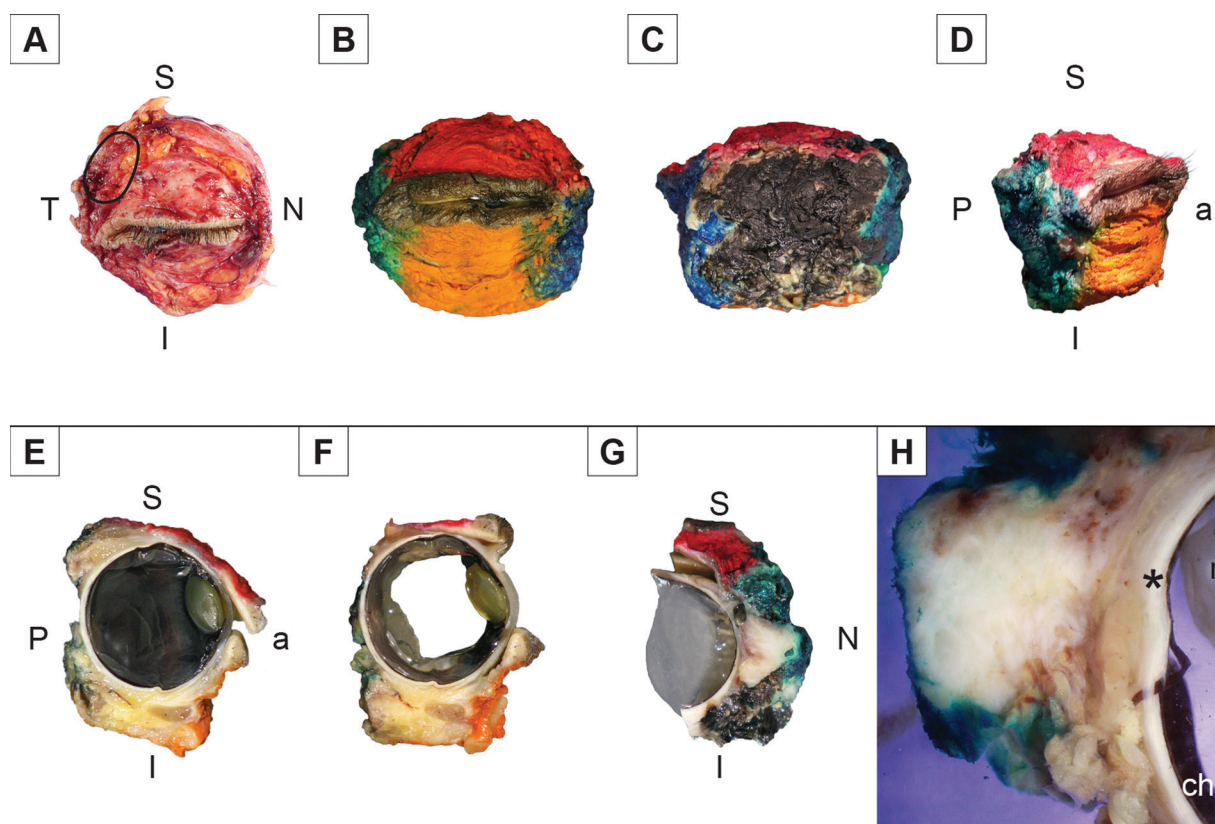


Figure 1-13

EXENTERATION SPECIMEN

A: Right exenteration specimen with lid skin sparing approach received fresh. Eyelid skin is absent. The exenteration contains a superior temporal tumor (circle) from the lacrimal gland.

B: View of the specimen after fixation and inking of the margins: temporal-green; superior-red; nasal-blue; inferior-yellow. The red and orange inks extend to the edge of the eyelid margin.

C: View of the posterior portion of the specimen with the posterior margin inked in black.

D: The orbit has a cuboidal shape in this lateral view.

E: View of the cut section of the specimen after the first vertical section is made to remove the temporal calotte. The upper eyelid is on top, and the soft tissues of the orbit appear in relationship with the eye.

F: View of the central pupil-optic nerve section after removing the nasal calotte. This section can be submitted as a whole mount.

G: Temporal calotte after bread-loaf sections. Green ink surrounds the superior temporal tumor (white lesion) in contact with the sclera of the eye.

