

Tissue pathways for non-neoplastic ophthalmic pathology specimens

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NICE has accredited the process used by The Royal College of Pathologists to produce its Cancer Datasets and Tissue Pathways guidance. Accreditation is valid for 5 years from July 2012. More information on accreditation can be viewed at www.nice.org.uk/accreditation.
For full details on our accreditation visit: www.nice.org.uk/accreditation.

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. It may rarely be necessary or even desirable to depart from the guidelines in the interests of specific patients and special circumstances. The clinical risk of departing from the guidelines should be carefully considered by the reporting pathologist; just as adherence to the guidelines may not constitute defence against a claim of negligence, so a decision to deviate from them should not necessarily be deemed negligent.

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

The tissue pathway has been developed in consultation with the following professional stakeholders:

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

The evidence used to compile this tissue pathway was obtained from published material in journals (PUBMED), textbooks and from previous standard operating procedures held by the National Specialist Ophthalmic Pathology Laboratories. Published evidence was evaluated using the modified SIGN criteria. The level of evidence was grade C and D, or met the GPP (good practice point) criteria.

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

A formal revision cycle for all tissue pathways takes place on a four-yearly basis. However, each year, the College will ask the authors of the tissue pathways, in conjunction with the relevant subspecialty 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 two weeks for Fellows' attention. If Fellows do not object to the changes, the short notice of change will be incorporated into the pathways and the full revised version (incorporating the changes) will replace the existing version on the publications page of the College.

The pathway has been reviewed by the Working Group on Cancer Services and was on the College website for consultation with the membership from 6 January to 3 February 2015. 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 Vice-President for Communications.

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 Director of Clinical Effectiveness and are available on request. The authors of this document have declared that there are no conflicts of interest.

1 Introduction

This tissue pathway 'guideline' document deals with the handling of non-neoplastic ophthalmic pathology tissue. The main intention of this document is to facilitate and standardise the approach to cut up, block selection, microtomy and selection of special stains for non-neoplastic ophthalmic tissues. Non-neoplastic ophthalmic 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.

The temporal artery specimen has been dealt with comprehensively in *Tissue pathways for cardiovascular pathology* (www.rcpath.org/publications-media/publications/datasets/datasets-TP.htm) and is not included in this document.

1.1 Target users of the guideline

The target primary users of the tissue pathway are consultant cellular pathologists who routinely report ophthalmic pathology, advanced biomedical scientists engaged in cut up/ reporting and trainee cellular pathologists.

1.2 Staffing and workload

Please see a previous College publication, *Guideline for the reporting of ophthalmic pathology specimens*. Section 2 of that document, which was prepared in collaboration with The Royal College of Ophthalmologists, covers the key issue of who should report ophthalmic pathology specimens.¹

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 Royal College of Pathologists. For common specimen types, it is not the intention to provide detailed guidance in the tissue pathways.

Pathologists should:

- participate in audit
- participate in the College's continuing professional development (CPD) scheme, of which ophthalmic pathology CPD should be a part/whole.
- participate in the NEQAS (National External Quality Assessment Service) for ophthalmic pathology
- have access to specialist ophthalmic referral opinions (e.g. National Specialist Ophthalmic Pathology Service Laboratories in Liverpool, London, Manchester and Sheffield).

1.3 Facilities

The laboratory should:

- be equipped to allow the recommended technical procedures to be performed safely
- be enrolled with Clinical Pathology Accreditation (CPA) (UK) Ltd
- participate in the UK NEQAS for Cellular Pathology Technique
- 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 common special stains
- have access to immunocytochemistry
- have access to a genetic service
- have access to a microbiology and virology service
- have access to digital photographic equipment
- have access to transmission electron microscopy
- 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 that is indexed according to Systematised Nomenclature of Medicine Clinical Terms (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 (HRGs).

1.4 Specimen submission

Request forms must be completed fully. In order to conform to specimen acceptance policy, the following information should be supplied:

- patient forename and surname
- date of birth
- NHS number
- patient address
- clinician
- hospital location
- date specimen taken
- high-risk status
- specimen type
- brief relevant clinical information.

For fresh specimens, e.g. for frozen section, cytology or direct immunofluorescence (IF):

- advance notice of fresh specimens and sender contact information is required so that late/non-arrival of specimens can be investigated and tracked
- where rapid paraffin processing (e.g. for delayed reconstruction) is provided, 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
- conjunctival specimens for IF may be received in Michel's transport medium or gel transport tubes. Receipt of fresh conjunctival samples for IF may be acceptable by laboratories located close to ophthalmology services, which can provide delivery immediately after a sample is taken.

Note: Frozen sections and IF cannot be performed on high-risk specimens.

For fixation and containers:

- 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
- 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).

Please also refer to The Royal College of Ophthalmologists'/Royal College of Pathologists' guidance on referral of ophthalmic pathology specimens. This is a comprehensive document that informs the users (ophthalmologists) of an ophthalmic pathology service and which specimens to submit and not to submit for assessment.²

1.5 Specimen dissection

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

1.6 Decalcification

See the College's tissue pathways for bone and soft tissue pathology (www.rcpath.org/publications-media/publications/datasets/datasets-TP.htm).^{2,3}

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 a pathologist's advice. It is responsibility of both 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.

2 Small lid biopsy⁴

The main indications for eyelid and periocular skin biopsies are: non-pigmented lesions (e.g. cyst, papilloma), differential diagnosis of pigmented lesion, suspected epithelial dysplastic/neoplastic process, inflammatory/granulomatous lesions (e.g. recurrent chalazion, pyogenic granuloma, inflammatory skin condition, infection) and vascular lesions.

2.1 Specimen submission and dissection

- Specimens are submitted in formalin.
- Skin ellipses and incisional biopsies of skin can be handled as standard skin specimens.
- If orientation marks are present, the margins of the specimen should be inked to facilitate histological orientation.
- The presence of lid margin or tarsal conjunctiva should be noted.

2.2 Sectioning

- Sections cut at three levels. Some laboratories cut a single level and cut further levels only if there is a clinico-pathological conflict or if it is not possible to make a diagnosis on a single haematoxylin and eosin (H&E).

