

## Tissue pathways for non-neoplastic neuropathology specimens

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## Contents

|                                                                                                                         |    |
|-------------------------------------------------------------------------------------------------------------------------|----|
| Foreword .....                                                                                                          | 3  |
| 1 Introduction .....                                                                                                    | 3  |
| 2 Skeletal muscle.....                                                                                                  | 6  |
| 3 Peripheral nerve .....                                                                                                | 9  |
| 4 Central nervous system (brain, spinal cord, meninges) incisional or excisional biopsy for non-neoplastic lesion ..... | 11 |
| 5 Brain biopsy for dementia .....                                                                                       | 12 |
| 6 Examination of tissue resections for the treatment of refractory epilepsy due to non-neoplastic lesion .....          | 14 |
| 7 Temporal artery biopsy .....                                                                                          | 16 |
| 8 CSF for cytological assessment.....                                                                                   | 17 |
| 9 Bone and soft tissue from skull or axial skeleton.....                                                                | 18 |
| 10 Samples for investigation of genetic disorders including neurometabolic and paediatric disorders.....                | 18 |
| 10.1 Skin and subcutaneous tissues .....                                                                                | 18 |
| 10.2 Peripheral blood .....                                                                                             | 19 |
| 10.3 Bone marrow .....                                                                                                  | 20 |
| 10.4 Hair .....                                                                                                         | 20 |
| 10.5 Urine .....                                                                                                        | 21 |
| 10.6 Other samples .....                                                                                                | 21 |
| 11 Criteria for audit of the tissue pathway .....                                                                       | 22 |
| 12 References .....                                                                                                     | 22 |
| Appendix A AGREE compliance monitoring sheet .....                                                                      | 23 |
| Appendix B Summary table – Explanation of grades of evidence .....                                                      | 24 |



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](http://www.nice.org.uk/accreditation).  
For full details on our accreditation visit: [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

## Foreword

The tissue pathways published by The Royal College of Pathologists (RCPATH) are guidelines which 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 stakeholders consulted for this document were The British Neuropathological Society and representatives of the British Myology Society.

As with many areas of diagnostic cellular pathology, evidence for these recommendations is not based on systematic, controlled studies. Evidence was evaluated using modified SIGN guidance. The evidence is predominantly classified as: "Good practice point (GPP): Recommended best practice based on the clinical experience of the authors of the writing group" (see Appendix B).

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 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 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.

This pathway has been reviewed by the Working Group on Cancer Services and was placed on the College website for consultation with the membership from 6 October to 3 November 2014. All comments received from the Working Group and membership were addressed by the author to the satisfaction of the Working Group Tissue Pathway Coordinator and the 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 document covers tissue pathways for specific areas within diagnostic neuropathology. The guidance in the first edition 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

Point (see appendix B) is typically expert opinion without explicit critical appraisal, or 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 section 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 cases are covered in The Royal College of Pathologists' *Dataset for tumours of the central nervous system, including the pituitary gland (3rd edition)*.

Post-mortem brain examination is covered by a set of documents have been developed as part of The Royal College of Pathologists. These guidelines are currently being rewritten and are due to be published later in 2015.

The target primary users of the tissue pathway are trainee and consultant cellular pathologists and, on their behalf, the suppliers of IT products to laboratories. The secondary users are surgeons and oncologists, cancer registries and the National Cancer Intelligence Network. Standardised cancer reporting and multidisciplinary team (MDT) working reduce the risk of histological misdiagnosis and help to ensure that clinicians have all of the relevant pathological information required for tumour staging, management and prognosis. Collection of standardised cancer specific data also provides information for healthcare providers, epidemiologists, and facilitates international benchmarking and research.

## **1.1 Staffing and workload**

Central nervous system 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 in order 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.

## **1.2 Laboratory and workplace facilities**

Routine light microscopy, immunohistochemistry, special histochemistry and access to electron microscopy 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. In order to reduce the risk of erroneous diagnoses, the reporting pathologists should have sessional time to attend regular multidisciplinary meetings, during which the pathological findings can be correlated with radiological and clinical findings.

There should be facilities for storage and archiving of fresh-frozen material at -80°C, or in liquid nitrogen, and 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. cytopsin, 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.

