



Tissue pathways for non-neoplastic neuropathology specimens

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Comments	This is an abridged review that supersedes the 2020 edition of <i>Tissue pathways for non-neoplastic neuropathology specimens</i> . All amendments made are minor and reflect slight technical advances, obsolescence of certain antibodies and stains, and change in practice, but none of those can be considered as major. Changes made are in sections 2.4, 3.5, 4.6, 5, 5.7, 6.6, and 19. In accordance with the College's pre-publications policy, this document was on the Royal College of Pathologists' website for consultation from 18 January to 1 February. Responses and authors' comments will be available to view on publication of the final document. Dr Brian Rous, Clinical Lead for Guideline Review (Cellular Pathology)



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NICE has accredited the process used by the Royal College of Pathologists to produce its tissue pathways. Accreditation is valid for five years from 25 July 2017. 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. 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:

- The British Neuropathological Society
- The British Paediatric Neurology Association
- The British Myology Society
- The British Peripheral Nerve Society
- The Association of British Neurologists
- The Society of British Neurological Surgeons.

No major organisational changes or cost implications have been identified that would hinder the implementation of the tissue pathway. The information used to develop this tissue pathway was collected from electronic searches of the medical literature, previous recommendations of the RCPATH and local guidelines in the UK. PubMed searches up to June 2019 in English language were included. Search results related to non-human samples were excluded. Published evidence was evaluated using modified SIGN guidance (see Appendix A). Consensus of evidence in the tissue pathways was achieved by expert review. Gaps in the evidence were identified by College Fellows via feedback received from consultation. The sections of this tissue pathway that indicate compliance with each of the AGREE II standards are indicated in Appendix B.

A formal revision cycle for all tissue pathways takes place on a five-yearly basis. However, each year the College will ask the author(s) of the tissue pathways, in conjunction with the relevant subspecialty advisor 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 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 publications page of the College website.

The pathway has been reviewed by the Clinical Effectiveness team, Working Group on Cancer Services and Lay Advisory Group and was placed on the College website for consultation with the membership from 18 January to 1 February. 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 Clinical Effectiveness team and are available on request. The authors have declared no conflicts of interest.

1 Introduction

This document covers tissue pathways for specific areas within diagnostic neuropathology. The guidance in the previous editions of the *Tissue pathways for non-neoplastic neuropathology specimens* has been piloted in laboratory practice, either by the authors or in the laboratories of those responding to the consultation.

As with many areas of diagnostic cellular pathology, evidence for these recommendations is not based on systematic, controlled studies. Such evidence, classified as good practice points (GPP; see Appendix A), is typically expert opinion without explicit critical appraisal or is based on physiology, bench research or 'first principles'. In view of this, references cited in support of the evidence are mainly drawn from textbooks.

There are few studies that document specificity and sensitivity for the range of diagnoses being considered in this area of diagnostic neuropathology. Users of these guidelines are recommended to consult PubMed to see if any original publications exist in cases where knowing the specificity and sensitivity of a biopsy technique would influence a clinical decision to undertake a procedure.

Neoplastic neuropathology is covered in RCPATH's *Dataset for histopathological reporting of tumours of the central nervous system in adults, including the pituitary gland (5th edition)*.¹

1.1 Target users and health benefits of this guideline

The target primary users of the tissue pathway are trainee and consultant neuropathologists, general histopathologists and, on their behalf, the suppliers of IT products to laboratories. The secondary users are neurologists, surgeons, oncologists, rheumatologists, general practitioners, cellular pathology laboratory managers and biomedical scientists. Health benefits for patients are realised through standardisation of examinations, use of agreed test portfolios and thus interchangeability of samples, preventing unnecessary additional interventions. Health benefits for laboratory personnel are realised through evidence-based laboratory safety precautions and guidelines for decontamination.

1.2 Staffing and workload

Central nervous system (CNS) biopsies should be reported by neuropathologists or histopathologists with a special interest in neuropathology who participate in the UK national neuropathology external quality assessment (EQA) scheme.

Muscle biopsies should be processed by accredited departments that participate in the national muscle technical EQA scheme.

Ideally, two or more neuropathologists in a unit should have the requisite skills, knowledge and externally validated diagnostic competence to provide continuity of service for periods of leave. In centres that have only one neuropathologist, arrangements with other units should be agreed to allow continuity of service, making use of telepathology if available and appropriate.

The minimum and maximum number of neurosurgical biopsies for maintenance of diagnostic accuracy, without risking errors, currently lacks an evidence base. All procedures should be carried out in adequately equipped and staffed centres by teams with experience in removing, handling and processing these samples.

Any on-call service offered to support neurosurgery should be staffed by neuropathologists who contribute to the routine neuropathology service and hold EQA evidence of competence.

1.3 Laboratory and workplace facilities

Routine light microscopy, immunohistochemistry, special histochemistry and access to electron microscopy (EM) are all required for a comprehensive service encompassing nerve, muscle and CNS (brain, spinal cord and meningeal) biopsies. There should be facilities to review and discuss radiological findings with local specialists in this area.

There should be facilities for storage and archiving of fresh-frozen material at -80°C or in liquid nitrogen. Furthermore, there should be facilities and resources to enable the transport of samples on dry ice using courier services for specialised investigation.

Processing of unfixed 'high-risk' specimens (e.g. cytospin, intraoperative smear and frozen sections or routine frozen sections such as muscle biopsies) and any decisions on whether to provide a service dealing with such material should be based on local risk assessments, the availability of appropriately trained staff and any necessary laboratory containment facilities.

1.4 Specimen submission: general considerations

Biopsies should be accompanied by adequate clinical, laboratory and radiological information to focus the differential diagnosis and choice of special investigations. All National Specialised Commissioning Team (NSCT; formerly National Commissioning Group [NCG] or National Specialised Commissioning Group [NSCAG]) referrals must be accompanied by a current NSCT form (completed by the referring clinician), up-to-date clinic letters, prior biopsy reports where relevant and all relevant available information pertaining to the case.

Further details and forms are available to download from the websites of the NSCT centres:

- [Dubowitz Neuromuscular Centre, Great Ormond Street Hospital and the UCL Institute of Child Health](#)
- [John Walton Muscular Dystrophy Research Centre, Newcastle University](#)
- [Rare Mitochondrial Disorders Service, Newcastle.](#)

Peripheral nerve and muscle biopsies should include:

- information about the biopsy site and laterality
- details of onset, distribution and progression of the disorder
- involvement of other organs
- results of nerve conduction studies and electromyography
- drug history
- occupational exposure(s)
- past and current personal and family medical history
- plasma creatine kinase (CK) level (essential for muscle biopsy)
- plasma and cerebrospinal fluid (CSF) lactate levels (muscle biopsies) and other biochemical investigations
- results of brain, spinal cord and adjacent nerve root or muscle magnetic resonance imaging (if performed and as relevant).

When applicable, specimens and their request sheet should be labelled with a 'Risk of infection' sticker.

