REVIEW

The ophthalmic pathology cut-up—Part 2

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Summary

In this second of two articles, we explore the range of common smaller specimens that present to the ophthalmic histopathology laboratory. Some of these specimens are quite small and difficult to handle, with a proven rate of loss during processing. This article contains techniques to overcome such difficulties. The full spectrum of biopsy material is covered, from full-thickness eyelid pentagonal resections, through to aspirations of intraocular tumours and handling tiny epiretinal membranes.

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Specimen fixation

For all ophthalmic specimens requiring routine paraffin processing, standard 10\% neutral buffered formalin gives excellent results. If the specimen requires electron microscopy, 3\% glutaraldehyde in 0.1 m sodium cacodylate buffer is the fixative of choice.

Specimens such as muscle biopsies or conjunctival biopsies requiring direct immunofluorescence studies should be sent fresh to the histopathology department.

Eyelid and periocular skin excisions\textsuperscript{1–5}

The main indication for an eyelid or periocular skin excision is a malignant neoplasm. The more common malignant neoplasms include basal cell carcinoma, squamous cell carcinoma and sebaceous carcinoma.\textsuperscript{6–9} Tumours can be removed either as a simple skin ellipse (if not involving the lid margin) or as a partial (usually cutaneous aspect only) or full-thickness (cutaneous, lid margin and conjunctival aspect) lid resection.

Skin ellipse

This is handled in the same way as any standard lesional skin ellipse (see the Royal College of Pathologists Minimum Dataset for the Reporting of Skin Cancers).\textsuperscript{10}
Full-thickness lid resections

These are usually pentagonal shaped and comprise the lid margin, anterior skin and posterior conjunctival surfaces.

- The vertical dimension of the skin and conjunctival surfaces are recorded, along with the usual medial–lateral and thickness dimensions. Any obvious lesion is described, as for skin ellipses.
- The presence or absence of eyelashes is noted as malignant lesions often efface the lashes.
- Note the position of any orientation sutures.
- The lateral and medial halves of the specimen should be painted in different colours to aid orientation at microscopy.
- Full thickness, consecutive 2–3-mm-thick vertical slices should be taken to show both conjunctival and skin surfaces. The medial and lateral slices should have their thicknesses recorded; this will facilitate estimation of the lesion’s distance from these margins (see below).
- Paraffin sections are cut as three levels through each block of tissue, except for the medial and lateral slices. These are levelled through the block at regular intervals. Knowing the initial thickness of the medial and lateral slices allows a good approximation of the distance of the lesion from these end slices (Figs. 1–3).

Partial-thickness lid resections

These are approached in a very similar way to full-thickness lid resection specimens (see above).

Corneal tissue

Corneal disc

Corneal tissue is removed during a penetrating keratoplasty or an anterior/posterior lamellar
keratoplasty, to produce a full- or partial-thickness disc of tissue, respectively. The principal indications for removing cornea are chronic decompensation (usually pseudoaphakic bullous keratopathy), a dystrophy, failed corneal graft, after inflammatory, traumatic or infective scarring, and for a perforation (Fig. 4). The cornea should be handled gently, with minimal trauma to the anterior and posterior surfaces. Formalin fixation will impart a degree of cloudiness to the specimen that may obscure some features. Examine the cornea using a stereo dissecting microscope against both dark and light backgrounds, and use retroillumination to highlight any areas of opacity. Note the diameter of the disc and whether it is full or partial thickness. Partial-thickness corneal tissue tends to round up or fold and may have an irregular outline.

The following features are noted:

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- Formalin fixation will impart a degree of cloudiness to the specimen that may obscure some features.
- Examine the cornea using a stereo dissecting microscope against both dark and light backgrounds, and use retroillumination to highlight any areas of opacity.
- Note the diameter of the disc and whether it is full or partial thickness. Partial-thickness corneal tissue tends to round up or fold and may have an irregular outline.

Figure 4 A full-thickness corneal disc showing a paraxial perforating bacterial keratitis. Note the white infiltrate around the perforation. The bottom right inset figure shows how it can be sliced.

- Opacities, which may be indicators of a corneal dystrophy. Their shape, colour and distribution should be noted.
- Ulceration.
- Perforations, in terms of their size, shape and position. Iris tissue may have become incarcerated into the perforation, appearing as a brown interposed pigment.
- The measurement and location of all scars. Scars from previous corneal graft surgery may be visible. Any suture material remnants should be noted.
- Vascularization of the cornea, with or without associated lipid keratopathy, which a sign of previous corneal hypoxia, triggered mostly by non-infective and infective inflammatory aetologies.
- Variations in stromal thickness. This is appreciated on the cut surface and occurs in ectatic dystrophies such as keratoconus. A ring of deposited iron (Fleischer ring) may be visible around the thinned area.
- The cornea should be bisected through any areas of interest axially or paraxially. If the region of interest is very small, the cut is made to one side of it in order to prevent loss of the area during block trimming. The bisection should pass at right angles to wounds and scars.
- If the specimen is precious, a central 1–2 mm strip may be taken for routine histology, leaving two halves behind for ancillary investigations, principally electron microscopy and polymerase chain reaction for detecting infective agent RNA/DNA (e.g. herpes simplex).