2.3 Routine staining

- H&E for initial assessment.

2.4 Further investigations

According to clinical indication or pathological findings:

- special stains for micro-organisms (e.g. Gram, Grocott, PAS, ZN, etc.)
- Congo red or Sirius red or immunohistochemistry for amyloid
- immunohistochemistry for differential diagnosis of unexpected malignancy.

2.5 Report content

- Clinical information.
- Biopsy dimensions and macroscopic description.
- Microscopic findings.
- Specific findings or Interpretation of findings leading to specific or differential diagnosis.
- Any recommendation for supra-regional referral.
- Conclusion.
- SNOMED codes.

The level of evidence was grade C and D, or met the GPP/good practice point criteria.

3 Full thickness lid resection^{4,5}

Indication for full thickness pentagonal/wedge lid resection is predominantly excision of a localised tumour but may also include other benign conditions (as mentioned for eyelid skin biopsies) if involving lid margin and or conjunctival aspect.

3.1 Specimen submission and dissection

- Specimens are received in formalin.
- Correct orientation with identification of cutaneous and conjunctival surfaces is key.
- Dissection protocols may vary among laboratories, however these should primarily facilitate orientation at embedding and microscopic interpretation. A suggestion for dissection is inking lateral and medial halves with two different colours. The specimen is then serially sliced parallel to the lid margin in 2–3 mm thick sections and embedded on their cut surfaces to allow, whenever required, microscopic measurement of the distance of the lesion to both medial and lateral margins, as these tend to be closest margins in eyelid resections. If applicable, assessment of margins in the last block (superior margin for upper eyelids and inferior for lower eyelids) can be done by cutting levels or re-embedding the tissue turned 180°. Some laboratories may wish to embed lateral and medial aspects of the specimen in separate cassettes, making colouring obsolete. Some laboratories will slice the specimen sagittally rather than horizontally.
- Some laboratories advocate embedding one slice per cassette; some advocate more than one slice per cassette. This tissue pathway is not prescriptive.
- A diagram or photograph of the specimen indicating the sections taken may be useful for microscopic correlation in complex cases.

3.2 Sectioning

- Three levels from the first block (lid margin) and the last block (inferior margin for lower eyelid and superior margin for upper eyelid). A single section from each of the

intermediate blocks. Some laboratories may not wish to cut levels and only do so for further clarification of the pathology.

3.3 Routine staining

- H&E for initial assessment.

3.4 Further investigations

According to clinical indication or pathological findings:

- special stains for micro-organisms (e.g. Gram, Grocott, PAS, ZN)
- EVG
- Congo red, Sirius red or immunohistochemistry for amyloid
- immunohistochemistry for differential diagnosis of unexpected malignancy.

3.5 Report content

- Clinical information.
- Biopsy dimensions and macroscopic description.
- Microscopic findings.
- Interpretation of findings leading to specific or differential diagnosis.
- Any recommendation for supra-regional referral.
- Conclusion.
- SNOMED codes.

The level of evidence was grade C and D, or met the GPP/good practice point criteria.

4 Corneal specimens^{4,5}

A wide range of corneal specimens can be submitted for histopathological analysis. These include small/punch biopsies, epithelial scrapings, corneal buttons (anterior/posterior lamellar or penetrating keratoplasties), corneal 'membranes' (endothelial keratoplasty) and donor corneal-scleral specimens.^{4,5}

4.1 Specimen submission and dissection

- Specimens are typically received in formalin. Please note that fixation in formalin may induce some degree of cloudiness to the specimen.
- In case of suspected corneal dystrophy or any research interest, an axial strip can be fixed in glutaraldehyde for electron microscopy if indicated.⁶
- Corneal specimens should be handled gently to avoid undesirable damage to anterior or posterior surfaces.
- Measure the diameter of the disc/biopsy and describe any variation in overall thickness. In corneal-scleral donor specimens, the width of the attached scleral rim should be measured.
- Macroscopic examination of corneas can be facilitated by using a dissecting microscope against both dark and light backgrounds.

- Corneal discs may contain suture material from previous keratoplasty or local repair. Some specimens may also show peripheral radial marks or a central dot to help orientation for the surgical procedure.
- Description of any of the following:
 - a. texture of anterior and posterior surfaces
 - b. any defect, ulceration or perforation including respective location and measurement
 - c. any opacity, loss of transparency or scars including distribution, shape, colour and measurements
 - d. 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.
- Both button halves should be embedded on their 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 three parallel sections. When only the scleral rim remains, this can be sectioned radially in 4 mm wide pieces to be embedded on their edges.

4.2 Sectioning

- Initially three levels are cut. It may require more careful stepwise approach and saving unstained spare sections if very focal pathology. Some laboratories will cut one section only and do further levels for clarification.
- An initial single section would be acceptable for scleral rims.

4.3 Routine staining

- H&E and PAS

4.4 Further investigations

According to clinical indication or pathological findings:

- special stains for micro-organisms (e.g. Gram, Grocott, Giemsa, ZN, modified ZN. A modified ZN also permits the detection of Nocardia)
- immunohistochemistry/PCR for HSV I and II
- Alcian blue for mucopolysaccharide deposits
- Congo red or Sirius red or immunohistochemistry for amyloid deposits
- Von kossa for suspected dystrophic calcification
- Perls' for haemosiderin or Fleischer ring
- immunohistochemistry (cytokeratins) if suspected posterior polymorphous dystrophy
- immunohistochemistry (e.g. CK3, CK19, CK7, CK12, CK13) for suspected limbal stem cell deficiency
- transmission electron microscopy (EM) – in some instances EM may be indicated for confirmation of a rare dystrophy⁶ or an unusual microorganism (e.g. microsporidium).

4.5 Report content

- Clinical information.
- Biopsy 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.

The level of evidence was grade C and D, or met the GPP/good practice point criteria.

5 Conjunctival biopsies

The main indications for conjunctival biopsies are:

- non-pigmented lesions (e.g. pterygium/pingueculum, cyst, dermoid,/choristoma)
- inflammatory lesions (infectious or non-infectious) including ocular cicatricial pemphigoid
- differential diagnosis of lymphoid lesions.