H: Temporal calotte segment under the stereoscopic microscope reveals the adhesion of the tumor to the sclera (*). The choroid (ch) and the retina (r) are tumor free.

specimen positioned on the cutting board on the anterior surface (eyelids in contact with the cutting board surface). In this way, it is possible to obtain a section through the PO, with the surrounding orbital tissue and both eyelids in the central section. In order to generate this section, the optic nerve is first identified, so the sectioning starts at the edge of the nerve, with an anterior-downward movement to enter the edge of the cornea. This maneuver results in a section that shows the interior of the eye, the

coats of the eye, and the relationships of these with the orbital and eyelid tissues (fig. 1-13E).

After describing the findings, especially if the tumor infiltrates the eye, the other side (calotte) of the eye is removed. After placing the specimen on the previous cut surface, the other side of the optic nerve is cut parallel to the cutting board with an anterior movement. Shortly after penetrating the globe, the specimen is turned down on the anterior surface to finish the cut through the cornea and eyelids,

obtaining a central PO section (fig. 1-13 F). The temporal and nasal calottes are then cut in an anterior-posterior direction in segments (bread loaf) to obtain sections that have the eye and the orbital tissues, maximizing the surface for histopathologic examination (fig. 1-13G,H).

The PO section is ideally submitted as a whole mount (similar to the prostate whole mount) in one large cassette or wrapped in a porous bag for long processing (including longer times in formalin, alcohols, and xylenes). The calottes are also ideally submitted in these large cassettes, so the specimen is not further sectioned. However, if this processing is not available, the large sections may be further segmented, maintaining the anatomic relationships and submitted in multiple sections (e.g., anterior, central, and posterior portions of the segments). The most important sections are those that show the tumor in a relationship with margins and the globe. Large whole mount preparations allow the entire specimen to be submitted in only three large cassettes.

An alternative technique may be useful if the indication for exenteration is not orbital involvement by the transscleral spread of an intraocular tumor. The orbital tissues are dissected away from the eyeball by making horizontal sections through orbital tissue until the eye is encountered, and then dissociating the eye from the conjunctiva. The lab then processes the eye as described above. The margins of soft tissues of the superior and inferior aspects of the orbit can be painted with various colors of ink to identify the location within the orbit. This alternative technique generates more tissue sections than the conventional method described above and does not generate an aesthetic section containing the eyeball, eyelids, and orbital soft tissue. On the other hand, this technique eliminates the need for large cassettes and blocks, and provides for accurate mapping of the tissues involved. Both enucleation and exenteration specimens require experienced, specialized histotechnologists for adequate embedding and sectioning.

Orbital Excisional Biopsies

The excision of an orbital lesion serves as both a diagnostic and therapeutic maneuver. The indications are usually masses that cause proptosis or intraocular changes. In children,

the most common lesions are benign dermoid cysts and hemangiomas. Rhabdomyosarcoma is the most common malignant primary orbital tumor in children. In adults, excisional biopsies are used for cavernous hemangiomas (usually retrobulbar, intraconal), solitary fibrous tumors, and secondary tumors including metastases. Optic nerve tumors (gliomas in children and meningiomas in adults) may be dissected from the eye and excised from the orbit.

Excisional biopsies in formalin should be measured and described, inked, and entirely submitted on cross sections for examination. In cases where there is suspicion of malignancy, especially in children, the specimen is submitted fresh for evaluation of the adequacy of sampling and tumor retrieval for genetic and molecular testing. The adequacy of sampling is evaluated by cryostat sections or by touch imprints (see below).

Incisional Orbital Biopsies

A biopsy performed for diagnostic purposes only (not therapeutic) is used in cases of large infiltrative lesions, extraocular muscle lesions, and lesions suspicious for lymphoma. In these cases, the biopsies are usually submitted for intraoperative evaluation to access adequacy of the sample, keeping the specimen fresh for molecular studies and flow cytometry. These biopsies are small and are better handled by touch imprints rather than cryostat sectioning because touch imprints preserve most of the tissue for permanent histopathology and testing (see below).

Occasionally, ophthalmologists obtain a biopsy from an optic nerve tumor. In these cases, the biopsy is usually small and obtained through the meninges, but occasionally, if the eye is already blind secondary to the tumor, the surgeon may select to obtain a segmental biopsy of the optic nerve with the lesion. In these samples, the margins (distal and proximal to the eye) should be submitted in different cassettes, the specimen inked and cross sections perpendicular to the axis of the optic nerve obtained to evaluate the tumor relationship with the meninges. This tissue is fixed in formalin.