### **1.3 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 NCG or NSCAG) referrals must be accompanied by a current NSCT form (completed by the referring clinician), up-to-date clinic letters, muscle biopsy reports and all relevant available information pertaining to the case. Further details and forms are available to download from the websites of the NSCT centres:

[www.gosh.nhs.uk/medical-conditions/clinical-specialties/neuromuscular-information-for-parents-and-visitors/about-us/muscle-biopsy-service/](http://www.gosh.nhs.uk/medical-conditions/clinical-specialties/neuromuscular-information-for-parents-and-visitors/about-us/muscle-biopsy-service/)

[www.newcastle-hospitals.org.uk/services/northern-genetics\\_specialist\\_newcastle-muscle-centre.aspx](http://www.newcastle-hospitals.org.uk/services/northern-genetics_specialist_newcastle-muscle-centre.aspx)

Peripheral nerve and muscle biopsy should include:

- information about the biopsy site and laterality
- age at biopsy (essential)
- details of onset, distribution and progression of the disorder
- involvement of other organs
- results of nerve conduction studies and electromyography or MRI of brain, spinal cord and adjacent nerve roots or muscle (if performed)
- drug history
- occupational exposure/s
- past and current personal and family medical history
- plasma creatinine kinase (CK) level (essential)
- plasma and CSF lactate levels, other biochemical investigations and results of brain, spinal cord and adjacent nerve roots or muscle MRI (if performed).

Where 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 taken
- fresh brain: within 15 minutes of being taken

- nerve: see below
- muscle biopsies must ideally be transported immediately to the lab fresh at room temperature, either wrapped in gauze lightly moistened in saline or wrapped in cling film to prevent drying out. For histopathological studies, this is acceptable for samples with total transit time less than two hours from the time of collection. Samples with transit time exceeding two hours must be frozen on site and transported on dry ice. Samples for electron microscopy 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 no later than 15 minutes to avoid loss of enzyme activity
- 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
- ideally skin samples for histopathological studies should be immediately transported to the lab fresh
- CVS samples for histopathology should be transported in sterile standard culture medium at room temperature within 24 hours.

#### **1.4 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 above). In practice, intra-operative 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.<sup>1</sup>

## **2 Skeletal muscle<sup>1,2</sup>**

### **2.1 Specimen submission and dissection**

- a) Formalin-fixed specimens: these will usually constitute the minority in a specialist neurosciences centre. All specimens in which there is a risk of infection should be formalin-fixed. 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 (see above). After measurement, submit formalin-fixed muscle for paraffin processing with embedding in both transverse and longitudinal orientations. Barring high-risk samples and large open biopsies where it may be impractical to freeze down the entire specimen volume, the routine formalin fixation of biopsies is discouraged as muscle pathology can be focal and formalin fixation can limit histopathological testing, thereby hindering a diagnosis.
- b) The majority of specimens in a clinical neurosciences setting will be delivered either:
  - i) in a fresh state: subdivide for cryostat-sectioning, molecular genetic, biochemical and electron microscopy studies. Fresh samples for cryostat sectioning must be orientated transversely (preferably using a dissecting microscope)
  - or
  - ii) on dry ice, having been snap-frozen (with or without an accompanying piece of tissue in EM fixative): separate pieces (or subdivide) for cryostat-sectioning, molecular genetic and biochemical studies. A minimum specimen diameter of

3–4 mm (size of an orange pip) is recommended for genetic and biochemical studies.

For both i) and ii) above, note the interval between removal of the tissue from the patient and freezing. This will affect the interpretation of histochemical and biochemical (respiratory chain) data.

## **2.2 Embedding options**

- a) Water-based mountant (e.g. 'optimal cutting temperature' OCT) with sample orientated transversely.
- b) Resin for electron microscopy, as appropriate, with at least some samples longitudinally orientated.

## **2.3 Sectioning**

- a) Frozen tissue: cryostat at 8–12  $\mu\text{m}$  (according to stain).
- b) Resin sections: ultra-microtome set at ~0.6–1  $\mu\text{m}$  for semi-thin sections and ~50–90 nm for ultra-thin sections.

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

Cryostat sections: routinely for haematoxylin and eosin (H&E); Gomori 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), PAS/DPAS, acid phosphatase, ATPase at pH 9.5, 4.6 and 4.3, and myosin heavy chain immunohistochemistry (slow, fast, fetal [neonatal] and developmental) and MHC-class I. Myosin immunolabelling has the advantage of identifying fibres expressing more than one isoform and determining if a biopsy is normal or abnormal. (Depending on the preference of the diagnostic service, immunohistochemistry may be used instead of histochemical stains for fibre typing.)