Caruncular biopsies can also be performed for pigmented or non-pigmented lesions and suspected neoplasia. Incisional mapping conjunctival biopsies consist of multiple biopsies usually performed to estimate the extent of a melanocytic or epithelial intraepithelial neoplasia.^{4,5}

5.1 Specimen submission and dissection

- Specimens are typically received in formalin, except those for direct IF which should be received fresh or in an appropriate transport medium (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 these are taken to allow that they fix flattened.
- Macroscopic examination should include measurements and description of any visible abnormality.
- Margins of excisional conjunctival or caruncular biopsies with appropriate clinically indicated orientation 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.^{7,8}

5.2 Sectioning

- Processed and paraffin embedded tissue: three to four levels. Some laboratories cut just one section initially with further levels for clarification.
- Frozen samples for direct IF: one or two sections per slide.

5.3 Routine staining

- H&E for initial assessment of all sample types.
- Antibodies used on frozen samples for direct IF: IgA, IgG, IgM, C3c and fibrinogen.

5.4 Further investigations

According to clinical indication or pathological findings:

- PAS for assessment of basement membrane and goblet cells
- special stains for micro-organisms (Gram, PAS, Grocott, ZN)
- EVG
- Congo red, Sirius red or immunohistochemistry for amyloid
- Masson Fontana
- immunohistochemistry for differential diagnosis of unexpected malignancy.

5.5 Report content

- 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. cytogenetics, direct IF) if not available locally).
- Conclusion.
- SNOMED codes.

The level of evidence was grade C and D, or met the GPP/good practice point criteria.

6 Trabecular meshwork⁵

6.1 Specimen submission and dissection

Trabecular meshwork samples are usually submitted for histopathological analysis for research purposes. The following instructions are suggested:

- fixation in formalin
- 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
- the specimen can be bisected perpendicularly to the trabecular meshwork.

6.2 Sectioning

- Ten serial sections mounted on five slides.

6.3 Routine staining

- H&E on three of five slides. Two unstained slides can be saved for special stains.

6.4 Further investigations

- Special stains if suspected extracellular material (Masson trichrome, EVG, Alcian blue, PAS).
- Transmission EM.

6.5 Report content

- Clinical information.
- Biopsy dimensions and macroscopic description.
- Microscopic findings.
- Interpretation of findings.
- Any recommendation for supra-regional referral.
- Conclusion.
- SNOMED codes.

The level of evidence was grade C and D, or met the GPP/good practice point criteria.

7 Iris, ciliary body and choroid^{4,9}

7.1 Specimen submission and dissection

The main indication for a non-neoplastic iris, ciliary body or choroidal/retino-choroidal biopsy is for cysts, glaucoma associated anterior chamber syndromes (essential iris atrophy and ICE syndrome) infectious and non-infectious inflammatory pathologies

- The biopsies should generally be fixed in formalin. However, if there is a suspected viral aetiology (e.g. viral retino-choroiditis), a small piece needs to be fixed in glutaraldehyde for transmission electron microscopy. Ensure that in any suspected infectious case, the clinicians send off material for microbiology (culture, PCR/molecular testing) before fixing the specimen.
- These biopsies are quite small and are best placed into sterile Eppendorf tubes containing formalin in theatre and not standard formalin pots.
- After description, the biopsies are best wrapped carefully in paper and then placed in a nylon tissue bag or biopsy wrap or cell-safe, to minimise risk of loss during processing.

7.2 Sectioning

- Serial sections or close levels with many spares in between are recommended, so that focal pathology is not missed.

7.3 Routine staining

- H&E.

7.4 Further investigations

- Gram (bacteria).
- PAS and Grocott (fungi and parasites; macrophages containing liquefied lens material-phacogenic uveitis; vacuolated iris pigment epithelium, thickened ciliary body basement membrane in diabetes, exfoliation material).

- Congo red (amyloid).
- Modified ZN (mycobacteria and nocardia).
- Warthin Starry (spirochetes).
- Perls' (haemorrhage).
- Immunohistochemistry for viruses (herpes virus family).
- PCR on paraffin sections (for infectious agent speciation).
- Transmission electron microscopy (for infectious agents mainly).

7.5 Report content

- Clinical information.
- Biopsy dimensions and macroscopic description.
- Microscopic findings.
- Interpretation of findings.
- Any recommendation for supra-regional referral.
- Conclusion.
- SNOMED code.

The level of evidence was grade C and D, or met the GPP/good practice point criteria.

8 Lens^{10,11,12}

8.1 Specimen submission and dissection

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

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

- Natural lens fragments should be fixed in formalin +/- glutaraldehyde if transmission electron microscopy is required. 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.
- IOL can be submitted dry unless there are attached intraocular contents, in which case formalin is required.
- In the case of an IOL, identify its make (clinical notes or IOL catalogue). Note the integrity of the optics and haptics and the colour and distribution of any opacification noted.

8.2 Sectioning

- In the case of a natural lens tissue, three levels. Some laboratories would cut one initial level only.

8.3 Routine staining

- For natural lens tissue, H&E and PAS.

- IOL can be stained whole with H&E. The 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.

8.4 Further investigations

- Gram, PAS, Grocott, ZN and WF if suspected infection. The whole-mounted IOL can be removed, decolourised of HE and re-stained.
- 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 electron microscopy (especially Alport's syndrome).
- Scanning electron microscopy (for IOLs).

8.5 Report content

- Clinical information.
- Biopsy dimensions and macroscopic description.
- Microscopic findings.
- Interpretation of findings.
- Any recommendation for supra-regional referral.
- Conclusion.
- SNOMED codes.

The level of evidence was grade C and D, or met the GPP/good practice point criteria.

9 Vitreous^{13–16}

9.1 Specimen submission and dissection

Non-neoplastic vitreous samples may be submitted for assessing the presence of a variety of cellular and non-cellular pathologies including: asteroid hyalosis, cholesterol crystals, amyloid, haemorrhage, inflammatory infiltrates (infectious and non-infectious related), infectious agents (bacterial, fungal, viral, parasitic, spirochaetes).