Specimens should be delivered to the laboratory in the appropriate state for the intended

investigations, for example:

- CSF for cytology: within one hour of being taken²
- fresh brain: within one hour of being taken. No specific guidelines exist for brain biopsies and we suggest recommendations for other cancer biopsies are followed.³
- nerve (see section 3)
- muscle biopsies must be transported immediately to the lab fresh at room temperature, either wrapped in gauze that is lightly moistened in saline or wrapped in cling film to prevent drying out. For histopathological studies, a total transit time of less than two hours from the time of collection is acceptable for samples. Samples with a transit time exceeding two hours must be frozen on site and transported on dry ice. Samples for EM should be fixed in glutaraldehyde within 15 minutes of being taken. Samples collected for mitochondrial respiratory chain enzyme analysis must be frozen immediately (at the bedside or in theatre) or within minutes to avoid loss of enzyme activity.^{4,5}
- skin and chorionic villus samples (CVS) for culturing should be transported to the lab in sterile standard culture medium at room temperature within 24 hours
- skin samples for histopathological studies should be immediately transported to the lab fresh
- CVS samples for histopathology should be transported in sterile standard transport medium at room temperature within 24 hours.

1.5 Specimen dissection: general considerations

Handling should be appropriate to the category of risk indicated and in line with local guidelines and health and safety regulations (see sections 1.3 and 1.4). In practice, intraoperative frozen sectioning of known or suspected Hazard Group 3 pathogens, such as *Mycobacterium tuberculosis*, should be avoided. Given that a proportion of biopsied lesions from the CNS turn out to be undiagnosed infective conditions, intraoperative diagnostic material should be handled as a potential category 3 pathogen risk until proven otherwise. A risk assessment should be performed on a case-by-case basis and local policy should inform of the procedure how to handle samples from patients with infection diseases, such as HIV or MRSA. Please see section 5 regarding prion disease.

2 Skeletal muscle

2.1 Specimen submission, dissection and preparation

The majority of specimens in a clinical neurosciences setting will be delivered fresh (unfixed) to the laboratory.

2.1.1 Risk assessment

When there is a risk of infection specimens should be risk assessed for frozen processing. In all other situations, the recommended practice is to examine snap-frozen tissue with the optional addition of a formalin-fixed specimen, if there is sufficient material. Risk assessment for transmissible infections is performed by the clinical team prior to the procedure and high-risk biopsies should be flagged up with the laboratory as they will be processed differently (formalin fixation, see below).

2.1.2 Data recording

Record the condition in which the sample was received, sample size (recommended ≥ 50 mm³), and/or weight, biopsy site and the interval between removal of the tissue from the patient and freezing. This information is essential for the correct interpretation of histochemical and biochemical (respiratory chain) data.

2.1.3 Laboratory procedures

The sample is subdivided into smaller pieces for the following procedures:

- histology (to be frozen in isopentane, cooled in liquid nitrogen to -160 C°)
- protein, biochemical and molecular genetic studies (snap-frozen in liquid nitrogen)
- EM (to be fixed in 4% glutaraldehyde in 0.1 M cacodylate buffer).

Needle biopsies yield smaller pieces and will require orientation under a dissecting microscope such that the myofibers are in a transverse plane for cryostat sectioning.

Individual pieces of muscle selected for freezing for histological analysis should not exceed 8 mm^3 , since larger pieces may freeze slowly risking the introduction of freezing artefacts.

Routine sampling of a portion of fresh muscle for formalin fixation and paraffin embedding (FFPE) is currently practiced in several laboratories. Preferably, the majority of the sample would be frozen and only a small portion of the sample fixed in formalin. FFPE of muscle has numerous disadvantages, including loss of biochemical enzyme activities, DNA/RNA and protein degradation, suboptimal morphology, loss of tinctorial staining properties of inclusions and protein aggregates and loss of immunoreactivities due to epitope masking and protein cross-linkages. In the current era of genomic, transcriptomic and proteomic testing for inherited neuromuscular disorders, there is considerable requirement for fresh tissues and FFPE material is suboptimal for most of these tests. In this environment, routine fixing of fresh muscle tissue in formalin is discouraged, except when the specimen is considered high risk or when it would be impractical to freeze the sample in its entirety due to its size. For the latter, consider formalin fixation of residual muscle only after preparing several additional snap-frozen blocks.⁶

2.2 Embedding options

- Frozen sectioning: water-based mountant (e.g. optimal cutting temperature [OCT medium]) with sample orientated transversely.
- Semi-thin and EM: epoxy resin, with at least some samples longitudinally orientated.
- FFPE.

2.3 Sectioning

- Frozen tissue: cryostat at $8\text{--}12\text{ }\mu\text{m}$ for histology and histochemistry and $5\text{--}7\text{ }\mu\text{m}$ for immunohistochemistry, as antibodies and immunoperoxidase do not penetrate thicker sections well.
- Epoxy resin sections: ultra-microtome set at $\sim 0.6\text{--}1\text{ }\mu\text{m}$ for semi-thin sections and $\sim 50\text{--}90\text{ nm}$ for ultra-thin sections.
- FFPE: microtome set at $3\text{--}5\text{ }\mu\text{m}$ for paraffin sections.

2.4 Typical routine staining (which may be adapted according to the clinical circumstances of a case)

Cryostat sections should be stained routinely with haematoxylin and eosin (H&E); Gömöri trichrome (Engel and Cunningham); cytochrome oxidase (COX); succinate dehydrogenase (SDH); combined COX/SDH; NADH-TR; acid phosphatase; lipid stains (e.g. Oil Red O or Sudan Black); periodic acid-Schiff (PAS)/PAS-diacetate (DPAS); acid phosphatase; myosin heavy chain immunohistochemistry (slow, fast, fetal [neonatal] and embryonic [developmental]); NCAM and MHC class I. Myosin immunolabelling has the advantage of identifying fibres expressing more than one isoform, identifying very small fibres less than $5\text{ }\mu\text{m}$

µm, identifying regenerating fibres that are not basophilic and determining if a biopsy is normal or abnormal by the presence of fetal myosin. It is now recommended that immunohistochemistry is used for fibre typing instead of ATPases, given the advantages described above. It is recommended that laboratories list the exact antibody clones and method of detection used for myosin immunolabelling given that differences have been described.

When staining muscle biopsies, it is good practice to stain in batches. If this is not possible, the use of good controls (clearly labelled if on the same slide) is particularly important. It should be noted that a diagnostic service may prefer to perform a subset of these routine stains first and then, on the basis of the initial histological analysis and consideration of clinical features in a case, decide on which additional routine investigations to perform alongside those listed in section 2.6. For resource planning, it is anticipated that most cases will require application of this panel to determine if a sample is normal or abnormal and to differentiate between various possible diagnoses. All tests should be interpreted with the aid of positive and negative age-appropriate biopsies, which may include age-matched normal and/or disease controls and with a control sample stained at the same time.