Orbital Fine-Needle Aspiration Biopsy

Surgeons only rarely use FNAB to diagnose orbital tumors because the potential complications may outnumber the benefits. Excisional biopsies

better treat many of the lesions that ophthalmologists might sample by FNAB. Nevertheless, some experienced orbital surgeons choose to use FNAB, and in these cases, the procedure is aided by imaging to avoid complications. Indications include either anteriorly located lesions (palpable) or large lesions with anterior access with a presumptive differential diagnosis of lymphoproliferative lesion or metastasis. Vascular or cystic lesions and lacrimal gland tumors are contraindications to this approach (8).

The disadvantages of orbital FNAB include poor cellular yield, a cytopathologic and not a histologic diagnosis, difficulty in interpreting the specimen, and low cellular yields requiring another biopsy procedure. The potential complications are retrobulbar hemorrhage, globe perforation, ptosis, extraocular motility dysfunction, and inadvertent entry intracranially. The samples are managed similarly to other FNAB specimens, either with rapid on-site evaluation (touch imprints, liquid-based cytology, RPMI, molecular analysis) or received prepared by the surgeon (smears and in fixative for further analysis).

Orbital Touch and Scrape Preparations

Cytologic evaluation by touch or scrape preparation is a constructive approach to handling small orbital biopsies. This technique allows for the processing and interpretation of small amounts of the sample, while allowing for preservation of most of the tissue for definite histopathologic diagnosis or other diagnostic tests, such as molecular pathology, flow cytometry, and cultures.

Clinical differential diagnoses that include lymphoma, rhabdomyosarcoma, and metastases to the orbit are rare indications for small incisional or excisional biopsies. If the specimen is an excisional biopsy, the margins are inked before bisecting the tissue. It is often helpful to obtain cells from the cut surface by picking up the tissue with forceps and gently placing the cut surface on a glass slide. This maneuver may be repeated on the same slide, adjacent to the previous touch specimen. It is customary to stain these touch preparations with a quick H&E stain, the same as used for frozen sections.

For larger specimens with firm, fibrous surfaces, it may be best to scrape the surface with a blade and to generate smears with the material

obtained. If small pieces detach during this process, then another slide is placed on top of the first slide and pressed gently on the tissue as the second slide is drawn toward the end of the first slide. This “squash technique” generates a smear, and the slide against which the tissue is initially pressed usually contains most of the material that will be useful to generate a diagnosis.

In cases of lymphoproliferative lesions, material in RPMI is suitable for flow cytometry and the rest in formalin for histopathologic examination. In tumors in children, especially rhabdomyosarcoma, the tissue is divided into frozen samples for molecular and genetic testing and formalin for histopathologic examination (fig. 1-14).

LACRIMAL GLAND AND LACRIMAL SAC

Excisional Biopsies

Surgeons generally perform excisional biopsies of the lacrimal gland to avoid violating the capsule in benign mixed tumors, thereby minimizing the likelihood of seeding orbital tissue and promoting recurrence. These specimens are then measured, described, and inked before cross sectioning, to allow for the evaluation of margins and maintaining the integrity of the specimen. Occasionally, malignant tumors invade bone, and the specimen may necessitate decalcification.

Excisional biopsies of the lacrimal sac are rare. Epithelial tumors such as squamous papillomas or carcinomas usually fill the lumen of the sac. The specimen is measured and inked, and margins submitted in separate cassettes (nasal-distal and ductal-proximal margins). The remainder of the specimen is cross sectioned, and depending on the size of the lesion, may need to be further sectioned and embedded on edge to obtain proper orientation in the histologic sections.

Incisional Biopsies

Ophthalmologists frequently obtain biopsies from the palpebral lobe of the lacrimal gland to establish or rule out inflammatory lesions and lymphoma. Fresh tissue is sent for flow cytometry if touch imprints reveal adequate cellularity and suggest a diagnosis of lymphoma.

Surgeons often perform incisional biopsies of the lacrimal sac during a dacryocystorhinostomy procedure for lacrimal drainage obstruction.