- Vitreous biopsies may be submitted in many ways. The two most common ways in which they are obtained is as a vitreous tap or as a diagnostic vitrectomy specimen.
- It is paramount that the clinicians be informed that in suspected infectious cases, portions of unfixed vitreous be sent for the appropriate microbiological investigations before fixation.
- Vitreous specimens are best fixed in a 'cytology fixative'/preservative fluid, of which there are a wide variety, preferably in theatre.
- Vitreous taps may be received in a capped syringe or in a universal tube or equivalent. They may be unfixed and will require a 'cyto-fix' fluid adding in the histo/cytopathology laboratory.

- Diagnostic vitrectomy specimens are placed into bags or large vitrectomy cassettes in balanced salt solution. These specimens are best fixed promptly in theatre by adding an equal volume of 'cyto-fix' fluid. If the bag or cassette is received unfixed, fixation can be done in the histopathology laboratory with the usual safety precautions.
- Record the volume and consistency of the fluid and any tissue fragments.
- Solid tissue fragments can be removed from the vitreous fluid and fixed in formalin and processed to paraffin wax in the usual way.
- There are many techniques for preparing vitreous specimens. The two most common methods employed include cell block and cytopins.

9.2 Sectioning

- Cytopins are prepared onto glass slides.
- Cell blocks require very careful serial section ribbons with spares cut for ancillary investigations. A useful protocol is to cut 20 serials with H&E at level 10 and 20 to assess the pathology. Further serials may be required.

9.3 Routine staining

- H&E/Giemsa.

9.4 Further investigations

- Congo red (amyloid and sub-typing staining at National Amyloidosis Centre, Royal Free Hospital, if required).
- Gram (bacteria).
- PAS (bacteria (Whipple's disease), fungi, parasites and asteroid hyalosis).
- Silver stains (fungi).
- Warthin starry (spirochaetes).
- Modified ZN (mycobacteria and nocardia).
- Perls' (haemorrhage).
- Immunohistochemistry for herpes family viruses.
- PCR for infectious agents.
- Transmission electron microscopy for infectious agents.

9.5 Report content

- Clinical information.
- Biopsy dimensions and macroscopic description.
- Microscopic findings.
- Interpretation of findings.
- Any recommendation for supra-regional referral.
- Conclusion.
- SNOMED codes.

The level of evidence was grade C and D, or met the GPP/good practice point criteria.

10 Epiretinal membranes^{15,17}

10.1 Specimen submission and dissection:

- Should be fixed in formalin in a vessel much smaller than a conventional formalin pot. An excellent vessel is a sterile Eppendorf tube in which the membrane can be visualised easily. This arrangement needs liaison with the vitreoretinal surgeons and theatre staff.
- These minute samples may have been stained (e.g. using trypan blue) during surgical procedure. When a sample had not been inked upon receipt, two drops of 20% crystal violet solution (or equivalent non-precipitating dye) are added to the Eppendorf tube. Adding too much dye will make it difficult to visualise the membrane. Leave the membrane to take up the dye for a minimum of two hours.
- Under a dissecting scope, remove the dyed membrane from the Eppendorf tube with fine forceps and place on a small square piece of tracing paper within a pencilled circle. Fold the tracing paper over the membrane securely and place it into a processing bag and then into a cassette.
- If no tissue is seen after addition of dye, check around lid of Eppendorf tube. If still not visualised, use a cell block technique to spin down contents.
- Routine overnight processing required.

10.2 Sectioning

- A serial ribbon is cut. Ensure tissue is cut into by visualising the tissue on the slide before staining.

10.3 Routine staining

- H&E and PAS. The PAS allows for visualisation of native inner limiting membrane neo-basement membrane, vessels, fungi and photoreceptor outer segments.

10.4 Further investigations

- Further levels if first set of serials reveal no tissue.
- Immunohistochemistry: CK7 (retinal pigment epithelium, RPE), CD31/CD34/factor VIII (neo-vessels), CD68/CD45 (macrophages and lymphocytes), GFAP for astroglial elements, smooth muscle actin for myofibroblasts, collagen 4 for neo-basement membrane.

10.5 Report content

- Clinical information
- Biopsy dimensions after dye.
- Typing of epiretinal membrane.

The following is a simple classification scheme for epiretinal membranes:¹⁵

- simple (surface wrinkling retinopathy membranes)
- complex, which can be divided into ischaemic type (diabetic or vein occlusion) or those occurring in the setting of a retinal hole (proliferative vitreoretinopathy or macular holes)
- idiopathic epiretinal membranes
- inflammatory epiretinal membranes.

- Subretinal proliferative vitreoretinopathy (PVR) – cores or lengths of PAS positive basement membrane surrounded by a cuff of RPE cells.

Subretinal neovascular membranes (SRNVM) membranes can be difficult to sub-classify neatly into type 1 and type 2, particularly as the RPE can migrate away from its normal location onto the underside of a subretinal membrane. Most will comprise RPE cells with varying degrees of metaplasia, Bruch's membrane, neo-basement membrane, degenerate and viable outer photoreceptor segments and inter-photoreceptor matrix and varying content of inflammatory cells, erythrocytes, haemosiderin laden macrophages, sub-RPE deposits, vessels¹⁵.

The level of evidence was grade C and D, or met the GPP/good practice point criteria.

11 Enucleations^{18,19,20}

11.1 Specimen submission and dissection:

This protocol below does not cover the ophthalmic pathology of paediatric non-accidental injury cases.

Enucleations require fixation in at least 60 ml of 10% neutral buffered formalin for at least 24 hours prior to dissection. Windows should not be cut into enucleation specimens.

First, orientate the eye and confirm the specimen corresponds with the clinical details on the form, i.e. right or left eye. Several factors assist in the orientation of the eye:

- ciliary arteries: these indicate the horizontal plane
- superior oblique muscle: fine tendinous insertion into superotemporal pole
- inferior oblique muscle: thick muscular insertion posterotemporal side, below the temporal long ciliary artery
- cornea: is normally longer horizontally than vertically
- optic nerve: lies closer to the nasal than temporal limbus.

