

Tissue pathways for non-neoplastic ophthalmic pathology specimens

October 2026

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Unique document number	G141
Document name	Tissue pathways for non-neoplastic ophthalmic pathology specimens
Version number	4
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Date active	October 2026 (to be implemented within 3 months)
Date for full review	October 2031
Comments	This document will replace the 3rd edition of <i>Tissue pathways for non-neoplastic ophthalmic pathology</i> specimens. In accordance with the College's pre-publications policy, this document was on the Royal College of Pathologists' website for consultation from 1 September to 30 September 2026. Responses and authors' comments are available to view at www.rcpath.org/profession/publications/documents-in-development.html Dr Brian Rous and Sarah Davies, Clinical Leads for Guideline Adjudication



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Foreword

The tissue pathways published by the Royal College of Pathologists (RCPATH) are guidelines that enable pathologists to deal with routine surgical specimens in a consistent manner and to a high standard. This ensures that accurate diagnostic and prognostic information is available to clinicians for optimal patient care and ensures appropriate management for specific clinical circumstances. This guideline has been developed to cover most common circumstances. However, we recognise that guidelines cannot anticipate every pathological specimen type and clinical scenario. Occasional variation from the practice recommended in this guideline may therefore be required to report a specimen in a way that maximises benefit to the patient.

The guidelines themselves constitute the tools for implementation and dissemination of good practice.

The following stakeholders were contacted to consult on this document:

- British Association for Ophthalmic Pathology (BAOP)
- National Specialist Ophthalmic Pathology Service (NSOPS).

The information used to develop this tissue pathway was obtained by undertaking a systematic search of medical literature (Pubmed, BMJ and Cochrane Library databases). Key terms searched included ‘ophthalmology histology tissue handling’, ‘tissue processing’, ‘macroscopy’, ‘grossing’, ‘cut up’, ‘biopsy processing’ and ‘resection processing’. Dates searched were between June 1980 and August 2026. In addition, information was used from previous recommendations of the RCPATH and local guidelines held by the NSOPS laboratories in the UK. Published evidence was evaluated using modified SIGN guidance (see Appendix A). Consensus of evidence in the guideline was achieved by expert review. Gaps in the evidence will be identified by College members via feedback received during consultation. The sections of this tissue pathway that indicate compliance with each of the AGREE II standards are indicated in Appendix B.

No major organisational changes or cost implications have been identified that would hinder the implementation of the tissue pathway.

A formal revision cycle for all tissue pathways takes place on a 5-yearly basis. However, each year, the College will ask the author/s of the tissue pathways, in conjunction with the relevant sub-specialty adviser to the College, to consider whether or not the document needs to be updated or revised. A full consultation process will be undertaken if major revisions are required. If minor revisions are required, an abridged consultation process will be undertaken whereby a short note of the proposed changes will be placed on the College website for 2 weeks for members' attention. If members do not object to the changes, the changes will be incorporated into the pathways and the full revised version (incorporating the changes) will replace the existing version on the College website. All changes will be documented in the data control section of the relevant pathway.

The pathway has been reviewed by the Professional Guidelines team, Working Group on Cancer Services and Lay Governance Group. It was placed on the College website for consultation with the membership from 1 September to 30 September 2026. All comments received from the Working Group and membership were addressed by the authors to the satisfaction of the Chair of the Working Group and the Clinical Lead for Guideline Review.

This pathway was developed without external funding to the writing group. The College requires the authors of tissue pathways to provide a list of potential conflicts of interest; these are monitored by the Professional Guidelines team and are available on request. The authors have declared no conflicts of interest.

List of abbreviations used in this document.

Abbreviation	Definition
NHS	National Health Service
NSOPS	National Specialist Ophthalmic Pathology Service
RCPATH	Royal College of Pathologists
BAOP	British Association for Ophthalmic Pathology
CPD	Continuing professional development
EQA	External Quality Assessment
UKAS	United Kingdom Accreditation Service
NEQAS	National External Quality Assessment Services
EM	Electron microscopy
IF	Immunofluorescence
H&E	Haematoxylin and eosin
PAS	Periodic acid-Schiff
ZN	Ziehl-Neelsen

EVG	Elastic van Gieson
PCR	Polymerase chain reaction
HSV	Herpes simplex virus
IgA, IgG, IgM	Immunoglobulin A, Immunoglobulin G, Immunoglobulin M
C3c	Complement C3
HPV	Human papilloma virus
IOL	Intraocular lens
MGG	May-Grunwald Giemsa
α SMA	Alpha smooth muscle actin
GFAP	Glial fibrillary acidic protein
PO	Pupil-optic nerve
CD68	Cluster of differentiation 68
CK	Cytokeratin
GPP	Good practice point

1 Introduction

This document provides guidance on handling and reporting of non-neoplastic ophthalmic pathology specimens. The tissue pathways are important as they provide a consistent approach to managing ophthalmic pathology samples and highlight ancillary techniques when appropriate. The aim of this document is to facilitate and standardise the approach to dissection, block selection, microtomy and selection of special stains for non-neoplastic ophthalmic tissues. Such specimens can originate from the eyelids, ocular surface, ocular coats, cornea, eyeball (evisceration and enucleation), orbit (including lacrimal gland and optic nerve), lacrimal drainage system and temporal artery.

Temporal artery specimens are comprehensively covered in *Tissue pathways for cardiovascular pathology* and are not included in this document.¹ In addition, guidelines for the handling and reporting of specimens for conjunctival or uveal melanoma, ocular retinoblastoma as well as skin tumours can be found in the respective RCPATH cancer datasets.² This document replaces previous versions that included the handling of ophthalmic samples.

Reference is made to the importance of clinical information in the accurate diagnosis of ocular and periocular disorders. The authors consider this to be integral to the assessment and diagnosis of ophthalmic specimens, and therefore encourage close working relationships between pathologists and surgeons/clinicians whenever possible to allow effective and accurate reporting of ophthalmic specimens to achieve best patient care. The need for second opinions and expert opinions is recognised and should be encouraged where required.