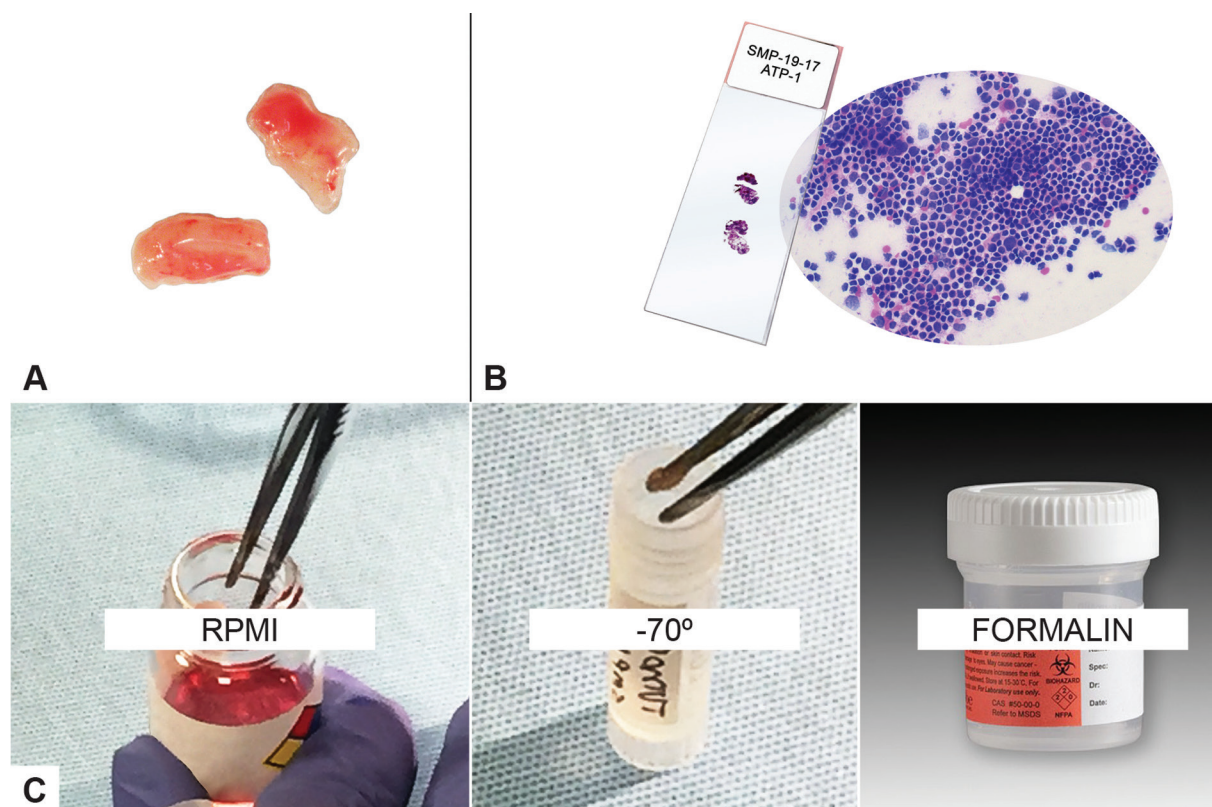


Figure 1-14

BIOPSY OF THE ORBIT

A: A small specimen is bisected fresh and the cut surface used to prepare a touch imprint.

B: The touch imprint shows the shape of the two pieces of the specimen. Cytologic preparation shows a cellular lymphocytic infiltrate.

C: A portion of the specimen is in RPMI solution for flow cytometry, and another piece is in formalin for histopathologic diagnosis. If needed, the specimen may be saved frozen (-70° centigrade) for possible molecular studies.

The pathology laboratory usually receives a few pieces of tissue from the lacrimal sac in formalin.

Fine-Needle Aspiration Biopsy

FNAB is seldom used in these areas because of the potential complications and the preference for excisional therapeutic and diagnostic biopsies.

PRETISSUE PROCESSING GEL EMBEDDING TECHNIQUE

Pretissue processing gel embedding is used in small specimens to secure the orientation of the specimen and to prevent tissue loss in processing (fig. 1-15). Histogel® or agar is used (9)

The gel is heated to achieve a liquid form, using a warm bath (with the tube in glassware containing hot water) or warmed by a hot

plate or a microwave oven. Using a metal small embedding mold (for paraffin embedding), the liquid gel is poured into the well. The mold is at room temperature and in proximity to the stereoscopic microscope (preferably). Under microscopic observation (or using a loupe), the specimen is embedded into the gel in the desired position (usually on edge). The gel should be mildly firm while positioning the specimen so that it is held in place and not floating or falling on its side.