Before any cuts are made into the eye, the maximum dimensions of the globe are measured in the following order:

- antero-posterior (normal 22–23 mm)
- horizontal (normal 22–23 mm)
- vertical (normal 22–23 mm).

An increase in anterior-posterior dimensions (28–30 mm) indicates axial myopia, or glaucomatous enlargement due to uveo-scleral bulging (staphyloma formation). Isolated or multiple staphylomata can also increase the horizontal and vertical dimensions.

A decrease in ocular dimensions (15–18 mm) indicates senile atrophy of the globe and shrinkage (phthisis or atrophía), which occurs after prolonged loss of pressure in the eye. Ocular hypotonia occurs after inflammatory damage to the ciliary processes or leakage of intra-ocular fluids through a defect in the cornea-scleral envelope.

Measure the cornea. A normal cornea measures approximately 12 mm horizontally and 11 mm vertically. Make a note of the shape of the cornea.

Comment on the transparency of the cornea. The limbus should have an even surface and limbal blood vessels do not normally pass into the corneal stroma, except superiorly where a superficial degenerate pannus can develop with age.

Comment on the following.

- Is there epithelial separation?
- Is there evidence of pyogenic ulcer or perforation?
- Are there any crescentic opaque scars (often found at the superior limbus where surgery is most common, and sutures that are often buried and almost invisible)?
- Is there a filtration bleb of glaucoma surgery is often found at the limbus between 11 and 1 o'clock?
- Is there any stromal pigmentation? Brown pigmentation is most commonly due to blood staining, however brown staining of the inner corneal surface is usually due to adherent uveal melanin.
- Is there Arcus lipidis in the stroma immediately internal to the limbus?
- Is there a superficial white oval area below the horizontal line? This is probably caused by post-inflammatory deposition of calcium salts in the superficial cornea and is known as 'band keratopathy'.
- Is there any stromal vascularisation, particularly at the periphery (pannus)?
- Are there any perforations?
- Is there an infiltrate?
- Are there deposits in the cornea?
- Any other unusual features should be noted.

Normally the anterior chamber is deep and contains clear colourless aqueous humour.

Look for:

- shallow chamber
- blood – hyphaema following trauma
- pus – hypopyon following infection
- tumour – pseudohypopyon in retinoblastoma
- iris defects. Ectropion of the iris pigment epithelium: a manifestation of a neovascular membrane on the anterior iris surface (*rubeosis iridis*) and often indicates diseases that cause retinal ischaemia (e.g. diabetes, inflammation and intra-ocular tumours).

The conjunctiva consists of a small rim remaining around the cornea. The features to observe and record are:

- congested vessels
- pigmented/non-pigmented tumours
- sutures
- fibrosis
- cysts
- pterygium and pingueculum.

Episclera and sclera

The features to observe and record are:

- sentinel vessels
- staphylomas (thinning and stretching)
- trauma wounds
- fibrosis
- thinning
- surgical intervention
- sutures: record type, colour, number and status
- transcleral spread of intraocular tumour
- previous surgery could include: vitrectomy ports, plastic indentation bands, explants, valves, seton tubes, biopsy/resection scars and tantalum discs from proton beam therapy.

Describe the shape, size and location of the features in relation to clock hours and in relation to fixed anatomical points (limbus, optic nerve).

A sclerotomy may be present representing tumour sampling prior to fixation; note its location and size.

Pupil

- measure the size
- note the position
- note distortions in shape, size and position; this assessment is aided by retro-illumination.

Iris

Look for:

- ectropian uveae
- entropian uveae
- rubeosis iridis
- atrophy
- iridectomy sites.

Optic nerve

Normally the optic nerve measures 4 mm diameter.

- Measure length and diameter.
- Note features of interest, i.e. atrophy with associated meningeal redundancy, demyelination, infarction and tumour.
- Sample the nerve by cutting a 3–4 mm length of tissue, perpendicular to the length. This can easily be achieved by resting the nerve on the edge of the dissection board and making a clean cut.

Place the tissue in Cassette A.

If possible, leave a small length of nerve after sampling to assist in microtomy. If this is not possible, ink the optic nerve so that it is easier to detect it during microtomy.

Vortex veins*

- Locate the vortex veins. Vortex veins are located approx 6–8 mm from the optic nerve and arranged in the four quadrants of the eye at 45° to the vertical/horizontal planes.
- Blood clot or other soft tissues may obscure the veins, and require dissection with fine forceps.
- The canals for the veins are oblique slits.
- Cut the veins flush to the sclera. Place them into moistened tissue wrap and a tissue bag and place into Cassette B. Embed longitudinally.
- If the veins are not identified, a slice of sclera along the canal is removed, so as to de-roof the intra-scleral part of the vortex vein. Often, the de-roofed vein is attached to the sclera and comes away with it. If not, the vein can be carefully lifted out and embedded longitudinally.

* Whilst vortex veins are sampled in cases of intraocular malignancy, outside the remit of this non-neoplastic tissue pathway, they are included in case an unexpected intraocular malignancy is encountered.

- Palpation: gently palpate the globe with the thumb and fingers.
- Assess the tension of the globe surface.
- Globally firm, e.g. glaucoma, intraocular calcification, massive intraocular haemorrhage.
- Globally soft, e.g. atrophica bulbi.
- Focally firm, e.g. focal mass lesions.
- Focally soft, e.g. staphylomas.

Trans-illumination/retro-illumination of the globe

Trans-illumination detects the presence of intraocular masses or areas of lucency.

- Position a bright light source (fibre optic) adjacent to the cornea.
- The eye will glow red due to the highly vascularised choroid.
- Any thickened areas (intraocular lesions) cast a shadow.
- Using a permanent ink marker pen, draw a line representing the location of the shadow on the outer scleral surface. This helps assist in the orientation of the first cut into the globe.
- Any areas of stromal thinning will allow the passage of increased light and will appear brighter.

Retro-illumination is when the light source is placed alongside the optic nerve on the sclera. It is used to detect iris pathology and to observe pupil alterations.