1.1 Target users and health benefits of this guideline

The target primary users of the tissue pathway are consultant cellular pathologists who routinely report ophthalmic pathology, trainee cellular pathologists, advanced biomedical scientists engaged in dissection and reporting, and, on their behalf, the suppliers of IT products to laboratories. The secondary users are cellular pathologists from other subspecialties (e.g. dermatopathology, head and neck, neuropathology, haematopathology, musculoskeletal and paediatric) who sporadically might be sent ophthalmic specimens.

It is anticipated that the recommendations could also be used by ophthalmologists in the clinical setting in the selection of biopsy sites, as well as indicating the importance of clinical information in aiding accurate clinicopathological correlation.

1.2 Staffing and workload

The key issue of who should report ophthalmic pathology specimens was covered in section 2 of *Best practice recommendations: Reporting ophthalmic pathology specimens*.³

The diagnostic laboratory should have sufficient pathologists, biomedical scientists and clerical staff to cover all of its functions. In general, staffing levels will follow the workload guidelines of the College. Tissue pathways do not provide detailed guidance for common specimen types.

It is suggested that pathologists reporting ophthalmic specimens:

- participate in auditing
- participate in the College's continuing professional development (CPD) scheme, of which ophthalmic pathology CPD should be a part/whole
- have access to specialist ophthalmic pathology services for referrals as required³
- participate in an appropriate external quality assessment (EQA) scheme, such as the National Ophthalmic Pathology EQA scheme.³

It is recognised that there is significant overlap between ophthalmic pathology and other areas of pathology. For example, eyelid with dermatopathology; lacrimal gland and drainage apparatus with ear, nose and throat pathology; optic nerve, sheath and optic chiasm with neuropathology; and orbital tissues and bony wall of orbit with musculoskeletal pathology.³

Pathologists reporting samples from these sites will not invariably be ophthalmic pathologists and will not necessarily participate in the ophthalmic pathology EQA scheme. However, they should have appropriate experience and should participate in an EQA scheme that is relevant to the samples they report.

1.3 Laboratory facilities

The laboratory should:

- be equipped to allow the recommended technical procedures to be performed safely

- be enrolled with United Kingdom Accreditation Service (UKAS) accreditation to ISO 15189 standard
- participate in the UK National External Quality Assessment Services (NEQAS) for cellular pathology technique and immunofluorescence (IF), if applicable
- participate in the UK NEQAS for immunocytochemistry and in situ hybridisation (when these techniques are used in the diagnostic pathway)
- have access to light microscopy and commonly used special stains
- have access to immunohistochemistry, including IF
- have access to a genetics service
- have access to a microbiology and virology service
- have access to digital photographic equipment
- have access to transmission electron microscopy (EM)
- have access to molecular pathology facilities.

Reports should be held on an electronic database that has facilities to search and retrieve specific data items and is indexed according to SNOMED CT or older versions of SNOMED T, M and P codes. It is acknowledged that existing laboratory information systems may not meet this standard; however, the ability to store data in this way should be considered when laboratory systems are replaced or upgraded.

Workload data should be recorded in a format that facilitates the determination of the resources involved and which, if applicable, is suitable for mapping to Healthcare Resource Groups.

1.4 Specimen submission

Request forms must be completed fully to comply with the specimen acceptance policy. The following information must be supplied:

- patient forename and surname
- date of birth
- NHS number
- patient address
- clinician

- hospital location
- date specimen taken
- high-risk status
- specimen type
- relevant clinical information.

Specimens should be submitted in appropriate containers. Most specimens will require fixation in 10% neutral buffered formalin. The volume of fixative (and therefore size of specimen pot) should be appropriate to the size of specimen. For minute biopsies (e.g. retina), it may be more appropriate to place the specimen and formalin within a small container (e.g. Eppendorf tube or similar).

Patient identification and specimen details must be completed on each specimen pot submitted. Multiple specimens from the same patient should be placed in different, individually identified containers and noted on request forms.

Rapid paraffin processing (e.g. for delayed reconstruction) is not typically required for non-neoplastic conditions; however, if intended, this should be arranged by prior discussion between clinician and laboratory. As with frozen sections, when a verbal report is issued, a written record should be made including exact wording, the recipient and time the report was communicated.

For fresh specimens (e.g. frozen sections, cytology or direct IF), advance notice of the arrival and the sender's contact information are required so late/non-arrival of specimens can be investigated and tracked.

Conjunctival specimens for direct IF may be received in Michel's transport medium or gel transport tubes. Receipt of fresh conjunctival samples for direct IF may be acceptable by laboratories located close to ophthalmology services, which can provide delivery immediately after a sample is taken.

Please note that frozen sections and IF cannot be performed on high-risk specimens.

Please also refer to the Royal College of Ophthalmologists' guidance on ophthalmic pathology. This comprehensive document informs the users (ophthalmologists) of an ophthalmic pathology service and the specimens to be submitted for assessment.⁴

1.5 Specimen dissection

Handling of specimens should be appropriate to the risk category indicated and in line with local guidelines and health and safety regulations.

1.6 Decalcification

For further information, please see *Tissue pathways for bone and soft tissue pathology*.⁵

1.7 Research

Ophthalmic samples may be required for research purposes. In such circumstances where the specimen is required both for diagnostic and research purposes, it is advisable for the ophthalmologist to seek advice from the pathologist involved. It is the responsibility of both the ophthalmologist and pathologist to ensure that the handling of the specimen does not significantly compromise diagnosis. Handling and processing of ophthalmic specimens taken exclusively for research purposes should follow appropriate research protocols.

1.8 Key changes to previous guideline

Section	Explanation
1 Introduction	Additional details added regarding desirable clinical information to be provided on the pathology request form with an emphasis on the importance of clinical information in the accurate diagnosis of ocular and periocular disorders.
1.1 Target users and health benefits of this guideline	Additional details added regarding the target users of this document.
2.1 Small eyelid biopsies – Specimen submission	Additional details added regarding desirable clinical information to be provided on the pathology request form.
2.2 Small eyelid biopsies – Dissection and processing	Additional details added regarding who is appropriate to conduct dissection of specimens. Additional details added regarding how to dissect specimens.
2.3 Small eyelid biopsies – Sectioning and staining	Additional details added regarding further levels and serial sectioning.
2.4 Small eyelid biopsies – Further investigations	Additional investigations included.
3.1 Full thickness lid resection – Specimen submission	Additional details added regarding desirable clinical information to be provided on the pathology request form.
3.2 Full thickness lid resection – Dissection and processing	Additional details added regarding who is appropriate to conduct dissection of specimens.