2.5 Further investigations depending on initial results and phenotype

According to clinical indications or pathological findings, supplementary investigations might include:⁵

- histochemistry for phosphorylase, phosphofructokinase, myoadenylate deaminase, aldolase and menadione nitro blue tetrazolium (NBT) (without substrate)
- immunohistochemistry for dystrophy-associated proteins (e.g. dystrophin using antibodies to epitopes against N-terminal, rod and C-terminal domains; utrophin; NNOS; alpha-, beta, gamma- and delta-sarcoglycan; caveolin-3; emerin; dysferlin; laminin α 2 [80 and 300 kDa], laminin α 5 and laminin β 1; and other appropriate dystrophin axis-associated proteins); beta-spectrin and laminin γ 1 for assessing sarcolemmal integrity
- immunohistochemistry for desmin, myotilin, alpha-B-crystallin, FHL-1, BAG-3, filamin C if a myofibrillar myopathy is suspected
- immunohistochemistry for complement membrane attack complex (complement C5b-9 components); and CD3, CD4, CD8, CD20 or CD79a, CD68 and MxA
- immunohistochemistry for respiratory chain enzyme complexes, especially complex I and IV⁶
- Congo red, ubiquitin and p62 if inclusion body myositis or protein aggregation myopathy are suspected or for exclusion. If rimmed vacuoles are present, EM may also be undertaken.
- EM for indications including mitochondrial cytopathy, nemaline myopathy, cores, inclusion body myositis, juvenile dermatomyositis and clarification of features observed with light microscopy. Muscle tissue to be preserved in fixative containing 2.5–6% glutaraldehyde in 0.1 M cacodylate or phosphate buffer.
- respiratory chain enzyme analysis for suspected mitochondrial cytopathy
- molecular genetic studies for indications including mitochondrial cytopathy
- western blot analysis for indications including various muscular dystrophies with raised creatine kinase
- specific single-enzyme studies for indications including glycogen storage diseases
- referral to NHS Highly Specialised Services (HSS):
 - for suspected congenital myopathy or congenital muscular dystrophy, patients should be referred to Dubowitz Neuromuscular Centre in London. Repertoire of tests includes immunoanalysis of dystroglycans, laminins and collagen VI in muscle, skin

and CVS and fluorescent activated cell sorting quantification in cultured fibroblasts.

- if CK is raised and phenotype suggests limb girdle muscle dystrophy, or a myofibrillar myopathy is suspected, patient should be referred to the Newcastle Limb Girdle Muscular Dystrophy referral service
- if a mitochondrial disorder is suspected, patients should be referred to the [NHS HSS for rare mitochondrial disorders](#) (centres in London, Newcastle, Oxford)
- if a myasthenic syndrome is suspected, patients should be referred to the HSS mitochondrial service in Oxford
- if an ion channel disorder is suspected, patients should be referred to the HSS mitochondrial service in London
- with the reconfiguration of the genomic testing landscape in the UK the above pathways may change and whole genome sequencing testing solutions may be available via local Genomic Laboratory Hubs.

2.6 Report content

The report should include:

- clinical and demographic information received with the biopsy (gender, date of birth, age at biopsy)
- biopsy dimensions and time of stations in handling, especially time to freeze
- description of the microscopic findings
- interpretation of the findings, with indication of limitations imposed by available clinical information, amounting to specific or differential diagnosis
- comparison with any previous biopsy
- any recommendation for re-biopsy if inadequate for diagnosis
- any recommendation for supra-regional referral (see section 2.5)
- diagnosis (if appropriate)
- optional: comment
- SNOMED codes.

[Level of evidence GPP – the recommendations in this section are based on the expert opinions of the authors.]

3 Peripheral nerve

The specimen should be obtained from an affected nerve. Most neuropathies show distal accentuation. The sural nerve is purely sensory in more than 90% of patients and contains only a few motor fibres in the remaining patients.⁷ It is easily accessible to surgery and therefore most frequently chosen for biopsy. It usually contains between five and ten nerve fascicles. In a large autopsy study, 3,300–8,000 myelinated and 10,500–45,500 unmyelinated nerve fibres were found in subjects without history of disease or ingestion of drugs known to affect peripheral nerve.⁸ Nerve biopsies should ideally be performed by a clinician experienced in peripheral nerve biopsies. The nerve segment must be excised and handled carefully to minimise mechanical injury to the sample. Squeezing or stretching of the nerve should be strictly avoided and excessive removal of epineural fat or connective tissue should not be attempted. Nerve fibres are very sensitive to mechanical injury.⁹

3.1 Specimen submission and dissection⁹

The recommended length of the biopsied nerve segment is 3–5 cm. Removal of a shorter segment will reduce diagnostic accuracy but will leave an identical sensory deficit. Formalin and glutaraldehyde fixation of nerve segments is essential, while freezing an unfixed segment is optional.^{10,11}

The majority of specimens derived from clinical neuroscience centres will be delivered fresh. However, the fresh tissue has to be immediately transversally divided: one part fixed in formalin and the other in 3% glutaraldehyde-containing fixative. Hospitals should work towards a latency of 10 minutes between surgical removal to fixation, where possible. It is acknowledged that the transfer time can be longer and, in such circumstances, immersion into fixative locally prior to transfer should be considered (see below). Delays in transferring nerve biopsies into fixative (e.g. by storing on damp gauze) can result in irreversible artefacts in myelin and axons.

Submissions received from non-specialist centres must be sent in fixative: one part of the nerve biopsy fixed in formalin and a separate piece in EM fixative (containing 2.5–6% glutaraldehyde in 0.1 M cacodylate or phosphate buffer). Material fixed in glutaraldehyde-containing fixative will be processed for semi-thin resin sections and potentially for EM and teased fibres. Formalin-fixed material will be processed into paraffin wax for histochemical and immunohistochemical studies.

3.2 Embedding options

Subdivide the sample into four segments for the following embedding options (in order of priority):

- formalin fixation and paraffin embedding: one piece oriented longitudinally (a length of at least 8 mm is recommended) and the remainder transversally (at least two segments are recommended)
- glutaraldehyde fixation and epoxy resin embedding: the glutaraldehyde-fixed specimen should be cut transversally (additional longitudinal orientation is optional depending on the length of the sample)
- glutaraldehyde fixation for teased fibre preparation (a length of at least 8–10 mm is recommended)
- optional: snap-freezing of fresh material.

3.3 Sectioning

For microtome sectioning, nominal thickness should be at 3–4 μm . The tissue should be cut at three levels, preserving sections mounted on slides suitable for immunohistochemical stains between levels (further levels may be performed depending on the histological examination). Ultra-microtome should be set at approximately 0.6 μm for semi-thin sections and ~50–90 nm for ultra-thin sections.

3.4 Routine staining

3.4.1 Paraffin sections

Paraffin sections should be routinely stained with H&E and Congo red. Elastic van Gieson (EVG; or other suitable trichrome) and Perls' iron stain are optional.