Note: A diagnostic service may prefer to perform a subset of these routine stains first and, based on an initial histological analysis and consideration of clinical features in a case, decide on which additional routine investigations to perform alongside those considered in the next section (below). 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 in the light of experience with 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 may include:

- a. histochemistry for phosphorylase, phosphofructokinase, myoadenylate deaminase, aldolase, menadione NBT (without substrate)
- b. 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  $\alpha 2$  [80 and 300 kDa], laminin  $\alpha 5$ , laminin  $\beta 1$ , and other appropriate

- dystrophin axis associated proteins); beta-spectrin and laminin  $\gamma$ 1 immunohistochemistry for assessing sarcolemmal integrity
- c. Congo red, ubiquitin and p62 if inclusion body myositis or protein aggregation myopathy are suspected or for exclusion, or if rimmed vacuoles are present. Electron microscopy may also be undertaken
  - d. immunohistochemistry for desmin, myotilin, alpha-B-crystallin, FHL-1, BAG-3, filamin A/C if a myofibrillar myopathy is suspected
  - e. immunohistochemistry for MHC Class I (HLA-ABC or  $\beta$ 2-microglobulin); complement membrane attack complex (complement C5b-9 components); CD3; CD4; CD8; CD79a; CD68
  - f. electron microscopy; indications including mitochondrial cytopathy, nemaline myopathy, cores, inclusion body myositis and clarification of features observed with light microscopy. Fixative containing 2.5–6% glutaraldehyde in 0.1M cacodylate or phosphate buffer
  - g. respiratory chain enzyme analysis for suspected mitochondrial cytopathy
  - h. molecular genetic studies; indications including mitochondrial cytopathy
  - i. Western blot analysis; indications including various muscular dystrophies with raised creatine kinase
  - j. specific single-enzyme studies; indications including glycogen storage diseases
  - k. referral to National Specialist Commissioning Team (NSCT) services, as follows:
    - i) Dubowitz Neuromuscular Centre (DNC), London – for suspected congenital myopathy or congenital muscular dystrophy; repertoire of tests includes immunoanalysis of dystroglycans, laminins and collagen VI in muscle, skin and chorionic villus samples; fluorescent activated cell sorting (FACS) quantification in cultured fibroblasts
    - ii) Newcastle: if CK raised and phenotype suggests limb girdle muscle dystrophy, or a myofibrillar myopathy is suspected
    - iii) Oxford: if a myasthenic syndrome is suspected
    - iv) Queen Square, London: if an ion channel disorder is suspected
    - v) NSCT mitochondrial service (Queen Square London, Newcastle, Oxford) if mitochondrial disorder is suspected.

## 2.6 Report content

To include:

- clinical information received with the biopsy
- biopsy dimensions and time of stations in handling, especially time to freeze
- age at biopsy
- 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 above)
- any required pre-referral form



- suggested diagnosis
- conclusion
- SNOMED codes.

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

### 3 Peripheral nerve

#### Introduction and general remarks

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 few motor fibres in the remaining patients.<sup>3</sup> Moreover, 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, 3300–8000 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.<sup>4</sup> The nerve segment should be excised and handled with much care in order to minimise mechanical injury to the sample. Squeezing or stretching the nerve should be strictly avoided and excessive removal of fat or connective tissue should not be attempted. Nerve fibres are very sensitive to mechanical injury.

#### 3.1 Specimen submission and dissection

The recommended length of the biopsied nerve segment is 4 cm. Removal of a shorter segment will be less useful diagnostically and can hamper a proper analysis, but will leave an identical sensory deficit.

- a) Formalin and glutaraldehyde fixation of nerve segments is essential, freezing an unfixed segment is optional.
- b) Submissions received from non-specialist centres can be in fixative, with one part in formalin and a separate piece in EM fixative (containing 2.5–6% glutaraldehyde in 0.1M cacodylate or phosphate buffer). Material fixed in glutaraldehyde containing fixative will be processed for semithin resin sections and potentially for EM and teased fibres. Formalin fixed material will be processed into paraffin wax for histochemical and immunohistochemical studies.
- c) The great majority of specimens derived from clinical neuroscience centres will be delivered fresh. However, the fresh tissue has to be fixed in both formalin and 3% glutaraldehyde containing fixative immediately (at most a 5-minute delay). Therefore the tissue sample should not be transported in the fresh state (unfixed) when the courier time would exceed 5 minutes.
- d) Sub-divide into four segments (in order of priority):
  - formalin-fixation (or equivalent fixatives) and paraffin embedding; with one piece oriented longitudinally and the remainder transversally
  - glutaraldehyde fixation and resin embedding; the glutaraldehyde fixed specimen is cut transversally (longitudinal optionally in addition)
  - glutaraldehyde fixation for teased-fibre preparation
  - optional: snap-freezing.