3.3 Full thickness lid resection – Sectioning and staining	Number of levels cut per block should be guided by histological findings.
3.4 Full thickness lid resection – Further investigations	Additional investigations included.
3.5 Full thickness lid resection – Report content	Record of the components present in the microscopic findings to be included in the report.
4.1 Corneal specimens – Specimen submission	Additional details added regarding desirable clinical information to be provided on the pathology request form.
9.3 Vitreous specimens and cytology – Routine staining	Additional details added recommending either MGG or Papanicolaou stains to be used when preparing cytopins.
9.4 Vitreous specimens and cytology – Further investigations	Modifications made to recommended further investigations (Removed transmission EM from routine investigation).
10.4 Epiretinal membranes – Further investigations	Modifications made to recommended further investigations (removed CK7, CD34 and CD68).
11.4 Eucleations – Further investigations	Modifications made to recommended further investigations (Removed modified ZN, added Masson–Fontana).
14.2 Exenteration – Dissecting and processing	Additional details added regarding the orientation of specimens.

2 Small eyelid biopsies

This section refers to eyelid and periorcular skin biopsies for non-pigmented lesions (e.g. cyst, papilloma), differential diagnosis of pigmented lesions, benign epithelial neoplastic processes, inflammatory/granulomatous lesions (e.g. recurrent chalazion, pyogenic granuloma, inflammatory eyelid skin condition, infection) and vascular lesions.⁶

2.1 Specimen submission

Specimens should be submitted in formalin.

Relevant clinical information is required. The clinical history should include the site and duration of the issue under investigation as well as details on any systemic or topical treatment and factors that might result in immune deficiency.

2.2 Dissection and processing

The handling and dissection of eyelid biopsies can be performed by appropriately trained biomedical scientists and medical personnel trained in histopathology and ophthalmic pathology.

Skin ellipses, shaves or incisional biopsies can be handled as standard skin specimens.⁷

The macroscopic description should include:

- type of specimen
- 3-dimensional measurements
- description of any lesion identified (e.g. size, type, colour and shape)
- description of the anatomical lid margin or tarsal conjunctiva if present.

Orientation markings are unusual on clinically benign specimens; however, if present, the dissector may consider inking the specimen accordingly to facilitate histological orientation.

The tissue must be embedded with the skin surface perpendicular to the plane of section.

2.3 Sectioning and staining

Sections are typically cut at 3 levels. Some laboratories cut a single level and only cut further levels if there is a clinicopathological conflict or if it is not possible to make a diagnosis from a single haematoxylin and eosin (H&E)-stained section. Further deeper levels or serial sectioning of the block are recommended in cases where the histological findings are expected to be focal or subtle.

H&E staining should be used for initial assessment and to inform the use of further special stains or immunohistochemistry as required.

2.4 Further investigations

According to clinical indications or pathological findings, supplementary investigations might include:

- special stains for microorganisms (e.g. Gram, Grocott, Periodic acid–Schiff [PAS], Ziehl–Neelsen [ZN])
- elastic van Gieson (EVG) for elastic fibres
- Congo red, Sirius red for amyloid
- immunohistochemistry for differential diagnosis of unexpected malignancy
- polymerase chain reaction (PCR) analysis for viral genomes if applicable.

2.5 Report content

The report should include:

- clinical information
- biopsy dimensions and macroscopic description
- microscopic findings with a record of the components present
- specific findings or interpretation of findings leading to specific or differential diagnosis
- any recommendation for supra-regional referral
- conclusion
- SNOMED codes.

[Level of evidence – GPP.]

3 Full-thickness lid resection

Indication for full-thickness pentagonal/wedge lid resection is predominantly excision of a localised tumour (not covered in this document). However, if the anatomical lid margin and/or conjunctival aspect is involved by a benign condition, as mentioned above for small eyelid biopsies, full-thickness lid resection might be required.^{6,8}

3.1 Specimen submission

Specimens should be submitted in formalin.

Relevant clinical information is required. The clinical history should include the site and duration of the issue under investigation, as well as details on any systemic or topical treatment and factors that might result in immune deficiency.

3.2 Dissection and processing

The handling and dissection of full-thickness lid resections can be performed by appropriately trained biomedical scientists and medical personnel trained in histopathology and ophthalmic pathology.

Correct orientation with identification of cutaneous and conjunctival surfaces is important.

Dissection protocols may vary among laboratories; however, these should primarily facilitate orientation at embedding and microscopic interpretation. The following dissection approaches are not prescriptive but described as examples.

3.2.1 Horizontal eyelid sectioning

To aid histological assessment, the lateral and medial edges of the specimen can be inked with 2 different colours before serially slicing the specimen parallel to the anatomical lid margin (horizontally) in 2–3 mm thick sections (Figure 1). Each slice is then processed in a separate block and embedded on its cut surface.

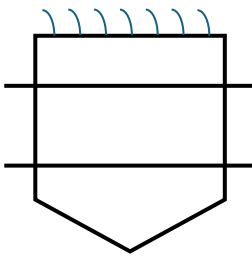


Figure 1. Horizontal eyelid sectioning.

3.2.2 Vertical eyelid sectioning

Alternatively, the specimen can be serially sliced across the lid margin (vertically/sagittally) in 2–3 mm thick sections, processed in separate blocks and embedded in on their cut surface (Figure 2).

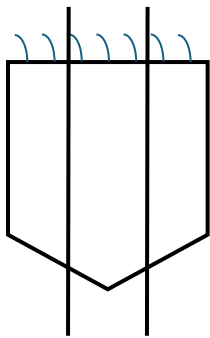


Figure 2. Vertical eyelid sectioning.

A diagram or photograph of the specimen indicating the sections taken may be useful for microscopic orientation in complex cases or in case of any unexpected malignancy.

[Level of evidence – GPP.]

3.3 Sectioning and staining

This document is not prescriptive on the number of initial sections cut per block; however, levels are recommended for further clarification of the pathology as required.