Immunohistochemistry should be performed for neurofilament protein (e.g. neurofilament cocktail, SM31 or any other pan-neurofilament marker), myelin basic protein (e.g. SM194), CD68 (macrophages), CD3 (T cells) and CD20 (B cells). It is recommended that a consistent

panel of stains is implemented (e.g. to establish a baseline for histopathological interpretation of the immunostains). Immunostainings should be used instead of tinctorial or histochemical stains for axons and myelin sheath since they are more specific, give a better signal to noise ratio and are usually easier to implement into the histopathological laboratory workflow.^{9,11,12}

3.4.2 Resin sections

It is highly recommended that semi-thin resin sections are routinely prepared from glutaraldehyde-fixed material (e.g. stained with Toluidine blue or methyl blue aniline–basic fuchsin) for each nerve biopsy. These sections allow for far superior morphological assessment of a large range of pathological features.

3.5 Further investigation

Depending on clinical indications or pathological findings, the following stains and preparations are optional:

- histochemistry for fibrin
- Wade-Fite (for leprosy)
- epithelial membrane antigen (EMA) or smooth muscle actin antigen for assessment of the perineurium. EMA is useful for the identification of perineurioma.
- immunohistochemistry for further immune cell subsets, e.g. CD79a (B cells), CD8, CD4, (T cells) and CD138 (plasma cells)
- EM
- teased fibres.

3.6 Report content

The report should include:

- clinical and demographic information received with the biopsy (gender, date of birth, age at biopsy)
- biopsy dimensions
- description of the histological findings
- morphometric data if relevant (e.g. density of myelinated fibres as well as other morphometric data)
- interpretation of the findings, with indication of limitations imposed by available clinical information, amounting to specific or differential diagnosis
- comparison with any previous biopsy
- any recommendation for re-biopsy if inadequate for diagnosis
- any recommendation for supra-regional referral
- diagnosis
- optional: comment
- SNOMED codes.

[Level of evidence B – the recommendations in this section are based on a body of evidence demonstrating consistency of results and comprising mainly high-quality systematic reviews of case studies with a very low risk of confounding or bias (see References).]

4 Central nervous system (brain, spinal cord, meninges) incisional or excisional biopsy for non- neoplastic lesion

4.1 Specimen submission and dissection

Specimens are usually received either in formalin or fresh. This is determined mainly, but not exclusively, by a requirement for intraoperative diagnosis. Specimens should be resected as a single piece, measuring at least 1 × 1 cm and should contain leptomeninges, cortex and subcortical white matter. Even if the clinical and radiological impression suggests a diffuse white matter process, it is strongly advised to include leptomeninges and cortical grey matter to maximise diagnostic accuracy.

4.2 Immediate handling and intraoperative diagnosis¹³

The biopsy of non-neoplastic lesions requires a different intraoperative approach than those of neoplastic or presumed neoplastic lesions. Generally, intraoperative diagnosis should not be requested for diagnostic biopsies of non-neoplastic lesions unless the result affects the intraoperative approach. Intraoperative assessments give only very limited information and should be avoided to preserve the maximum possible amount of diagnostic material.

Prior to biopsy, check for infection risk label and assess risk from the clinical information provided. If the request for intraoperative diagnosis is firm, consider smear preparation and/or frozen sections. Frozen sections will have a relatively greater value if the tissue texture precludes making an effective smear. Furthermore, the block of frozen tissue can be fixed and processed through paraffin afterwards.

For intraoperative diagnosis, a verbal report should be issued, along with a written record of the exact wording of the discussion, the recipient and time and date of when the report was communicated.

If there is a risk of infection, ensure the surgeon has submitted separate material to microbiology. If no material has been submitted and the operative field has been closed and material is still unfixed, do so oneself.

Tissue should be set aside for:

- freezing (e.g. for further morphological investigations on frozen tissue, molecular genetics and tissue banking)
- EM
- paraffin processing (the sizes for each fixation type are determined by diagnostic needs and terms of consent).

4.3 Embedding options

- Routine stains and immunohistochemistry: formalin fixation and paraffin processing.
- Semi-thin resin sections and EM: epoxy resin processing.
- High-risk biopsies: treatment with formic acid prior to embedding in paraffin is necessary on a proportion of the sample if prion disease is in the differential diagnosis (the other half/portion should be left in formalin until prion disease is either confirmed or excluded).

4.4 Sectioning

Microtome should be set at 4 µm and sections should be taken at multiple levels. Ultramicrotome should be set at ~0.6 µm for semi-thin sections and ~50–90 nm for ultra-thin

sections.

4.5 Routine staining

Microtome sections should be routinely stained for H&E.

4.6 Further investigation

Depending on clinical indications or pathological findings, the following stains might be required:

- Congo red and/or A β for evacuated blood clot. A β immunohistochemistry is strongly recommended for amyloid β angiopathy since it is more sensitive than Congo red. A β should be considered for diagnosing early onset cerebral amyloid β angiopathy (CAA), while Congo red enables detection of genetic CAA caused by misfolded proteins other than A β peptide.^{14,15}
- Perls, EVG or equivalent for diagnosing vascular malformative lesions
- for non-neoplastic space occupying lesions:
 - use Ziehl-Neelsen, Gram, Grocott, DPAS and add toxoplasma immunohistochemistry if lesion looks infective/inflammatory
 - consider viral aetiology, e.g. using immunohistochemistry to diagnose herpes simplex virus, cytomegalovirus, measles, Epstein-Barr virus, JC virus (SV40 or equivalent) and human immunodeficiency virus. Since the immunohistochemical investigation of biopsies with suspected viral aetiology is limited, it is strongly recommended that material is submitted (fresh/frozen) for viral sequencing using next-generation sequencing (NGS) approaches. NGS can cover a wide range of viral pathogens, including a precise definition of the viral strain, if necessary.
 - if there is evidence of demyelination, use myelin (myelin basic protein, e.g. immunohistochemistry: SMI94; histochemistry: Luxol fast blue/cresyl violet) and axonal (neurofilament protein) stain; CD68; and consider glial fibrillary acidic protein (GFAP). If progressive multifocal leukoencephalopathy is suspected clinically or pathologically, perform immunohistochemistry or in situ hybridisation for polyoma virus (e.g. SV40 immunohistochemistry).
 - consider EM if material is available in appropriate fixative (glutaraldehyde fixed, to be embedded into epoxy resin) in patients with small vessel disease with or without infarcts, or clinical suspicion of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), to look for granular osmiophilic material in the blood vessel walls. The use of electron microscopy in the diagnosis of viral diseases has lost its relevance since the availability of molecular pathology techniques, such as NGS, and sampling of frozen material may be considered of higher priority for the diagnosis of viral infections (see below).
 - preserve a representative sample for freezing to allow molecular assays for an extended range of pathogens (see assays for viral aetiology above). DNA/RNA extraction can be performed for viral, bacterial (e.g. *M. tuberculosis*) and fungal PCR. (Note that while frozen tissue is preferred, DNA/RNA extraction is also possible from paraffin-embedded, non-formic acid-treated material.)

4.7 Report content

The report should include:

- clinical and demographic information received with the biopsy (gender, date of birth, age at biopsy)
- biopsy dimensions

- description of the histological findings and, where relevant, histochemical and immunohistochemical stains
- interpretation of the findings amounting to specific or differential diagnosis
- comparison with any previous biopsy
- any recommendation for re-biopsy if inadequate for diagnosis
- any recommendation for supra-regional referral (e.g. fungal, bacterial or viral reference lab) or any other diagnostic work-up
- diagnosis
- optional: comment
- SNOMED codes.