The metal mold containing the specimen embedded in the gel is gently moved on to a cold plate or ice pack with a flat surface to allow for cooling and solidification of the gel. A cassette with the case number is placed on top to identify the specimen at all times, especially if

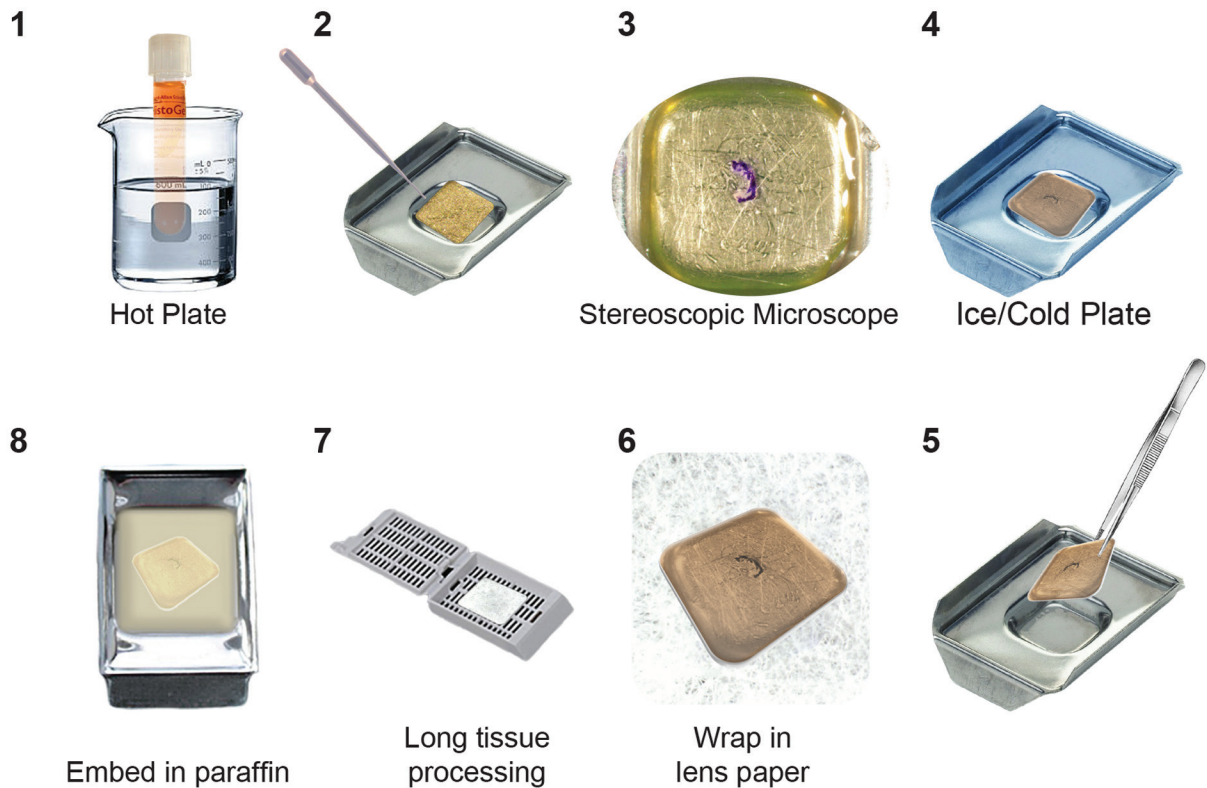


Figure 1-15

PRETISSUE PROCESSING GEL EMBEDDING

1. The gel (Histogel or agar) is melted in a hot bath using a hot plate or microwave oven. 2. Fluid gel fills the well of a small-diameter metal mold. 3. The tissue is in the gel with the desired orientation. 4. The embedded tissue in the metal mold is transferred to a cold surface to allow hardening of the gel. 5. The gel block is dislodged from the metal mold. 6. The gel block is wrapped in lens paper to prevent loss of tissue if the gel breaks or dissolves during processing. 7. The wrapped gel block is submitted in a cassette for a long tissue processing schedule to allow penetration of the reagents into the tissue. 8. The processed gel block is embedded in paraffin to obtain a paraffin block for sectioning.

the technique is repeated to embed other pieces of the specimen.

After the gel becomes solid, the gel block is gently dislodged and removed from the metal mold. The gel block is then wrapped in lens paper to prevent any loss of tissue should the gel fracture or melt during processing.

The wrapped gel block is then processed in a long processing schedule (not the short biopsy

program) to allow for adequate infiltration of the reagents into the tissue. The processed gel block is positioned on the bottom of the well of the metal mold, with the flat surface down, to maintain the orientation of the tissue in the gel. The gel block then is embedded into paraffin to create the paraffin block that is sectioned to obtain the histologic slides.

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