H&E staining should be used for initial assessment.

3.4 Further investigations

According to clinical indications or pathological findings, supplementary investigations might include:

- special stains for microorganisms (e.g. Gram, Grocott, PAS, ZN)
- EVG for elastic fibres
- Congo red, Sirius red for amyloid
- immunohistochemistry for differential diagnosis of unexpected malignancy
- PCR analysis for viral genomes may be performed if applicable.

3.5 Report content

The report should include:

- clinical information
- biopsy dimensions and macroscopic description
- microscopic findings with a record of the components present
- specific findings or interpretation of findings leading to specific or differential diagnosis
- any recommendation for supra-regional referral
- conclusion
- SNOMED codes.

[Level of evidence – GPP.]

4 Corneal specimens

A wide range of corneal specimens can be submitted for histopathological analysis. These include small/punch biopsies, epithelial scrapings (superficial keratectomies), corneal

buttons (anterior/posterior lamellar or penetrating keratoplasties), corneal ‘membranes’ (endothelial keratoplasties) and donor corneal-scleral specimens.^{6,8}

4.1 Specimen submission

Specimens should typically be received in formalin. It should be noted that fixation in formalin may induce some degree of cloudiness in the specimen.

Relevant clinical information is essential, which may include onset of the condition, uni/bilaterality, details about any clinical therapies, previous corneal grafts, history of recent travel, or trauma and family history. Clinical differential diagnoses should be provided whenever available.

In cases of suspected corneal dystrophy or if specimens are required for research purposes, an axial strip can be fixed in glutaraldehyde for EM if indicated.⁹

4.2 Dissection and processing

Corneal specimens should be handled gently to avoid damage to anterior or posterior surfaces. Macroscopic examination can be facilitated by using a dissecting microscope against both dark and light backgrounds, if available.

Measurements of corneal discs/biopsies should be taken. In corneal–scleral donor specimens, the width of the attached scleral rim should also be measured.

Corneal discs may contain suture material from previous keratoplasty or local repair. Some specimens may also show peripheral radial marks or a central inked dot to help orientation for the surgical procedure.

A description of any relevant macroscopic findings should be included, for example:

- any defect, ulceration or perforation including respective location and measurements
- variation in overall thickness
- change in texture of anterior and posterior surfaces
- any opacity, loss of transparency or scars, including distribution, shape, colour and measurements
- any signs of neovascularisation.

In most cases, a corneal disc can be bisected through areas of interest and, whenever possible, at right angles to wounds and scars. This can be safely done with the concave

posterior surface upwards to minimise any possible artefactual endothelial detachment from Descemet's membrane.

Button halves should be embedded on their cut edges. Corneal biopsies can be processed whole and should be embedded on their edges.

Donor corneal–scleral specimens may or may not include a central defect corresponding to the trephined area. If the cornea or part of it is present, these specimens can be cut in 3 parallel sections. When only the scleral rim remains, this can be sectioned radially in 4 mm wide pieces to be embedded on their edges.

[Level of evidence – D.]

4.3 Sectioning and staining

This document is not prescriptive on the number of initial sections cut per block; however, 3 levels would be suggested. If pathology is very focal, a more careful stepwise approach may be required and unstained spare sections might be kept. Some laboratories will cut 1 section; however, further levels would be cut for clarification if required. An initial single section is acceptable for scleral rims from unused donor specimens.

H&E paired with PAS staining should be done for all cases.

4.4 Further investigations

According to clinical indications or pathological findings, supplementary investigations might include:

- special stains for microorganisms (e.g. Gram, Grocott, Giemsa, ZN, modified ZN; a modified ZN also permits the detection of Nocardia and microsporidia)
- immunohistochemistry or PCR for herpes simplex virus (HSV) I and II
- Alcian blue for mucopolysaccharide deposits¹⁰
- Masson's trichrome for differential diagnosis of granular/keratohyaline deposits¹⁰
- Congo red or Sirius red for amyloid deposits¹⁰
- von Kossa for suspected dystrophic calcification
- Perls' for haemosiderin or Fleischer ring
- immunohistochemistry (cytokeratins) for suspected posterior polymorphous dystrophy

- immunohistochemistry (e.g. CK3, CK19, CK7, CK12, CK13) for suspected limbal stem cell deficiency
- transmission EM (in some instances EM may be indicated for confirmation of a rare dystrophy or an unusual microorganism [e.g. microsporidium]).⁹

[Level of evidence – C. Use of special stains facilitates morphological differential diagnosis of suspected corneal dystrophies.]

4.5 Report content

The report should include:

- clinical information
- specimen dimensions and macroscopic description
- microscopic findings
- interpretation of findings leading to specific or differential diagnosis
- any recommendation for supra-regional referral (expert opinion or further tests [e.g. EM, PCR] if not available locally)
- conclusion
- SNOMED codes.

[Level of evidence – D, GPP.]

5 Conjunctival biopsies

The main indications for non-neoplastic conjunctival or caruncular biopsies are:

- non-pigmented lesions (e.g. pterygium/pinguecula, cyst, dermoid/choristoma, benign tumour/papilloma)
- inflammatory lesions, infectious or non-infectious (e.g. mucous membrane pemphigoid, sarcoid, chalazion)
- differential diagnosis of lymphoid lesions.

Incisional mapping conjunctival biopsies consist of multiple biopsies typically performed to estimate the extent of an epithelial or melanocytic intraepithelial neoplasia^{6,8} (not covered by this document).

5.1 Specimen submission

Specimens are commonly submitted in 10% neutral buffered formalin, except those for direct IF, which should be received in Michel's transport medium or gel transport tubes. Fresh specimens for direct IF may be accepted if prompt delivery is achievable (see section 1.4).

Conjunctival specimens are usually very thin and as such tend to curl. This can be minimised by laying the biopsies on a piece of sponge or card immediately after being taken to allow them to fix flattened.

5.2 Dissection and processing

Macroscopic examination should include measurements and a description of any visible abnormality.

If orientation markings are provided, margins of excisional conjunctival or caruncular biopsies should be inked whenever practical.