[Level of evidence GPP – the recommendations in this section are based on the expert opinions of the authors.]

5 Brain biopsy for diffuse central nervous system pathology, including neurodegenerative processes

5.1 General comments

These brain biopsies are typically considered when clinical or laboratory features suggest a reversible, tractable process such as vasculitis or infection in the differential diagnosis. Other possible diagnoses include neurodegenerative diseases, such as Creutzfeldt-Jakob disease (CJD) or neurodegenerative diseases associated with other misfolded protein deposition, inflammatory disease (including encephalitis or meningitis), cerebrovascular disease (genetic or acquired), neoplasm (primary and metastatic, including intravascular), leukodystrophy, storage disorders and undetermined encephalopathies. The diagnostic yield ranges from 22 to 84%.^{16–19}

5.2 Specimen submission and dissection

Specimens must be sent to the laboratory unfixed. Microbiological (bacteriological/mycological) and virological samples should be submitted separately from the operating theatres to respective laboratories.

5.3 Immediate handling

- Check for infection risk labels and assess risk from the clinical information provided. Handle in appropriate containment if prion disease (CJD) is a possibility. Even if no infection risk label is present, it is recommended that such biopsies from adults are considered high risk and the biopsy is managed/divided accordingly (see below regarding prion disease).
- Assess the specimen for adequacy: cerebral full thickness including leptomeninges and grey and white matter.
- Set aside tissue for freezing (e.g. for molecular genetics, prion biochemistry), EM and adjacent tissue paraffin processing, all according to diagnostic needs.
- If prion disease is in the clinical differential diagnosis, divide the specimen into three equal portions: two-thirds to be immediately fixed in formalin and one third to be frozen and preserved for immunoblotting, which will enable strain typing in a specialist centre, such as the National Creutzfeldt-Jakob Disease Surveillance Unit in Edinburgh. If the specimen is small, consider fixing the entire specimen in formalin to maximise the diagnostic yield. After overnight formalin fixation (12 hours or longer), immerse part of

the tissue for 1 hour in 98% formic acid to reduce (potential) prion infectivity and then fix with 10% buffered formalin for 1 hour.²⁰ Next, the tissue is processed through paraffin and used for routine H&E and immunohistochemical study to look for abnormal prion protein and, depending on findings followed by staining for other misfolded protein or inflammatory markers. While some markers may not work as the antigens may be denatured by formic acid treatment, all commonly used markers for neurodegenerative misfolded protein-associated diseases, such as A β , AT8, α -synuclein, TDP43, p62, FUS or inflammatory markers, such as CD68, CD3 and CD20, show preserved labelling. If prion disease is confirmed, the second tissue fragment will also have to be treated with formic acid and processed to paraffin for archiving as described above.^{16,17} If prion disease is excluded, the second formalin-fixed tissue does not require formic acid treatment and following paraffin embedding may be used for further histochemical and immunohistochemical tests to explore other differential diagnoses. The frozen tissue can be used for further tests such as NGS for viral nucleic acid sequences.

5.4 Embedding options

Paraffin processing for routine stains and immunohistochemistry and epoxy resin processing for EM.

5.5 Sectioning

Microtome should be set at 4 μ m and sections cut at multiple levels. Ultra-microtome should be set at ~0.6 μ m for semi-thin sections and ~50–90 nm for ultra-thin sections.

5.6 Routine staining

Microtome sections should be routinely stained for H&E.

5.7 Further investigation

Depending on clinical indications or pathological findings further investigation may include detailed further examination, such as genetic testing to look for pathogenic mutations associated with neurodegenerative diseases or to characterize the genetic make-up of identified neoplastic process. Further investigation may also include a referral to a microbiology or virology laboratory or for a specialist opinion in infectious diseases, soft tissue pathology or general histopathology.

5.8 Report content

The report should include:

- clinical and demographic information received with the biopsy (gender, date of birth, age at biopsy)
- biopsy dimensions
- description of the histological and immunohistochemical findings
- interpretation of the findings amounting to specific or differential diagnosis
- comparison with any previous biopsy
- any recommendation for re-biopsy if inadequate for diagnosis
- any recommendation for supra-regional referral (e.g. fungal or viral reference lab) or any further diagnostic work-up
- diagnosis
- optional: comment

- SNOMED codes.

5.9 Supra-regional referral

Contact the National Creutzfeldt-Jakob Disease Surveillance Unit in Edinburgh if prion disease is diagnosed or suspected.

[Level of evidence B – the recommendations in this section are based on a body of evidence demonstrating consistency of results and comprising mainly high-quality systematic reviews of case studies with a very low risk of confounding or bias (see References).]

6 Examination of tissue resections for the treatment of refractory epilepsy due to non-neoplastic lesions

6.1 Specimen collection

Specimens will typically be received by the laboratory either fresh or in formalin. Intraoperative smears may be required in cases where an underlying tumour is in the differential diagnosis. The specimens may be received in several parts. Common types of specimen include temporal lobectomy, hippocampectomy or cortical resection. Close correlation with diagnostic imaging and preoperative clinical consultation can aid tissue sampling and diagnostic interpretation, in particular regarding history of intracranial recordings/depth electrodes prior to resective surgery.

6.2 Intraoperative diagnosis and immediate handling

Check for infection risk label and assess risk from the clinical information provided. Representative tissue is set aside for freezing (molecular genetics, tissue bank) and EM according to diagnostic needs, terms of consent and local project protocols. Larger specimens will require orientation and coloured ink can be used to mark resection boundaries or specific gyri. Larger specimens may be weighed. Photography of the macroscopic specimen may also be considered. The bulk of the specimen is fixed in formalin. Large specimens, including temporal lobectomies, will require overnight fixation before slicing and sampling tissue for processing. Hippocampal specimens should be orientated and cut coronally. Ideally, the entire specimen should be embedded with selected representative slices in larger specimens. The International League Against Epilepsy (ILAE) has published guidelines regarding optimal sampling and protocols in epilepsy resections.^{21,22}

6.3 Embedding options

Paraffin processing for routine stains and immunohistochemistry and epoxy resin processing for EM.

6.4 Sectioning

Microtome should be set at 5 µm and sections cut at multiple levels. Thicker sections are useful for assessment of cortical architecture, for example in Nissl- and NeuN-stained sections in cases of suspected cortical dysplasia.

6.5 Routine staining

Microtome sections should be stained routinely with H&E stains. Luxol fast blue/Nissl stain can be used to delineate the anatomy of hippocampal subfields and the amygdala and assess the neocortical architecture when this is required according to the context of the case and adequacy of the material.