We routinely cut three levels through the bisected cornea. The routine ‘starter’ stains include haematoxylin and eosin (H&E) and a periodic acid–Schiff (PAS) stain. (See Ref. 1 for a good chapter on corneal special stains for identifying the various dystrophies and deposits.)

Corneal biopsy

- Small biopsies of cornea that comprise epithelium and superficial stroma are usually taken to aid the diagnosis of infection. These should be measured, described and processed whole, with ‘spares’ cut for ancillary investigations. If the biopsy arrives fresh, part of it should be sent to microbiology.
- As this type of sample is likely to harbour an infective agent, a full spectrum of infective agent stains is used.
Corneal smears

- These samples are unusual to receive but are performed to elucidate the cause of a corneal ulcer (usually infective). Air-dried smears should be stained with Giemsa, and alcohol-fixed smears with Papanicalou or H and E stains.
- A sufficient number of slides should be prepared for infective agent stains.

Donor corneal-scleral tissue

Ophthalmologists may submit the remnants of the donor corneal-scleral ring used during penetrating keratoplasty. This is to give an indication of the normality and quality of the donated cornea, especially the number and quality of the endothelial cells. The specimen usually arrives fresh, enabling part of the sample to be taken for microbiology (Fig. 5).

- After fixation, the whole specimen should be measured and the size of the trephined corneal circular defect should be noted. After microbiological sampling under sterile conditions, the remaining corneal-scleral tissue should be examined carefully. The remaining cornea should be interrogated as above. The exact composition of the sample (e.g. presence of trabecular meshwork, ciliary muscle) should be noted, together with the presence of any old operative scars.
- The donor rim of tissue should be divided into 4-mm-wide sectors and each processed to paraffin. These are embedded on their edges to show the full thickness of the sample.
- The sections are stained with H&E and PAS stains.

Conjunctival biopsy

Conjunctival biopsies (incisional or excisional) are performed for three main indications: non-pigmented lesions (e.g. pingueculum, pterygia, papillomas, actinic keratosis and conjunctival intraepithelial neoplasia, squamous carcinoma and sebaceous carcinoma), pigmented lesions (principally naevi, primary acquired melanosis, melanoma and non-melanocytic pigmented lesions) and for inflammatory lesions (infective or non-infective).

Incisional biopsies are often taken as part of a ‘mapping’ procedure to establish the extent of an intraepithelial disease process (e.g. primary acquired melanosis with atypia and pagetoid sebaceous carcinoma), so several incisional biopsies may be received per patient.

- Measure the size.
- Describe any lesional tissue.
- If the sample is an excision, the resection margins should be painted and the specimen dissected using consecutive slices perpendicular to the long axis. If the excision incorporates the limbus, the slices should pass at right angles through this.
- All suspected inflammatory-type conjunctival lesions should be embedded whole, along their longest axis; this maximizes the chances of detecting the relevant pathology, for example granulomas in sarcoidosis.
- In a suspected case of a classical blistering disorder affecting the conjunctiva (mucous membrane pemphigoid, epidermolysis bullosa acquisita, linear IgA disease, etc.), the specimen should be sent fresh to histopathology. The sample should be described as above and embedded in freezing mount, such as cryomatix, along its long axis.
- The sample is rapidly frozen, frozen sections prepared and stained by direct immunofluorescent techniques. Antibodies for IgG, IgA, IgM, C3 and fibrinogen are used, along with an H&E stain.
- These cases should only be handled in laboratories experienced in this type of investigation or...
where quick referral to an experienced dermatopathologist is available.

Local resection of iris or ciliary body tumour\textsuperscript{1–5}

Localized resections of the iris, ciliary body and choroid are sight-sparing procedures. These are performed in ocular oncology centres for localized benign and malignant neoplasms (Figs. 6 and 7).\textsuperscript{15,16}

Iris resections

- The pupillary-margin-to-peripheral and circumferential-to-circumferential margins and the thickness are measured.

Examine for the following:

- tumours—measure the size and describe in the usual way, with relations to the margins;
- iris atrophy, which can be seen by retroillumination of the specimen;
- ectropion uveae or entropion uveae;
- rubeosis iridis (neovascularization of the iris);
- previous biopsy sites.