Excisional specimens (usually larger than 6 mm in their long axis) should be serially sliced perpendicular to the long axis. Each slice should then be embedded on its cut surface.

Small biopsies can be processed whole and embedded on their longest edge.

Samples for direct IF should be embedded whole in freezing mount on their longest axis.^{11,12}

5.3 Sectioning and staining

This document is not prescriptive on the number of initial sections cut per block; however; 3 to 4 levels would be suggested. If pathology is very focal, a more careful stepwise approach may be required and unstained spare sections might be kept. Some laboratories will cut 1 section; however, further levels would be cut for clarification if required.

H&E staining should be used for initial assessment of all sample types.

For frozen samples for direct IF, cut 1 or 2 sections per slide. The following antibodies are typically used for investigation of mucous membrane pemphigoid: IgA, IgG, IgM, C3c and fibrinogen. In some cases, an additional buccal mucosa specimen may also be submitted for IF.

5.4 Further investigations

According to clinical indications or pathological findings, supplementary investigations might include:

- PAS for assessment of basement membranes and goblet cells
- special stains for microorganisms (Gram, PAS, Grocott, ZN)
- EVG for elastic fibres and assessment of pterygium/pinguecula
- Congo red, Sirius red or immunohistochemistry for amyloid (local conjunctival amyloidosis is most commonly caused by deposition of monoclonal immunoglobulin light chains [kappa or lambda])
- Masson–Fontana for melanin
- immunohistochemistry for differential diagnosis of unexpected malignancy.

[Level of evidence – D and GPP.]

5.5 Report content

The report should include:

- clinical information
- biopsy dimensions and macroscopic description
- microscopic findings (with correlation with respective location for mapping biopsies)
- interpretation of findings leading to specific or differential diagnosis
- any recommendation for supra-regional referral (for expert opinion or further tests [e.g. HPV PCR, molecular studies, direct IF] if not available locally)
- conclusion
- SNOMED codes.

6 Trabecular meshwork

Trabecular meshwork samples are usually submitted for histopathological analysis for research purposes. Such cases typically follow respective research protocol.⁸ The following instructions are suggested for a diagnostic context.

6.1 Specimen submission

Specimens are typically submitted in 10% neutral buffered formalin.

6.2 Dissection and processing

Macroscopic examination may require magnification. Most samples contain scleral tissue only. If present, the outflow system is usually pigmented. Any attached ciliary muscle may aid orientation.

Depending on size, the specimen can be bisected perpendicularly to the trabecular meshwork.⁸

6.3 Sectioning and staining

In view of the minute size of this type specimen, serial sections would be recommended to facilitate identification and assessment of the outflow system. The limited size of the processed tissue also allows multiple sections to be mounted on each slide. The following protocol is performed at research and specialist centres:

- 10 serial sections are cut per slide, usually mounted on 5 slides⁸
- 3 out of 5 slides are stained with H&E and 2 slides are kept unstained for further investigation as required.

The above protocol example is not prescriptive and can be adapted according to local practice.

[Level of evidence – D.]

6.4 Further investigations

Further investigations might include:

- special stains if suspected extracellular material (Masson Trichrome, EVG, Alcian blue, PAS)
- transmission EM.⁸

6.5 Report content

The report should include:

- clinical information
- biopsy dimensions and macroscopic description

- microscopic findings
- interpretation of findings
- any recommendation for supra-regional referral
- conclusion
- SNOMED codes.

7 Iris, ciliary body and choroid

The main indications for a non-neoplastic iris, ciliary body or choroidal/retino-choroidal biopsy are cysts, glaucoma-associated anterior chamber syndromes (essential iris atrophy and iridocorneal endothelial syndrome), and infectious and non-infectious inflammatory pathologies.

7.1 Specimen submission

Specimens are typically submitted in 10% neutral buffered formalin. However, if there is a suspected viral aetiology (e.g. viral retino-choroiditis), a small piece might be fixed in glutaraldehyde for transmission EM.

For any suspected infectious case, clinicians must send material for microbiology (culture, PCR/molecular testing) before fixing the specimen.

These biopsies are quite small, therefore they can be placed into Eppendorf tubes containing formalin in theatre rather than standard formalin pots.^{6,13}

7.2 Dissection and processing

After a description has been taken, the biopsies are best wrapped carefully in paper and placed in a nylon tissue bag, biopsy wrap or cell-safe capsule to minimise risk of loss during processing.^{6,13}

7.3 Sectioning and staining

Serial sections or close levels with unstained spare sections in between are recommended to ensure focal pathology is not missed.^{6,13}

H&E staining should initially be used.

7.4 Further investigations

According to clinical indications or pathological findings, supplementary investigations might include:

- special stains for microorganisms (Gram, PAS, Grocott, ZN, modified ZN, Warthin-Starry)
- PAS for phacogenic uveitis, exfoliation material, vacuolated iris pigment epithelium and thickened ciliary body basement membrane in diabetes
- Congo red or Sirius red for amyloid
- Perls' for haemosiderin
- immunohistochemistry for viruses (e.g. HSV family)
- PCR on paraffin sections (for infectious agent speciation)
- transmission EM (for unusual infectious agents mainly).^{6,13}

[Level of evidence – D.]

7.5 Report content

The report should include:

- clinical information
- biopsy dimensions and macroscopic description
- microscopic findings
- interpretation of findings
- any recommendation for supra-regional referral
- conclusion
- SNOMED code.

8 Lens

The natural lens is rarely submitted, but usual indications may be for congenital, traumatic, metabolic or infectious aetiologies, or suspected heavy metal deposition.

Intraocular lenses (IOL) may be submitted when there have been issues with mechanical trauma, inflammation, infection, wrong power or decentration.

8.1 Specimen submission

Natural lens fragments should be fixed in formalin $\pm$ glutaraldehyde if transmission EM is required.

IOL should be submitted in formalin and their make should be identified (clinical notes or IOL catalogue).

8.2 Dissection and processing

The lens may be received as fragments in a bag, containing balanced salt solution, post-phacoemulsification. Fixative can be poured into the bag to fix the fragments.

Note the integrity of IOL's optics and haptics, and the colour and distribution of any opacification.^{14–16}

8.3 Sectioning and staining

In the case of a natural lens tissue, 1 to 3 levels can be cut.^{14–16} Both H&E and PAS (for assessment of lens capsule) should be used in the first instance.