6.6 Further investigations

Special stains and immunohistochemistry might be required depending on the initial findings on H&E sections. For example:

- NeuN, neurofilament, GFAP, CD34 and vimentin stains are useful in the assessment of focal cortical dysplasia
- GFAP in the confirmation of hippocampal sclerosis, NeuN in the assessment of granule cell dispersion and subtype of sclerosis,^{21,22} and dynorphin or ZnT3 (zinc transporter 3) immunohistochemistry (in preference to Timms stain) in the identification of axon sprouting.
- if a low grade glial/glioneuronal tumour is suspected, tumour datasets and WHO Classification of Tumors of the Central Nervous System (CNS) 2022 guidelines²³ for appropriate immunohistochemistry and molecular biology tests (targeted mutations, methylation analysis or NGS) should be followed as appropriate based on histology.
- inflammatory markers in suspected encephalitis, and viral markers where appropriate
- genetic testing for mammalian target of rapamycin pathway mutations may be available in the diagnosis of focal cortical dysplasia (FCD) in some centres, as recommended in forthcoming ILAE classification scheme for FCD.²⁴

6.7 Report content

The report should include:

- clinical and demographic information received with the biopsy (gender, date of birth, age at biopsy)
- biopsy dimensions
- description of the histological findings
- interpretation of the findings amounting to specific or differential diagnosis and using ILAE classification schemes as appropriate^{21,22,25}
- comparison with any previous biopsy
- any recommendation for re-biopsy if inadequate for diagnosis; for example, in small, poorly orientated or incomplete and fragmented hippocampal specimens, confirmation or subtyping of the pattern of hippocampal sclerosis may not be possible¹⁵
- conclusion
- SNOMED codes.

[Level of evidence B – the recommendations in this section are based on a body of evidence demonstrating consistency of results and comprising mainly high-quality systematic reviews of case studies with a very low risk of confounding or bias (see References).]

7 Temporal artery biopsy

See the relevant section in the RCPATH's *Tissue pathways for cardiovascular pathology*.²⁶

8 Cerebrospinal fluid for cytological assessment

CSF cytology serves several different purposes and largely depends on the clinical settings and the expectations of the clinical teams. It can be used to determine if there are malignant

cells in the CSF, where it has an important role in the investigation, diagnosis and staging of suspected or established malignancy.²⁷ It is used for the staging of paediatric brain tumour, most notably medulloblastoma. CSF examination by microbiology, immunology and chemistry laboratories is important in the investigation of non-neoplastic disease. Other useful information can be obtained from CSF cytology in non-neoplastic disease, for example neutrophilia in most cases of meningitis, mixed neutrophils and lymphocytes in sarcoidosis, and tuberculosis or lymphocytes in encephalitis. In cases of suspected tuberculosis, the laboratory must be set up to handle high-risk samples.

8.1 Specimen submission and immediate handling

Specimens may be received as fluid from wards, theatre or from other diagnostic departments, when multiple tests are being performed on CSF (e.g. cell count, protein concentration). Specimens should be handled in a microbiological safety cabinet. For high-risk samples, consider decontamination steps following local procedures and policy. For the investigation of malignant cells in the CSF, it has been reported that false-negative CSF cytology results correlate with a small CSF volume being withdrawn.²⁸ A further finding of this and another study² was the importance of immediate submission to the laboratory for examination. Optimal assessment of CSF cytology and cell count requires immediate processing of fresh liquids, i.e. within one hour of lumbar puncture²⁹ owing to rapid lysis of the cells after two hours, even when kept at 4 °C. This contrasts with other CSF-based biochemical analyses that may be less time critical.

8.2 Preparation: options and routine staining

A standardised volume of CSF should be examined. The speed, time and acceleration of the machine must be adjusted to obtain an optimum yield of morphologically preserved cells in a monolayer. Additional slides may be prepared for subsequent immunohistochemical tests, for example to confirm the epithelial nature of atypical cells.^{30,31}

The number of additional slides can be adapted to the cell count, which should be done prior to the CSF cytospin.

The following should be considered:

- preparation of tinctorial staining such as H&E, Giemsa or other suitable preparations on one slide. Consider preparing an additional two or more slides and fix in methanol, when fluid volume is sufficient.
- if clinical differential diagnosis includes dissemination of a neoplasm into the CSF, prepare several spare slides, if possible, to enable subsequent immunohistochemical staining to characterise atypical cells
- high red blood cell counts are usually an indication of blood contamination
- save supernatant if larger volumes (e.g. exceeding 3 ml) are sent; this can be further used for molecular biology (e.g. NGS) or biochemistry.

8.3 Additional investigations

In some cases, further special stains (e.g. Gram, Periodic Acid-Schiff [PAS], Grocott and Ziehl-Neelsen) may be required for investigation of infectious diseases. Perls' stain should be used when there is clinical suspicion of superficial siderosis or subarachnoid haemorrhage. Mucicarmine, PAS or other adequate stains should be carried out when cryptococcal meningitis is suspected. Immunohistochemistry may be required for suspected lymphomas or other malignancies, e.g. metastatic carcinomas or glial tumours.

8.4 Report content

The report should include:

- clinical and demographic information received with the biopsy (gender, date of birth, age at biopsy)
- presence or absence of tumour and atypical cells
- optional: cell counts (total red blood cells and white blood cells)
- description of the cytological findings, such as increased granulocytes, macrophages and blood contamination
- interpretation of the findings amounting to specific or differential diagnosis. The report should include a descriptive report of the cells present and detail whether extraneous cells or contaminants are present (e.g. chondrocytes in lumbar puncture specimens or glial tissue in external ventricular drainage specimens).
- any recommendation for repeat CSF tap if inadequate for diagnosis. In cases where the specimen is insufficient in volume and a further specimen is necessary, this should be indicated on the report to alert the clinician. Furthermore, in cases where there has been any delay in processing (e.g. if the specimen has been misdirected) with deterioration of specimen, a note on the quality of the specimen should be included for the clinician and the possibility of an additional specimen may be suggested.
- comparison with concurrent or previous biopsy or CSF examination
- diagnosis
- optional: comment
- SNOMED codes.

[Level of evidence GPP – the recommendations in this section are based on the expert opinions of the authors.]

9 Bone and soft tissue from skull or axial skeleton

See RCPATH's *Tissue pathways for bone and soft tissue pathology*.³²

10 Samples for investigation of genetic disorders including neurometabolic and paediatric disorders

Histopathology from a variety of tissue samples can provide sensitive, specific and rapid diagnosis in neurogenetic disease and can be particularly helpful for metabolic diseases and diseases of childhood. The choice of sample should be the least invasive that can be used to diagnose the suspected disorder (e.g. many of the lysosomal storage disorders [LSDs] can be investigated by the examination of a blood film). The examination of some of these samples is highly specialised and referral to regional or national centres is recommended.