Radiological examination of the globe

Radiological examination may be used for the identification of a foreign body, or foci of calcification within the specimen. This simplifies localisation and removal of the foreign body or

signifies that decalcifying fluids should be used prior to cutting the histological sections. The location of an intraocular radio-opaque area is assisted by the insertion of a needle into the episclera prior to taking the X-rays, which should be done in two or more planes. It is then possible to achieve a section that illustrates all the relevant features.

Once all the external measurements of the globe have been made, it is time to assess where the first cut is made. The plane of sectioning is dependent on the findings of external examination and trans-illumination.

There are three important factors to consider:

- vertical cuts for superior and inferior located pathology
- horizontal cuts for macular pathology and horizontally located pathology
- oblique cuts may be used to include areas of particular interest.

The position of the first cut may be marked with a permanent marker pen on the scleral surface. The cut should begin 2 mm from the edge of the optic nerve and end 1 mm inside the rim of the cornea.

- Commence the first cut with a skin graft blade, using a gentle smooth action.
- The cut should travel from posterior pole towards the corneal rim to prevent dislocation of the lens.
- Once you have sliced through the eye, retain the loose piece of eye (the cap) as this may also need processing.
- The second cut should be parallel to the first, in an identical manner, along the opposite side of the eye. This block is known as the PO (pupil-optic nerve) block.
- Again retain the cap and process if necessary.

Internal examination

- Assess the anterior chamber angle. Is it open or closed?
If open, look for blood, abnormal blood vessels, pigment deposits and tumour.
- Lens – is it present or absent? Is the lens natural or artificial?
If it is artificial, do not remove as it will process effectively.
Look for decentration and bag opacification.
If a natural lens, look at the size, shape and colour and assess for cataracts.
- Ciliary body – look for effusions and cryotherapy marks and tumours.
- Vitreous – assess the consistency and texture and colour.
Comment on any unusual components within the vitreous.
- Retina – is it attached or detached?
A true retinal detachment shows pigment disturbance under the neural retina with or without subretinal exudate (that appears as clear yellow-brown gelatinous material).
Also look for:
 - retinal haemorrhages
 - yellow exudates
 - cotton wool spots
 - pigment disturbances
 - on the retinal surface look for white band-like or confluent white areas with irregular edges +/- pigment indicating wrinkling of retinal surface (epiretinal membranes)

- examine vessels for: silver wiring, emboli, ghost profiles and peri-vascular fluffiness (vasculitis)
- macula: oedema, holes, haemorrhage, disciform scars and drusen
- optic disc: papilloedema, atrophy, cupping and vascularisation.
- Choroid; haemorrhage, exudates, bone, white/grey areas indicating inflammation.

Embedding options

- Vortex veins should be embedded horizontally.
- Main globe block should be embedded in a deep mould
- Calottes may be embedded into either deep moulds or normal moulds, depending on the thickness of the tissue.
- IOLs, and retinal detachment external silicon bands will process and will be able to cut on a standard microtome blade. However, devices such as Ahmed valves will not process and will require removal.

11.2 Sectioning

This depends on the pathology under consideration. Usually, three levels through the PO block and three levels through callotes. However, a careful stepwise approach may be required if the pathology is very focal.

11.3 Routine staining (which may be adapted according to the clinical circumstances of case)

H&E, PAS (useful for all intra-ocular basement membranes and vessels) and Perls' (for detecting old haemorrhage, especially within the iris, vitreous and retina in the setting of rubeosis, vitreous haemorrhage, chronic, ischemic retinopathy (pre-proliferative or proliferative) and for siderosis.

11.4 Further investigations depending on initial results

- Further levels (suspected sympathetic ophthalmia).
- Infectious agent tinctorial stains (Gram, Grocott/MST, modified ZN).
- Immunohistochemistry (limbal stem cell deficiency, unexpected tumour, reactive cellular proliferations).
- Transmission electron microscopy if needed.
- PCR for infectious agents.

11.5 Report content

- Clinical information.
- Globe and nerve dimensions.
- Macroscopic findings.
- Microscopic findings.
- Interpretation of findings – the key in any enucleation is to exclude unsuspected malignancy and sympathetic ophthalmia.
- Recommendations for supra-regional referral.
- Conclusion.
- SNOMED codes.

The level of evidence was grade C and D, or met the GPP/good practice point criteria.

12 Eviscerations^{4,18,19,20}

Evisceration is carried out when the eye is blind and painful, from traumatic (non-surgical and repeated surgical procedures), non-infectious inflammatory and infectious inflammatory aetiologies when there is no prospect of saving sight.

The specimen will consist of usually a cornea with a scleral ring, with intraocular contents in varying quantities and condition, leaving the scleral shell in the patient.

12.1 Specimen submission and dissection

- Fix in formalin for 24 hours.
- Corneo-scleral ring is measured and any obvious pathology noted (e.g. perforation, vascularisation, band keratopathy, inflammatory infiltrates/abscess, dystrophic deposits, graft-donor interfaces, lamellar procedures). Bisect with consideration of keeping behind a central strip for ancillary investigations (transmission electron microscopy). See section 4, 'Cornea specimens'.
- Examine contents for natural lens tissue or intraocular lens prosthesis (IOL). Comment on damage or cataract changes. Comment on any pathology involving the IOL.
- The evisceration sac contents usually appear as clotted blood with disorganised iris, ciliary body, vitreous, retina and choroid. Record which tissues are identified and if possible the gross pathological alteration. Usually the sac can be bisected or gently bread sliced if the sac has been neatly removed by the surgeon. If the contents are scrappy fragments, place them without slicing into a cassette.
- Submit all tissue for examination.
- Decalcification may be required. IOLs will process and will be able to cut on a standard microtome blade.
- Embed cornea and evisceration sac in separate cassettes.

12.2 Sectioning

- Three levels on cornea and evisceration sac. May require more careful stepwise approach if very focal pathology. Some laboratories may cut a single initial level.