IOL can be whole mounted on a glass slide with a well using aqueous medium and coverslipped. This technique allows cellular material to be assessed clearly once stained whole with H&E.^{14–16}

8.4 Further investigations

Further investigations might include:

- Gram, PAS, Grocott, ZN and Wade–Fite if infection is suspected; the whole mounted IOL can be removed, decolourised of H&E and restained if required
- PAS for lens-induced uveitis (macrophages with eosinophilic liquified lens contents), membranes surrounding IOL and exfoliation syndrome
- Alizarin red or von Kossa for mineralisation of IOL
- Perls' for siderosis lentis
- Copper stains for chalcosis lentis
- transmission EM (especially for Alport's syndrome)
- scanning EM (for IOL).

[Level of evidence – D.]

8.5 Report content

The report should include:

- clinical information
- biopsy dimensions and macroscopic description
- microscopic findings
- interpretation of findings
- any recommendation for supra-regional referral
- conclusion
- SNOMED codes.

9 Vitreous and cytology

Clinical indications for vitrectomy include removal of opacities (e.g. vitreous haemorrhage) or in the course of other treatment (e.g. retinal detachment surgery or peeling of epiretinal membranes). Such material need not routinely be submitted for examination unless the clinical presentation is unusual.

9.1 Specimen submission

Where vitreous is intentionally sampled for laboratory examination, it may be obtained as an undiluted tap (submitted in a capped syringe or suitably sized receptacle) or as a vitrectomy specimen (submitted in a bag or cassette or decanted into a pot). The local laboratory and surgeon should agree in advance whether such specimens are submitted fixed or unfixed. Fixative used (whether at surgery or on receipt in the laboratory) will depend on local cytology protocols. If infection is suspected, ophthalmologists should be advised to send material for microbiological investigation before fixation.

Anterior chamber (aqueous) aspirates may occasionally be submitted for evaluation of, e.g. haemorrhage or inflammation. These aspirates are very low volume (less than 0.3 ml) and often submitted in a syringe.

9.2 Dissection and processing

Volume of fluid should be recorded and any tissue fragments noted. These can be removed, fixed in formalin and processed to wax.^{17,18}

Preparation of vitreous and cytology specimens will depend on individual laboratory protocols. 2 common methods are cytopins and cell blocks. Cytopins are prepared on glass slides. Cell blocks are processed to wax.

9.3 Sectioning and staining

MGG or Papanicolaou are used for cytopins.¹⁸

As the material is usually limited, if a paraffin block is available serial sections and unstained spare sections could be cut.¹⁷ Sections are initially stained with H&E to guide any further investigation if required.

9.4 Further investigations

According to clinical indications or morphological findings, supplementary investigations might include:

- Congo red for amyloid (the National Amyloidosis Centre, Royal Free Hospital, can undertake subtyping)
- special stains for pigment (e.g. Perls', Masson–Fontana)
- special stains for microorganisms (e.g. Gram, PAS, silver stains, [modified] ZN, Warthin–Starry)
- specific immunohistochemistry or PCR for certain infectious organisms.¹⁸

[Level of evidence – D.]

9.5 Report content

The report should include:

- clinical information
- specimen volume and presence of fragments
- microscopic findings
- interpretation of findings
- any recommendation for supra-regional referral (especially for further investigations that are not available locally)
- conclusion
- SNOMED codes.

10 Epiretinal membranes

10.1 Specimen submission

Epiretinal membranes are not usually histologically examined outside of a research setting. If such a specimen is submitted, it is likely to be very small.

Epiretinal membranes should be submitted in formalin in an appropriately sized receptacle, such as an Eppendorf tube.

10.2 Dissection and processing

If the specimen is transparent, a drop of dye such as Alcian blue may be added in the laboratory to aid visualisation.

Identify and measure the specimen fragment(s). Specimen wrap paper may be used to retain the specimen within the cassette. If the specimen cannot be seen in the receptacle, the formalin can be spun down and handled as cytology.⁶

10.3 Sectioning and staining

Serial sections with spares should be cut.⁶

Both H&E and PAS should be initially used.⁶ The PAS allows for visualisation of native inner limiting membrane, neo-basement membrane, vessels and photoreceptor outer segments.

[Level of evidence – D and GPP.]

10.4 Further investigations

Depending on the clinical indication for submission, immunohistochemistry may be used to highlight constituent cells (e.g. α SMA for myofibroblasts or GFAP for glial cells).¹⁹

10.5 Report content

The report should include:

- clinical information
- specimen dimensions
- microscopic findings
- interpretation of findings

- conclusion
- SNOMED codes.

11 Enucleations

11.1 Specimen submission

Note that this protocol does not cover paediatric suspected non-accidental injury cases.

Specimens should not be opened or injected with formalin in an attempt to improve fixation. They should be fixed in formalin for at least 24 hours before dissection.

11.2 Dissection and processing

The following process should be followed.

- Orientate the globe: the superior oblique muscle tendinous insertion lies superiorly. The optic nerve lies posteriorly and medially to the inferior oblique muscle insertion. Traumatized or phthisical globes may be too shrunken or distorted to orientate.
- Measure the globe in 3 dimensions: anteroposterior, horizontal and vertical. Typical measurements of each are 22–24 mm. There may be focal or generalised distortion, or discolouration or features of previous surgery (e.g. filtering blebs) or trauma.
- Measure the cornea horizontally (typically 12 mm) and vertically (typically 11 mm). Note any opacities.
- Note the pupil shape (e.g. round, oval) and size. Corneal opacity or blood in the anterior chamber may obscure the view.
- Measure the optic nerve length. If long enough, sample the cut end so a transverse section can be assessed. Inking of the (remaining) cut end helps to identify it during microtomy.
- Sample the vortex veins in case of unexpected intraocular malignancy.
- Transilluminate through the cornea/pupil to identify potential intraocular masses. If a mass is suspected, its position may influence how the globe is sliced.
- If no focal pathology is identified, the eye can be opened horizontally so macular histology can be assessed. Otherwise, vertical or oblique cuts can be made to demonstrate the pathology and produce the calottes (caps).