11 Skin and subcutaneous tissues

See RCPATH's *Tissue pathways for dermatopathology* for general diagnostic approaches.³³
Samples for investigation of suspected:

- neuronal ceroid lipofuscinosis (NCL; including Batten's disease, Kuf's disease)
 - fixation in glutaraldehyde with epoxy resin embedding for EM, skin formalin fixation

for wax embedding and immunohistochemistry for subunit C of mitochondrial ATP synthase and saposin D (sphingolipid activating protein D)

- Lafora body disease
 - fix skin-bearing apocrine glands (preferable) or eccrine glands in formalin followed by wax embedding and staining with PAS ± diastase and/or Lugol's iodine to detect inclusions
 - photography or digital slide scanning for documentation recommended as Lugol's stain may fade rapidly
 - fixation in glutaraldehyde with epoxy resin embedding for EM
- Niemann-Pick disease type C
 - frozen tissue for fibroblast culture (mandatory)
 - fixation in glutaraldehyde followed by epoxy resin embedding for EM
 - formalin fixation and paraffin embedding for light microscopy (to look for characteristic but non-specific foam cells), but only if there is plenty of tissue³⁴
- CADASIL
 - skin fixed in formalin and embedded in paraffin wax; fix in glutaraldehyde and embed in epoxy resin for EM to look for granular osmiophilic material
 - stain paraffin-embedded tissue with PAS to look for granular deposits in the media of small arteries and arterioles
 - immunostaining with an antibody to the extracellular domain of NOTCH3 is possible,³⁵ but may in clinical practice be gradually replaced by genetic screening³⁶
- rabies
 - nuchal skin biopsy and immunostaining for rabies virus. Skin biopsy specimens should be 5–6 mm in diameter, taken from the back of the neck at the hairline and contain at least ten hair follicles.³⁷ The biopsy specimen should be of sufficient depth to include the cutaneous nerves that are abundant at the base of the hair follicles, thus increasing the probability of detecting the rabies virus owing to its neurotropic nature. Processing of unfixed high-risk specimens from patients with suspected rabies and any decisions on whether to provide a service dealing with such material should be based on local risk assessments, the availability of appropriately trained staff and any necessary laboratory containment facilities. For more information on rabies in the UK, see *Rabies: risk assessment, post-exposure treatment, management*.³⁸

11.1 Report content

The report should include:

- clinical and demographic information received with the biopsy (gender, date of birth, age at biopsy)
- biopsy dimensions
- description of the histological, histochemical and immunohistochemical findings, as well as ultrastructural (if performed) findings
- interpretation of the findings amounting to specific or differential diagnosis
- any recommendation for further investigation (in all patients, a diagnostic test should be undertaken to confirm a specific diagnosis)
- diagnosis

- optional: comment
- SNOMED codes.

[Level of evidence B – the recommendations in this section are based on a body of evidence demonstrating consistency of results and comprising mainly high-quality systematic reviews of case studies with a very low risk of confounding or bias (see References).]

12 Skin biopsies for diagnosis of small fibre neuropathy

Small fibre neuropathy (SFN) is a structural abnormality of small fibres characterised by degeneration of the distal terminations of small fibre nerve endings (intraepidermal nerve fibres, IENFs). SFN is increasingly encountered, given improvements in its diagnosis and a rising prevalence of diabetes.³⁹ Small fibres (in this context) are narrow myelinated (A δ) and unmyelinated (C) nerve fibres of the peripheral nervous system.⁴⁰ Skin biopsy with IENF density measurements is the diagnostic modality of choice for SFN.

12.1 Specimen submission

A skin punch biopsy 3 mm in diameter can in principle be taken from any location on the body, but the recommended site for SFN diagnostic purposes is 10 cm proximal to the lateral malleolus.⁴¹ An additional biopsy site is the proximal thigh, 20 cm below the iliac spine, which in combination with the distal biopsy can help differentiate between a neuropathy and a neuronopathy.⁴²

12.2 Embedding and fixation options

After skin biopsy is performed, the specimen is immediately fixed in cold fixative for approximately 24 hours at 4 °C, then kept in a cryoprotective solution for one night and serially cut with a freezing microtome or a cryostat. Each biopsy yields approximately 50 vertical 50 μ m sections. The first and last few sections should not be used for nerve examination because of possible artefacts. Most studies for bright-field microscopy use 2% paraformaldehyde-lysine periodate, whereas most studies for indirect immunofluorescence with or without confocal microscopy used Zamboni's (2% paraformaldehyde, picric acid) fixative. Formalin fixation should be avoided owing to artefacts causing a fragmented appearance of nerve fibres.⁴³

As instructions for specific preparations, fixative and submissions to a referral centre can vary, it is essential to liaise with the laboratory. Some UK referral centres will accept individual biopsies from further afield, whereas others serve their own region and have service level agreements in place when supporting SFN clinics elsewhere in the country.

An example of a UK referral centre accepting individual samples from elsewhere can be found from King's College Hospital.⁴⁴

12.3 Staining and assessment

Either bright-field immunohistochemistry or immunofluorescence with or without confocal microscopy can be used; the technique does not affect the reliability of skin biopsy in assessing IENF loss in SFN.⁴⁵ Bright-field immunohistochemistry is readily available within the NHS setting and slides can be stored in the archive and reviewed, which may be an advantage. Most laboratories use bright-field microscopy for routine diagnostic purposes.⁴² Confocal microscopy allows analysing double-, triple- and even quadruple-stained sections. For example, PGP 9.5 and collagen IV double-stained sections can be used easily to visualise axons and basement membrane in order to trace intraepidermal nerve fibres from the site where they penetrate the basement membrane to their endings.⁴¹

IENFs are counted under the microscope at high magnification (i.e. 40x objective) or using software for image analysis. The epidermal surface length is measured; this is best achieved using software for biological measures. The density is calculated in at least three sections as the number of IENFs per length of the section (IENF/mm).⁴¹ The median is calculated. Adequate training in a well-established skin biopsy laboratory is needed and good practice includes intra- and interobserver variability testing and interlaboratory agreement on IENF counts.⁴¹ Normative reference values are available for bright-field immunohistochemistry.^{42,45}

Congo red staining can be added to screen for amyloid deposition given that there are treatment options for transthyretin familial amyloid polyneuropathy.⁴⁶

12.4 Report content

The report should include:

- clinical and demographic information received with the biopsy (gender, date of birth, age at biopsy and site of biopsy). Ethnicity can be helpful as bleaching the section prior to immunostaining may be required if the skin is very dark).
- biopsy dimensions
- description of the histological findings
- morphometric data (e.g. density of fibres as well as other morphometric data). An overview of the normative values of intraepidermal nerve fibre density has been published in a consensus paper.⁴²
- interpretation of the findings, with an indication of limitations imposed by available clinical information, amounting to specific or differential diagnosis (this may be difficult for the pathologist to accomplish given that important information is often missing and it may be preferable for the integration of the IENF density result to be undertaken by the clinician, typically a neurologist with an interest in SFNs in the context of an SFN specialist clinic), particularly given that there is emphasis on clinical features.³⁹ This could be emphasised in the report (e.g. 'Please correlate with the clinical and any other relevant findings').
- comparison with any previous biopsy
- any recommendation for re-biopsy if inadequate for diagnosis
- any recommendation for supra-regional referral (typically patients with SFN are seen at a specialist clinic prior to skin biopsy or the result is referred as part of a clinical specialist referral)
- diagnosis or median IENF density count (with recommendation for clinical correlation)
- optional: comment
- SNOMED codes.