The sample should be sliced as follows:

- The resection margins should be painted.
- The iris should be sliced into consecutive parallel blocks around the iris circumference, so that each contains both pupillary and peripheral margins.

Figure 6 A local resection of a ciliary body tumour. I, iris; CB, ciliary body expanded by melanoma; S, partial thickness sclera.

Local resection of ciliary body and choroid tumours

These usually comprise the ciliary body, choroid and often some iris root, with partial-thickness sclera.

- The anatomical structures included are noted and the specimen measured in three dimensions.
- Any obvious tumour lesion is measured and its position in relation to the margins noted.
- The resection margins are painted, and if there is sufficient tissue overhang, the scleral and, if included, iris resection margins are sampled and placed into separate cassettes.
- The specimen is then sliced, usually in the anteroposterior plane, and the cut surface of the tumour is described.

Figure 7 The sample as in Fig. 6, painted on its scleral aspect with yellow paint, showing the sampling of the scleral margins and bisection of the tumour.

Lens tissue\textsuperscript{1–5}

The natural lens is rarely submitted as a separate specimen, but it is sometimes submitted because of an interesting type of cataract, be it congenital, infective, traumatic or metabolic.\textsuperscript{1} The natural lens is usually part of an evisceration, enucleation or exenteration specimen.

- Natural lenses that are part of an evisceration, enucleation or exenteration should have their location, shape, size, colour and transparency noted. Note the colour of any opacities and their location within the lens matter, i.e. whether they are anterior, nuclear or posterior. Note the integrity of the zonules.
During microtomy, we suggest soaking the paraffin block prior to cutting in order to reduce artefactual cracking and rolling of the lens tissue.

Artificial intraocular lenses (IOLs) are encountered in two situations: either as part of an evisceration, enucleation or exenteration specimen, or submitted alone. The effects of the IOL on the surrounding tissues and vice versa can be studied easily.\textsuperscript{17,18}

IOLs are submitted alone because of intraocularly induced complications caused by mechanical trauma and inflammatory, infectious or optical problems, such as wrong power or decentration.\textsuperscript{19}

- Optimal handling involves identifying the make of lens, either from the notes or from an IOL catalogue. The lens is examined under light and phase-contrast optics, noting its integrity and measuring the haptics and optics. The colour and distribution of any opacification is noted.
- The IOL can be prepared for light or scanning electron microscopy. For light microscopy, it can be fixed in standard formalin and stained whole with H&E. It is then placed on a glass slide with a depression, dehydrated, cleared and mounted in standard xylene-based mount, before coverslipping.\textsuperscript{17,18} In our hands, this technique gives excellent results to assess cells and other material coating the IOL. If infection is suspected, the IOL can be decolourized and restained with infectious agent tinctorial stains.
- For electron microscopy, the IOL is fixed in standard glutaraldehyde and embedded in standard resin; sections are then cut.\textsuperscript{17,18}
- IOLs as part of eviscerations, enucleations or exenterations will dissolve during routine processing but the indentation of the haptic and optic will remain.\textsuperscript{18,19} This greatly facilitates the assessment of the effect of the IOL on the surrounding tissues.\textsuperscript{17,18}

Evisceration specimens\textsuperscript{1–5}

An evisceration removes the cornea and the intraocular contents, leaving the scleral shell behind. The indication for an evisceration is usually a blind, chronically painful eye, resulting from a traumatic or inflammatory aetiology or an intraocular pyogenic infection not responding to treatment.

The specimen consists of the cornea and limbal tissues together with the ocular contents (Fig. 8):

- Cornea—describe and bisect as for corneal biopsy.
- Lens—may be present or absent. Note any damage or cataract-type changes. See lens protocol above.
- Contents—usually appear as clotted blood together with disorganized iris, ciliary body, vitreous, retina and choroid. Recognizable tissues are noted.
- The entire contents should be bisected and submitted for paraffin processing.
- H&E and PAS are useful starter stains.

Orbital biopsy\textsuperscript{1–5}

Orbital biopsies (including lacrimal gland) are often received for the investigation of neoplastic or inflammatory processes. This may be a biopsy or an attempt to remove the whole lesion.