- Take the calottes by making 2 parallel anteroposterior slices, 1 on either side of the cornea and optic nerve. A skin graft or similar blade is useful to achieve a smooth cut. The non-calotte (middle) portion is the 'main block' or 'PO block' (pupil–optic nerve).
- The main block of an enucleation specimen should include cornea, pupil, optic nerve and representative pathology.
- Examine the interior of the eye. Note any shallowing of the anterior chamber, masses, haemorrhage or retinal detachment (which may have subretinal exudate). A crystalline lens or IOL may be present.
- The main block will probably require a deep or large cassette and mould. The calottes may be processed if they show macroscopic pathology.^{20–24}

11.3 Sectioning and staining

The main block may require 3 to 5 levels before the pupil and optic nerve are reached. Sufficient levels should be cut to demonstrate findings specific to the case

H&E and PAS should be used. The latter is useful to demonstrate the Descemet membrane, lens capsule, the retinal inner limiting membrane and Bruch's membrane, especially if structures are distorted.^{20–22}

[Level of evidence – D and GPP.]

11.4 Further investigations

Further investigations will depend on clinical context and microscopic findings. They might include:

- special stains for microorganisms (e.g. Gram, Grocott)
- Perls' and Masson–Fontana to differentiate between haemosiderin and melanin
- immunohistochemistry for reactive proliferations or tumours
- CD68 immunohistochemistry can help define granulomas if sympathetic endophthalmitis is suspected.^{20,22}

11.5 Report content

The report should include:

- clinical information

- globe, cornea and optic nerve macroscopic dimensions
- microscopic findings
- interpretation of findings (neoplasia or sympathetic endophthalmitis are of particular concern)
- any recommendation for supra-regional referral (especially for further investigations that are not available locally)
- conclusion
- SNOMED codes.

12 Eviscerations

12.1 Specimen submission

The specimen which usually includes cornea with a rim of sclera as well as the intraocular contents should be submitted in formalin. The specimen may be heavily calcified.

12.2 Dissection and processing

The following process should be followed.

- Fix in formalin for at least 24 hours.
- Measure any corneoscleral button. Note obvious pathology such as opacity, perforation, scars or sutures. Slice into 3 or 4 pieces to allow for embedding on edge.
- Measure the intraocular contents. They may be submitted as a sac or as piecemeal haemorrhagic and membranous fragments. Slice any sac. Note the constituents, e.g. retina, vitreous, crystalline lens or IOL and uveal tissue. There may be oil post-vitrectomy.
- Submit all tissue for processing. It may be easier to place the cornea in its own cassette.
- Process any IOL. It can be cut without damage to the microtomy blade.⁶

12.3 Sectioning and staining

Depending on the clinical information, a single section or 3 levels may be cut initially.^{6,20}

H&E and PAS should be used.^{5,13}

12.4 Further investigations

Further investigations will depend on clinical context and microscopic findings. They might include:

- special stains for microorganisms (e.g. Gram, Grocott)
- Perls' for old haemorrhage
- CD68 immunohistochemistry can help define granulomas if sympathetic endophthalmitis is suspected.^{20,22}

[Level of evidence – D and GPP.]

12.5 Report content

The report should include:

- clinical information
- specimen dimensions
- microscopic findings
- interpretation of findings (especially any unexpected neoplasia or features of sympathetic endophthalmitis)
- conclusion
- SNOMED codes.

13 Orbital and lacrimal gland biopsies

13.1 Specimen submission

Non-neoplastic orbital biopsies are often submitted for investigation of inflammatory disease. Dermoid cysts may also occur, especially in children.

13.2 Dissection and processing

The following process should be followed.

- Fix in formalin at least overnight.
- Measure the specimen in 3 dimensions. Piecemeal specimens may be measured in aggregate.

- Description may include texture, colour and any cyst contents.
- A discrete specimen may be inked if there is the possibility of neoplasia. Slice if required.⁶

[Level of evidence – GPP.]

13.3 Sectioning and staining

Depending on clinical information and specimen size, a single section or 3 levels may be taken. Spare unstained sections may be kept if the specimen is very small.²²

H&E should be used.

13.4 Further investigations

Further investigations will depend on clinical information and microscopic findings. They might include:

- special stains for microorganisms (e.g. Gram, PAS, Grocott)
- Congo red for amyloid
- immunohistochemistry for suspected IgG4-related disease.

13.5 Report content

The report should include:

- clinical information
- specimen dimensions
- microscopic findings
- interpretation of findings
- any recommendation for supra-regional referral (e.g. for further assessment of amyloid)
- conclusion
- SNOMED codes.

14 Exenteration

14.1 Specimen submission

Non-neoplastic orbital exenterations are usually performed for intractable symptoms such as chronic inflammation and/or fibrosis, or occasionally for infections. An exenteration includes the globe (if present) as well as orbital soft tissue and eyelids. A lid-sparing exenteration leaves eyelid tissue (usually skin) behind to aid reconstruction. Some exenterations include orbital bone.

14.2 Dissection and processing

The following process should be followed.

- Fix in an adequate volume of formalin which is likely to be more than that needed for other ophthalmic specimens. 48 hours should be sufficient.
- Orientate the specimen. The caruncle and puncta lie medially, and the upper eyelid is longer (vertically) than the lower. The optic nerve cut end lies posteriorly, although it may be hidden by fat.
- Measure the specimen in 3 dimensions: anteroposterior, horizontal (mediolateral) and vertical.
- Note the amount of skin and optic nerve. The optic nerve cut end may be sampled.
- Check for the presence of the globe and note any obvious abnormalities (e.g. disruption).
- Note any obvious soft tissue abnormalities (e.g. masses).
- If bone is included, consider removing it for separate decalcification and processing.
- Paint the external surfaces. Different colours for superior and inferior will aid microscopic orientation.
- The specimen is typically bread-sliced sagittally (medial to lateral or vice versa). The middle blocks will contain any globe, with any lacrimal gland lying superiorly in a lateral slice and any lacrimal sac lying inferiorly in a medial slice.
- Note the appearance of the cut soft tissue surface (e.g. scarring, haemorrhage, abscess). Note any globe disruption or intraocular abnormalities.
- If available, large blocks will allow slices to be processed and sectioned whole.^{21,23}

14.3 Sectioning and staining

Ensure that full faces are achieved.