### **3.2 Embedding options**

Paraffin-processing for routine stains and immunohistochemistry; resin for electron microscopy. Optional preparation of frozen sections.

### **3.3 Sectioning**

Microtome section at approximately 3–4 µm nominal thickness, at multiple levels. Ultra-microtome set at approximately ~0.6 µm for semi-thin sections and ~50–90 nm for ultra-thin sections.

### **3.4 Routine staining**

Microtome sections: routinely for H&E; EVG (or other suitable Trichrome); Congo red; Perl's. Immunohistochemistry for neurofilament protein (e.g. NF200), myelin basic protein (e.g. SMI94), CD68 (macrophages); CD3 (T cells), CD20 (B cells). It is recommended to use immunostainings instead of tinctorial stains for axons and myelin sheath, as they are more specific and give a better signal to noise ratio.<sup>5</sup>

It is highly recommended to prepare semithin sections from glutaraldehyde fixed material (stained for example with Toluidine blue or Methyl Blue Aniline–Basic Fuchsin [MBA-BF]) as standard, as it allows superior morphological assessment of a large range of pathological features.

### **3.5 Further investigations**

According to clinical indications or pathological findings, supplementary:

- a) histochemistry for fibrin
- b) epithelial membrane antigen for assessment of the perineurium
- c) immunohistochemistry for further immune cell subsets, e.g. CD79a (B cells), CD8, CD4, (T cells), CD 138 (plasma cells)
- d) electron microscopy
- e) teased fibres
- f) Wade-Fite (leprosy).

### **3.6 Report content**

To include:

- clinical information received with the biopsy
- biopsy dimensions and time of stations in handling
- 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
- any required pre-referral form

- conclusion
- SNOMED codes.

*[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 below) – Level of evidence B].*

## **4 Central nervous system (brain, spinal cord, meninges) incisional or excisional biopsy for non-neoplastic lesion<sup>2</sup>**

### **4.1 Specimen submission and dissection**

Usually, specimens will be received either in formalin or fresh, determined mainly but not exclusively by a requirement for intra-operative diagnosis. Specimens should be resected as a single piece, measuring at least 1x1 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 cortical grey matter in order to maximise the diagnostic options.

### **4.2 Immediate handling and intra-operative diagnosis**

Check for infection-risk label and also assess risk from clinical information provided.

If submitted material is minute, discuss with surgeon whether management will really be altered by intra-operative provisional diagnosis, bearing in mind that it might sacrifice material that would otherwise be utilised for a definite paraffin-based tissue diagnosis.

If request for intra-operative diagnosis is firm, consider smear-preparation and/or frozen section (FS). FS will have relatively greater value if the tissue texture precludes making an effective smear. Also, the block of frozen tissue can be fixed and processed through paraffin afterwards.

Issue a verbal report, making written record of the exact wording, the recipient and time the report was communicated.

If infection is possible, check that the surgeon has submitted separate material to microbiology. Do so oneself if this has not been done, the operative field has been closed and material is still unfixed.

Set aside tissue for freezing (further morphological investigations on frozen tissue, molecular genetics and tissue bank), electron microscopy and adjacent-tissue paraffin-processing, all according to diagnostic needs and terms of consent.

### **4.3 Embedding options**

Paraffin-processing for routine stains and immunohistochemistry; resin for electron microscopy. Formic acid treatment on a proportion of the sample with prion disease 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 section at 4 µm, at multiple levels. Ultra-microtome set at ~0.6 µm for semi-thin sections and ~50–90 nm for ultra-thin sections.

## 4.5 Routine staining

Microtome sections: routinely for H&E.