[Level of evidence A – 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.]

13 Peripheral blood

13.1 Clinical indication and specimen collection

The examination of blood films is only offered in a small number of specialist centres, but it provides a very rapid and, in several diseases, very specific test. The clinical context will usually be the investigation of suspected storage disorders, principally LSD (e.g. Batten's disease [NCL], Salla disease, I cell disease, β galactosidase deficiency [GM1 gangliosidosis], mucopolysaccharidoses, Niemann-Pick disease, fucosidosis, mannosidosis, Wolman's

disease and certain glycogenosis). Blood films can provide a specific diagnosis in Wolman's disease and some NCL, or they can provide a diagnosis when no biochemical test is available (e.g. for some forms of NCL). There is an increasing role in monitoring response to therapy in LSDs.

13.2 Specimen handling

A sample of ethylenediaminetetraacetic acid (EDTA)-preserved whole blood should be received by the laboratory. Heparinised whole blood is also acceptable. Check for infection risk label and assess risk from the clinical information provided. EDTA blood is stable for days at room temperature or at 4 °C, which facilitates transport to specialist centres. However, the sample must not be frozen, as this will cause artefacts. Several blood films (typically six) are prepared. For the initial assessment, one of the smears is prepared with a Romanowsky stain.

13.3 Routine examination

The blood film should be examined at the thin end of the film near the tail rather than the thicker region. The blood film should be examined in a systematic manner, with particular attention to the detection and morphological characteristics of the lymphocytes. The optimal number of lymphocytes to be examined is uncertain, but if there are fewer than 20 seen in the first film, a second film should be examined.⁴⁰

13.4 Ultrastructural examination

A buffy coat should be prepared for EM if vacuolated lymphocytes are seen in the blood film or in all cases of certain diseases (e.g. all suspected NCL cases should be examined by EM). Ultrastructural examination of inclusions in lymphocytes can provide a specific diagnosis and aid the identification of variant subtypes of NCL. At least 100 lymphocytes should be examined, since in some variants of NCL only a minority contain diagnostic material.⁴¹

13.5 Report content

The report should include:

- clinical and demographic information received with the biopsy (gender, date of birth, age at biopsy)
- description of the cytological findings (comments should be made as to the size and extent of the vacuolation)
- interpretation of the findings amounting to specific or differential diagnosis
- any recommendation for further investigation (in all patients, a diagnostic test should be undertaken to confirm a specific diagnosis)
- diagnosis
- optional: comment
- SNOMED codes.

[Level of evidence B – the recommendations in this section are based on a body of evidence demonstrating consistency of results and comprising mainly high-quality systematic reviews of case studies with a very low risk of confounding or bias (see References).]

14 Bone marrow

Bone marrow aspirate can be helpful in the diagnosis of some LSDs, for example when vacuolated lymphocytes are not a feature on blood films. In particular, it can assist in the

diagnosis of Niemann-Pick and Gaucher diseases. Aspirates should be prepared and stained by the standard haematological protocols.

[Level of evidence GPP – the recommendations in this section are based on the expert opinions of the authors.]

15 Hair

15.1 Clinical indication and specimen collection

The examination of hair samples is a very specialised test (for example, referrals to [Great Ormond Street Hospital](#)), but it may help in the diagnosis of a wide range of paediatric disorders. Abnormalities of hair shafts may be seen in conditions such as Menkes, Netherton's, trichothiodystrophy, Griscelli or uncombable hair syndrome.²⁴

Most abnormalities of the shaft are best diagnosed in cut samples of hair as plucking may cause unwanted breakages at the sites of trichorrhhexis invaginata in Netherton's syndrome, thus rendering the sample non-diagnostic. The hairs should be cut close to the scalp.

Disorders of the hair follicle can only be diagnosed on a sample of plucked hair containing the root. There are a number of suggested methods for obtaining such samples, e.g. hair samples may be obtained by plucking. This may be carried out either by gripping fewer than ten hairs between a finger and the thumb, or by gripping a couple of rows of hairs with a needle holder at the base and pulling sharply.⁴²

15.2 Immediate handling

Hairs are dry mounted (without mounting medium) by placing a rectangular frame with double sided sticky tape edges (e.g. Frame-Seal incubation chamber) on a microscope slide. The hairs are lined up in parallel order securing one or both ends to the sticky edge(s) of the frame. A cover glass is then placed over the Frame-Seal containing the strands of hair.⁴²

If Griscelli syndrome is suspected, a mountant (DPX) is used to give a clearer view of the specimen.

15.3 Routine examination

Samples are examined using routine light microscopy and under polarised light. It is important to examine as many strands of hair as possible because not every hair may demonstrate morphological abnormalities in the lengths examined.

15.4 Ultrastructural examination

In some centres, scanning EM is used to define the shape of the hair shaft, but this is technically challenging and not suitable for routine use.

15.5 Report content

The report should include:

- clinical and demographic information received with the biopsy (gender, date of birth, age at biopsy)
- description of the morphological findings
- interpretation of the findings amounting to specific or differential diagnosis. Morphological changes described as being compatible or indicating specific diagnoses must be interpreted in light of appropriate clinical information.

- any recommendation for further investigation (in all patients, a diagnostic test should be undertaken to confirm a specific diagnosis)
- diagnosis where applicable
- optional: comment
- SNOMED codes.

[Level of evidence B – the recommendations in this section are based on a body of evidence demonstrating consistency of results and comprising mainly high-quality systematic reviews of case studies with a very low risk of confounding or bias (see References).]

16 Urine

16.1 Clinical indication and specimen collection

Urine examination can provide a rapid and accurate diagnosis in metachromatic leukodystrophy. A 10 ml sample of fresh urine (not the first of the day) is recommended.

16.2 Immediate handling

Three cytopspins should be prepared and air-dried and fixed in formalin vapour. The samples should be stained overnight in 0.01% Toluidine blue.

16.3 Routine examination

Routine light microscopy reveals crystals of sulphatides, which appear red/brown and should be confirmed to show green birefringence.

17 Other samples

Brain biopsies are rarely undertaken in the investigation of genetic and metabolic disorders in modern practice, but they are occasionally encountered in paediatric patients with undiagnosed encephalopathic illnesses. The samples should be treated as described in section 5, but with an emphasis on preserving material for EM and for viral and other microbiological investigation.

Muscle biopsies are frequently taken in paediatric patients with complex undiagnosed neurological disorders to investigate for mitochondrial disease (see section 2).

[Level of evidence GPP – the recommendations in this section are based on the expert opinions of the authors.]

18 Criteria for audit

As recommended by the RCPATH as key assurance indicators ([Key assurance indicators for laboratory services, 2019](#))

- histopathology cases must be reported, confirmed and authorised within an agreed turnaround time between service providers and service users.

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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 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	2–17
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	2–17
16 The different options for management of the condition or health issue are clearly presented	2–17
17 Key recommendations are easily identifiable	2–17
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	2–17
20 The potential resource implications of applying the recommendations have been considered	Foreword
21 The guideline presents monitoring and/or auditing criteria	18
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