12.3 Routine staining (which may be adapted according to the clinical circumstances of case)

- Cornea: three levels H&E with PAS.
- Lens: H&E, PAS.
- Contents: three levels H&E, PAS, Perls'. The Perls' is for detecting old haemorrhage, especially within the retina in the setting of a chronic, ischaemic retinopathy (pre-proliferative or proliferative) and siderosis.

12.4 Further investigations depending on initial results:

- Further levels through cornea or sac contents (suspected sympathetic ophthalmia).
- Infectious agent tinctorial stains (Gram, Grocott/methenamine silver technique (MST), modified ZN).

- Immunohistochemistry (limbal stem cell deficiency, unexpected tumour).
- Transmission EM.

12.5 Report content

- Clinical information.
- Specimen dimensions.
- Microscopic findings.
- Interpretation of findings – the key in any evisceration is to exclude unsuspected malignancy and sympathetic ophthalmia.
- Recommendations for supra-regional referral.
- Conclusion.
- SNOMED codes.

The level of evidence was grade C and D, or met the GPP/good practice point criteria.

13 Orbital and lacrimal gland biopsies^{9,20}

Non-neoplastic biopsies of the orbit and lacrimal gland are received for the diagnosis of inflammatory pathologies. The biopsies may be excisional or incisional.

13.1 Specimen submission and dissection

- Most orbital biopsies require overnight fixation in formalin.
- Description of size, shape, colour, texture of tissue.
- Inking margins if an excisional biopsy.
- Slice the specimen taking into consideration the key margins if an excision.

Embedding:

- Ensure key margins are embedded into separate cassettes.

13.2 Sectioning

- Excision biopsies – three levels per block. Some laboratories may cut a single initial level.
- Incision biopsies – depends on size of tissue. May have to cut serials or three careful levels

13.3 Routine staining

- H&E.

13.4 Further investigations depending on initial results.

- Elastin and Perls' stains in suspected vasculitis cases.
- Gram, Grocott, PAS, ZN, Wade-Fite for suspected infectious pathology.
- Tinctorial or immunohistochemical stains for orbital amyloid deposits.
- Immunohistochemistry, e.g. for suspected IgG4 related disease.

13.5 Report content

- Clinical information.
- Biopsy dimensions.
- Microscopic findings.
- Interpretation of findings – lymphoid hyperplasia can progress to orbital lymphoma. This should be mentioned in the report as this will trigger careful follow up. Not to over-interpret the significance of a low level of IgG4 positive plasma cells. A diagnosis of IgG4 related disease depends on a combined approach of assessing the clinical presentation, serological data, histological changes and immunohistochemical criteria for IgG4 cut-offs of significance.
- Recommendations for supra-regional referral.
- Recommendations for re-biopsy.
- Conclusion.
- SNOMED codes.

The level of evidence was grade C and D, or met the GPP/good practice point criteria.

14 Orbital exenteration^{9,18,19,20}

Non-neoplastic exenterations may be partial or complete.

The clinical indications for a non-neoplastic exenteration include uncontrollable orbital inflammatory conditions such as fibrotic phase vasculitis.

14.1 Specimen submission and dissection

- Exenterations require around 48 hours for adequate fixation.
- Complete exenterations comprise of globe, upper and lower lids, optic nerve, extraocular muscles, orbit fat, periosteum and lacrimal gland. Incomplete exenterations usually are anterior eyelid skin sparing for cosmetic reconstruction.
- The anterior surface of the specimen will be eyelids. The upper lid shows longer lashes, is longer in the vertical plane and shows the lid crease.
- The medial part of the exenteration is identified by the caruncle and puncta. The lacrimal sac and proximal nasolacrimal duct comprise the antero-medial soft tissues. The surgeons often mark the proximal cut end of the nasolacrimal duct with a suture.
- Determine whether a complete or partial exenteration. Measure specimen in three dimensions.
- The eyelid skin and optic nerve length and diameter are recorded. Any obvious externally observed tumour is described in terms of shape, colour, size consistency and relationship to anatomical landmarks.
- The lids are prized open and the ocular surface is described.
- After the external description, the specimen is painted.
- The basic approach to slicing is to vertically bread-slice the specimens from side to side, generating even thickness slices from 5 to 10 mm. If required, the medial and lateral slices can be horizontally serially sliced to examine the distance of the lesion from these margins. Slicing gauges may be used to facilitate even thickness slice production.

- Describe the colour, shape, texture, size, location, relationships and proximity to surgical margins.
- Describe relevant intraocular pathology (as per enucleations protocol see above).

Embedding

Jumbo blocks are useful, with inlay sponges. The sponges stop the slices bending during processing.

14.2 Sectioning

Ensure that full faces are achieved.

14.3 Routine stain

H&E, PAS.

14.4 Further investigations depending on initial results

- Elastin and Perls' stains in suspected vasculitis cases.
- Gram, Grocott, PAS, ZN, Wade-Fite for suspected infectious pathology.
- Tinctorial or immunohistochemical stains for orbital amyloid deposits.
- Immunohistochemistry, e.g. for suspected IgG4 related disease.

14.5 Report content

- Clinical information.
- Specimen dimensions.
- Microscopic and macroscopical findings.
- Interpretation of findings: not to over-interpret the significance of a low level of IgG4 positive plasma cells. A diagnosis of IgG4 disease depends on a combined approach of assessing the clinical presentation, serological data, histological changes and immunohistochemical criteria for IgG4 cut-offs of significance.
- Recommendations for supra-regional referral.
- Recommendations for re-biopsy.
- Conclusion.
- SNOMED codes.

The level of evidence was grade C and D, or met the GPP/good practice point criteria.

15 Cytology^{13,14,21}

The main applications of cytological investigations in ocular pathology include the diagnosis of limbal stem cell deficiency and detection of intraocular inflammation +/- infectious agents.

15.1 Specimen fixation

Impression cytology for suspected limbal stem cell deficiency is usually carried out using 10 mm diameter impression discs mounted on a plastic ring mount that are then placed in neutral buffered formalin.

Intraocular fluids can be fixed in a variety of cytology media of choice depending on local preference.

If intraocular cytology specimens are received unfixed, liaise with the ophthalmologist to ensure that samples have been sent for PCR and culture if an infectious aetiology is suspected.