## 4.6 Further investigations

According to clinical indications or pathological findings, useful supplementary:

- a) if vascular malformation or evacuated blood clot, typically apply Congo red; Perls'; elastic van Gieson or equivalent. A $\beta$  immunohistochemistry for amyloid angiopathy is strongly recommended as it is more sensitive than Congo red
- b) if non-neoplastic space occupying lesion:
  - i) if looks infective/inflammatory: ZN, Gram, Grocott, PAS+D; add Toxoplasma immunohistochemistry, as appropriate
  - ii) consider viral aetiology: HSV, CMV, measles, EBV, HIV
  - iii) if there is evidence of demyelination: myelin (myelin basic protein e.g. IHC: SMI94; histochemistry: Luxol fast blue/cresyl violet) and axonal (NFP) stain; CD68; GFAP. If PML possible, perform IHC or *in-situ* hybridisation for polyoma virus (SV40)
  - iv) EM if material available in appropriate fixative (glutaraldehyde) and DNA/RNA extraction for viral, bacterial (e.g. M. tuberculosis), fungal PCR (note that also possible from paraffin-embedded material).

## 4.7 Report content

To include:

- clinical information received with the biopsy
- biopsy dimensions
- description of the histological 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, bacterial or viral reference lab)
- conclusion
- SNOMED codes.

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

## 5 Brain biopsy for dementia

### 5.1 General comments<sup>6-9</sup>

Brain biopsy for dementia is typically considered when clinical or laboratory features suggest a reversible process such as vasculitis or infection in the differential diagnosis. Possible diagnoses include Creutzfeldt-Jakob disease; other neurodegenerative dementias; inflammatory disease; cerebrovascular disease including cerebral amyloid angiopathy; multiple sclerosis; neoplasm; leukodystrophy; storage disorders; undetermined encephalopathies. Diagnostic yield ranges from 22–84%.<sup>4</sup>

## **5.2 Specimen submission and dissection**

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

## **5.3 Immediate handling**

- a) Check for infection-risk labels, and also assess risk from clinical information provided. Handle in appropriate containment if CJD is a possibility.
- b) Assess for adequacy: cerebral full thickness including leptomeninges, grey and white matter.
- c) Set aside tissue for freezing (molecular genetics; prion biochemistry), electron microscopy and adjacent-tissue paraffin-processing, all according to diagnostic needs.
- d) If prion disease is in the clinical differential diagnosis, divide specimen into three equal portions: two thirds to be immediately fixed in formalin and one portion to be frozen and preserved for immunoblotting, which will enable strain typing in a specialist centre, such as the CJD Surveillance Unit in Edinburgh. If the specimen is small, consider fixing the entire specimen in formalin in order to maximise morphological diagnosis. After overnight formalin-fixation, subject part of the tissue to one hour immersion in 98% formic acid and subsequent formalin post-fixation, to reduce (potential) prion infectivity. Next, process this tissue through paraffin and use it for immunohistochemical study with a wide range of markers including prion protein. Some markers may not work as the antigens may be denatured by formic acid treatment. If prion disease is confirmed, the second tissue fragment will have to be formic acid treated and paraffin processed for archiving. If prion disease was excluded, the second, formalin-fixed tissue may be used for further tests to explore other differential diagnoses.

## **5.4 Embedding options**

Paraffin-processing for routine stains and immunohistochemistry; resin for electron microscopy.

## **5.5 Sectioning**

Microtome section at 4 µm, at multiple levels. Ultra-microtome set at ~0.6 µm for semi-thin sections and ~50–90 nm for ultra-thin sections.

## **5.6 Routine staining**

Microtome sections: routinely for H&E.

## **5.7 Further investigation**

According to clinical indications or pathological findings:

- special histochemical stains:
  - Luxol fast blue/cresyl violet (neuronal architecture)
  - periodic acid-Schiff (granular deposits in the vessels)
  - MSB (fibrinoid necrosis of vessels)
  - Congo red (amyloid in the vessels and parenchyma)

- immunohistochemistry:
  - glial fibrillary acidic protein (GFAP)
  - Tau (for example antibody AT8)
  - neurofilament protein cocktail (70 and 200 kDa)
  - phosphorylated neurofilament
  - A $\beta$  peptide
  - prion protein
  - ubiquitin
  - p62, alpha-synuclein, TDP-43 and FUS protein.

Consider also CD20 B-cell marker, CD3 T-cell marker, CD68 (microglia/macrophage/monocyte marker).