- The tissue should be described to include its size, shape, colour and texture. It may be necessary to X-ray the tissue if bone or calcification is suspected. The surgical resection margins should be painted and the tissue sliced serially. The cut surface is described and slices taken of the lesion with the closest inked margins.
- If an orbital sarcoma is resected/debulked after biopsy diagnosis, it is worth having it submitted fresh, so that fresh tissue can be sampled for cytogenetics.
Temporal artery biopsy$^1-5$

Temporal artery biopsy is usually performed to confirm suspected cases of giant-cell arteritis. As this disease process can display a 'skip lesion' configuration,$^{20,21}$ the entire specimen should be processed. The longer the sampled artery, the higher the detection rate for temporal arteritis.$^{22-24}$

- The length and diameter of the artery should be noted (for audit purposes), together with the presence of attached connective tissue. The latter should not be detached as it will contain smaller vessels that may exhibit a vasculitis.
- Transverse cuts should be made along the length of the artery to produce 1.5–2-mm-thick discs.
- All the discs should be embedded to show cross-sections through the artery.
- Sections are cut through the entire specimen at regular intervals. An elastin stain is used to assess the status of the internal elastic lamina. This sectioning strategy ensures an optimal histological pick-up rate for temporal arteritis.$^{22-24}$

Muscle biopsy$^1-5$

Muscle biopsies may be received following squint surgery, ptosis surgery or for the diagnosis of thyroid eye disease (endocrine exophthalmos). In these cases, the sample is submitted in 10% neutral buffered formalin. The sample should be described (size, shape, colour and texture) as normal and processed to paraffin wax.

If a muscle biopsy is taken for a suspected neuropathic or myopathic process, it should be submitted fresh and handled in accordance with standard neuropathological protocol.

Epiretinal membranes$^1-5$

Vitreous and epiretinal membranes are contractile and can lead to tractional distortion and detachment of the retina.$^{25}$ These are removed and often sent to the ophthalmic pathologist to categorize them and to shed light on their aetiology. Epiretinal membrane biopsies are delicate and small; they are prone to getting lost at several key stages of harvesting (Figs. 9 and 10).$^{25,26}$

We employ the following protocol, which leads to well over 95% of all membranes being successfully harvested for histological examination.

- Epiretinal membranes are placed into sterile 0.5 ml Eppendorf tubes in theatre. The use of sterile tubes has enabled the surgeon to place the membrane directly into the tube, without the need to pass it to another member of theatre staff. This minimizes loss.
- A volume of 0.25 ml 10% neutral buffered formalin is added.
- After receipt, the tube is examined using the dissecting microscope for membrane material. The membranes are often translucent but may contain pigment from RPE cells. If no obvious membrane is visible, the addition of a small quantity of dye (cresyl violet) enables visualization within 5–10 min as the dye adheres to the membrane.
- Retrieval of the membrane is by using watchmaker's forceps or by sucking the membrane and
Intraocular aspiration cytology\(^1\)–\(^5\)

In our laboratory, fine-needle aspiration specimens are generated from (a) suspected solid intraocular tumours, requiring confirmation of a diagnosis prior to enucleation,\(^2\)\(^7\)–\(^29\) and (b) aqueous and vitreous specimens, mainly to detect an infective agent or malignancy.\(^3\)\(^0\),\(^3\)\(^1\)

Aspiration from a suspected solid intraocular tumour

- The sample arrives in a suitable transport medium such as cytospin collection fluid (Thermo Shandon) and is immediately sent to the laboratory.
- Following receipt, any visible solid pieces are removed with watchmaker’s forceps or a fine pipette and handled as for epiretinal membranes (see above).
- The fluid is centrifuged, resuspended and then cytospun to produce several slides.
- The cytospin specimens are stained with either H&E or Papanicalou stain. The spare cytospin preparations can be used for immunohistochemistry after methanol fixation.
- Equally valid techniques include processing the entire sample to a cell block in agar\(^2\)\(^5\) or using Millipore filters.\(^3\)\(^0\)

Aqueous and vitreous aspirates

- The surgeon should be encouraged to send some of the sample to microbiology for culture and polymerase chain reaction analysis if infection is suspected.
- We encourage surgeons to send as much neat vitreous as possible, in a capped syringe.
- On receipt, any visible tissue pieces are removed with fine watchmaker’s forceps or a fine-tip pipette. These fragments are processed as for epiretinal membrane (see above). The remaining vitreous can be smeared directly, or multiple cytospin slides can be prepared. Any remaining fluid is prepared as a cell block. Immunohistochemistry may be performed on the cytospin slides after methanol fixation, or on the cell block.
- If there is a delay in preparing the fluid, it is fixed with an equal volume of 10% formalin and stored in a fridge.
- Slides are stained with an H&E or a Papanicalou stain. The spare cytospin slides are used for infective agent stains and immunohistochemistry as required.
- Equally valid techniques for aqueous and vitreous processing include the use of agar cell blocks\(^2\)\(^5\) and Millipore filters.\(^3\)\(^0\)

References