Immunohistochemistry is performed on cytology impression discs whilst still attached to the plastic ring mounts.

Intraocular cytology fluids are cell blocked as they are small specimens and benefit from cellular concentration.

15.2 Sectioning

Impression cytology discs are removed from the plastic ring mounts and mounted whole and cover-slipped in a conventional way.

Cell blocks require careful step-wise sectioning, preferably serial sections with spares cut for ancillary investigations.

15.3 Routine stains

Impression cytology:

- corneal cells – CK3, CK12 immunohistochemistry
- conjunctival cells: CK7, CK13, CK19.

Cell blocks – H&E or Papanicolaou or Giemsa, depending on local preference.

15.4 Further investigations depending on initial results.

Cell blocks:

- Congo Red for vitreous amyloid
- PAS, alcian blue for asteroid hyalosis
- Gram, ZN, Wade-Fite, PAS, Grocott for intraocular infectious agents
- Perls' stain for old haemorrhage.

15.5 Report content

- Clinical information.
- Fluid volume/details of membranes received.
- Microscopic findings.
- Interpretation of findings.
- Recommendations for supra-regional referral.
- Recommendations for re-biopsy.
- Conclusion.
- SNOMED codes.

The level of evidence was grade C and D, or met the GPP/good practice point criteria.

16 Lacrimal sac biopsies/excision^{9,19,20}

Lacrimal sac contents may be submitted from a dacryolith or from an infective nidus (usually an actinomycetoma).

Whilst a lacrimal sac biopsy is usually performed for the exclusion of neoplasia, most show changes of chronic dacryocystitis.

Excision of non-neoplastic lacrimal sacs are performed by chronic obstruction from recurrent bouts of dacryocystitis.

16.1 Specimen submission and dissection.

Specimens require fixation in neutral buffered formalin.

Routine overnight processing.

For whole lacrimal sac, the structure will comprise a fusiform swelling with a proximal and distal part, the latter being identified by the presence of the naso-lacrimal duct margin. After taking the naso-lacrimal duct margin, the sac is serially sliced into a series of discs.

16.2 Sectioning

- For biopsies, three levels with spare sections for ancillary investigations. Some laboratories may cut a single initial level.

16.3 Routine stains

- H&E

16.4 Further investigations depending on initial results

- Gram, Grocott, PAS, ZN, Wade-Fite for suspected infectious pathology.
- Tinctorial or immunohistochemical stains for amyloid deposits.

16.5 Report content

- Clinical information.
- Biopsy dimensions.
- Microscopic findings.
- Interpretation of findings.
- Recommendations for supra-regional referral.
- Recommendations for re-biopsy.
- Conclusion.
- SNOMED codes.

The level of evidence was grade C and D, or met the GPP/good practice point criteria.

17 Criteria for audit of tissue pathway

Content and timeliness of histopathology reports should be audited against the recommendations in these guidelines.

The following are recommended by the RCPATH as key performance indicators (KPIs) – see *Key Performance Indicators – Proposals for implementation* (July 2013) on www.rcpath.org/clinical-effectiveness/kpi/KPI:

- Cancer resections must be reported using a template or proforma, including items listed in the English COSD which is, by definition, core data items in RCPATH cancer datasets. English Trusts were required to implement the structured recording of core pathology data in the COSD by January 2014.

Standard: 95% of reports must contain structured data.

- Histopathology cases that are reported, confirmed and authorised within seven and ten calendar days of the procedure.

Standard: 80% of cases must be reported within seven calendar days and 90% within ten calendar days.

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Appendix A Summary table – Explanation of grades of evidence

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

Grade (level) of evidence	Nature of evidence
Grade A	<p>At least one 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 cancer type</p> <p>or</p> <p>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.</p>
Grade B	<p>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 cancer type</p> <p>or</p> <p>Extrapolation evidence from studies described in A.</p>
Grade C	<p>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 cancer type</p> <p>or</p> <p>Extrapolation evidence from studies described in B.</p>
Grade D	<p>Non-analytic studies such as case reports, case series or expert opinion</p> <p>or</p> <p>Extrapolation evidence from studies described in C.</p>
Good practice point (GPP)	<p>Recommended best practice based on the clinical experience of the authors of the writing group.</p>

Appendix B AGREE compliance monitoring sheet

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

AGREE standard	Section of tissue pathway
SCOPE AND PURPOSE	
1. The overall objective(s) of the guideline is (are) specifically described.	Foreword
2. The clinical question(s) covered by the guidelines is (are) specifically described.	Foreword, 1
3. The patients to whom the guideline is meant to apply are specifically described.	1
STAKEHOLDER INVOLVEMENT	
4. The guideline development group includes individuals from all the relevant professional groups.	Foreword
5. The patients' views and preferences have been sought.	n/a*
6. The target users of the guideline are clearly defined.	1
7. The guideline has been piloted among target users.	Foreword
RIGOUR OF DEVELOPMENT	
8. Systematic methods were used to search for evidence.	Foreword
9. The criteria for selecting the evidence are clearly described.	Foreword
10. The methods used for formulating the recommendations are clearly described.	Foreword
11. The health benefits, side effects and risks have been considered in formulating the recommendations.	Foreword
12. There is an explicit link between the recommendations and the supporting evidence.	Throughout
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 are clearly presented.	Throughout
17. Key recommendations are easily identifiable.	Throughout
18. The guideline is supported with tools for application.	2–16
APPLICABILITY	
19. The potential organisational barriers in applying the recommendations have been discussed.	Foreword
20. The potential cost implications of applying the recommendations have been considered.	Foreword
21. The guideline presents key review criteria for monitoring and/audit purposes.	17
EDITORIAL INDEPENDENCE	
22. The guideline is editorially independent from the funding body.	Foreword
23. Conflicts of interest of guideline development members have been recorded.	Foreword

* The Lay Advisory Committee (LAC) of The Royal College of Pathologists has advised that there is no reason to consult directly with patients or the public regarding this tissue pathway because it is technical in nature and intended to guide pathologists in their practice. The authors will refer to the LAC for further advice if necessary.