H&E and PAS should be used. PAS is useful for evaluation of the globe.

14.4 Further investigations

These will depend on clinical information and findings on initial microscopy, and might include:

- special stains for microorganisms (e.g. Gram, Grocott)
- Congo red for amyloid
- immunohistochemistry for possible IgG4-related disease or suspected tumours.

[Level of evidence – D and GPP.]

14.5 Report content

The report should include:

- clinical information
- specimen dimensions
- microscopic findings
- interpretation of findings
- any recommendation for supra-regional referral (e.g. for investigation of amyloid)
- conclusion
- SNOMED codes.

15 Impression cytology

15.1 Specimen submission

Impression cytology specimens may be used when investigating suspected limbal stem cell deficiency. Sampling generally uses a commercially available disc/membrane. The specimen should be fixed in formalin overnight.²⁵

15.2 Sectioning and staining

No sectioning is required. The disc is removed from its plastic ring and mounted whole (en face) onto the glass slide.

Dual immunohistochemistry for corneal cells (CK3 and CK12) and conjunctival cells (CK13 and CK19) can be performed on a single disc. If immunohistochemistry or if there is an additional disc, PAS stain can be used.²⁵

[Level of evidence – D.]

15.3 Report content

The report should include:

- clinical information
- microscopic findings (cellularity, cell size, presence of goblet cells and immunohistochemical pattern)
- interpretation of findings (specially, whether the appearance supports conjunctivalisation or not)
- conclusion
- SNOMED codes.

16 Lacrimal sac biopsies/excision

16.1 Specimen submission

The specimen may be lacrimal sac contents (e.g. a dacryolith), a biopsy of the sac wall or a lacrimal sac excision (i.e. dacryocystectomy). The specimen should be fixed in formalin for 24 hours.

16.2 Dissection and processing

The following process should be followed.

- Measure the specimen which can be in aggregate if piecemeal.
- Wall biopsies and dacryoliths do not usually require slicing.
- A sac can be bread-sliced to reveal the lumen.

[Level of evidence – D.]

16.3 Sectioning and staining

- A single section or 3 levels may be taken.⁸
- H&E should be used.

16.4 Further investigations

This might include special stains for microorganisms (e.g. Gram, PAS, Grocott).⁸

16.5 Report content

The report should include:

- clinical information
- specimen dimensions
- microscopic findings
- interpretation of findings
- conclusion
- SNOMED codes.

17 Criteria for audit

The following are recommended by the RCPATH as key assurance and key performance indicators:^{26,27}

- histopathology cases that are reported, confirmed and authorised within 7 and 10 calendar days of the procedure
 - standard: 80% of cases must be reported within 7 calendar days and 90% within 10 calendar days.

18 References

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Appendix A

of evidence

Summary table – explanation of grades

(modified from Palmer K et al. BMJ 2008;337:1832)

Grade (level) of evidence	Nature of evidence
Grade A	At least 1 high-quality meta-analysis, systematic review of randomised controlled trials or a randomised controlled trial with a very low risk of bias and directly attributable to the target population or A body of evidence demonstrating consistency of results and comprising mainly well-conducted meta-analyses, systematic reviews of randomised controlled trials or randomised controlled trials with a low risk of bias, directly applicable to the target cancer type.
Grade B	A body of evidence demonstrating consistency of results and comprising mainly high-quality systematic reviews of case-control or cohort studies and high-quality case-control or cohort studies with a very low risk of confounding or bias and a high probability that the relation is causal and which are directly applicable to the target population or Extrapolation evidence from studies described in A.
Grade C	A body of evidence demonstrating consistency of results and including well-conducted case-control or cohort studies and high-quality case-control or cohort studies with a low risk of confounding or bias and a moderate probability that the relation is causal and which are directly applicable to the target population or Extrapolation evidence from studies described in B.
Grade D	Non-analytic studies such as case reports, case series or expert opinion or Extrapolation evidence from studies described in C.
Good practice point (GPP)	Recommended best practice based on the clinical experience of the authors of the writing group.

Appendix B AGREE II guideline monitoring sheet

The tissue pathways of the Royal College of Pathologists comply with the AGREE II standards for good quality clinical guidelines. The sections of this tissue pathway that indicate compliance with each of the AGREE II standards are indicated in the table.

AGREE standard	Section of guideline
Scope and purpose	
1 The overall objective(s) of the guideline is (are) specifically described	Introduction
2 The health question(s) covered by the guideline is (are) specifically described	Introduction
3 The population (patients, public, etc.) to whom the guideline is meant to apply is specifically described	Foreword
Stakeholder involvement	
4 The guideline development group includes individuals from all the relevant professional groups	Foreword
5 The views and preferences of the target population (patients, public, etc.) have been sought	Foreword
6 The target users of the guideline are clearly defined	Introduction
Rigour of development	
7 Systematic methods were used to search for evidence	Foreword
8 The criteria for selecting the evidence are clearly described	Foreword
9 The strengths and limitations of the body of evidence are clearly described	Foreword
10 The methods for formulating the recommendations are clearly described	Foreword
11 The health benefits, side effects and risks have been considered in formulating the recommendations	Foreword and Introduction
12 There is an explicit link between the recommendations and the supporting evidence	2–16
13 The guideline has been externally reviewed by experts prior to its publication	Foreword
14 A procedure for updating the guideline is provided	Foreword
Clarity of presentation	
15 The recommendations are specific and unambiguous	2–16
16 The different options for management of the condition or health issue are clearly presented	2–16

17 Key recommendations are easily identifiable	2–16
Applicability	
18 The guideline describes facilitators and barriers to its application	Foreword
19 The guideline provides advice and/or tools on how the recommendations can be put into practice	2–16
20 The potential resource implications of applying the recommendations have been considered	Foreword
21 The guideline presents monitoring and/or auditing criteria	17
Editorial independence	
22 The views of the funding body have not influenced the content of the guideline	Foreword
23 Competing interest of guideline development group members have been recorded and addressed	Foreword