Consider polyoma virus antigen immunohistochemistry or *in-situ* hybridisation if progressive multifocal leuko-encephalopathy (PML) is suspected.

## 5.8 Report content

To include:

- clinical information received with the biopsy
- biopsy dimensions
- description of the histological 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)
- conclusion
- 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; see section 3.6. and references below].*

# 6 Examination of tissue resections for the treatment of refractory epilepsy due to non-neoplastic lesion<sup>10</sup>

## 6.1 Specimen collection

Specimens will typically be received by the laboratory either in formalin or fresh. Intra-operative smears may be required in cases where 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 pre-operative clinical pathological consultation can aid tissue sampling and diagnostic interpretation, in particular regarding history of invasive EEG recordings prior to resective surgery.

## **6.2 Intra-operative diagnosis and immediate handling**

Check for infection-risk label and also assess risk from clinical information provided. Representative tissue is set aside for freezing (molecular genetics, tissue bank) and electron microscopy 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.

## **6.3 Embedding options**

Paraffin-processing for routine stains and immunohistochemistry; resin for electron microscopy.

## **6.4 Sectioning**

Microtome section at 4 µm, at multiple levels. Thicker sections are useful for assessment of cortical architecture, for example in Nissl and in NeuN stained sections in cases of suspected cortical dysplasia.

## **6.5 Routine staining**

Microtome sections: H&E stains as routine and LFB/Nissl stain to delineate the anatomy of hippocampal subfields, amygdala and assessment of neocortical architecture where this is required by the context of the case and adequacy of the material.

## **6.6 Further investigations**

Special stains and immunohistochemistry are carried out according to initial findings on H&E sections. As examples:

- a) NeuN, neurofilament, GFAP, MAP2, CD34 and vimentin stains are useful in the assessment of focal cortical dysplasia<sup>11</sup>
- b) GFAP in the confirmation of hippocampal sclerosis, NeuN in the assessment of granule cell dispersion and dynorphin immunohistochemistry in the identification of axon sprouting<sup>12</sup>
- c) if low-grade tumour is suspected, tumour datasets and appropriate immunohistochemistry panel should be followed
- d) inflammatory markers in suspected encephalitis, viral markers where appropriate.

## **6.7 Report content**

To include:

- clinical information received with the biopsy
- biopsy dimensions
- description of the histological 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; for example in small, poorly orientated or incomplete and fragmented hippocampal specimens confirmation or subtyping of the pattern of hippocampal sclerosis may not be possible<sup>12</sup>
- conclusion
- SNOMED codes.

*[Level of evidence B; see section 3.6. and references below].*

## **7 Temporal artery biopsy**

See the relevant section in The Royal College of Pathologists' *Tissue pathways for cardiovascular pathology* (2008).

### **7.1 Fixation and embedding options**

Fix in formalin and embed in paraffin. The artery should be oriented transversely.

### **7.2 Sectioning**

Microtome section at 4 µm, at multiple levels.

### **7.3 Routine staining**

Analyse the biopsy on multiple transversely oriented levels stained with H&E and elastin van Gieson (to look for vessel wall destruction and internal elastic lamina disruptions and duplications).

### **7.4 Further investigations**

If inflammation is identified characterise further by immunohistochemistry for the inflammatory markers (CD3, CD20, CD68), particularly for recognising steroid-treated temporal arteritis.<sup>13</sup>

### **7.5 Report content**

To include:

- clinical information received with the biopsy
- biopsy dimensions
- description of the histological 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
- conclusion
- SNOMED codes.

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



## **8 CSF for cytological assessment**

The purpose of CSF cytology is primarily 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. CSF examination by microbiology, immunology and chemistry laboratories has important roles in the investigation of non-neoplastic disease. CSF cytology can more accurately identify cell types, morphology and differential counts with direct visualisation and flow studies, which are important in the diagnosis of non-neoplastic disease, for example neutrophilia in most causes of meningitis, or mixed neutrophils and lymphocytes in sarcoidosis and tuberculosis, lymphocytes in encephalitis.

### **8.1 Specimen submission and immediate handling**

Specimens may be received as fluid from wards, theatre or from other diagnostic department, when multiple tests are being performed on CSF (e.g. cell count, protein concentration). Specimen should be handled in a microbiological safety cabinet. For high-risk samples, consider decontamination steps following local procedures and policy.

### **8.2 Preparation – options and routine staining**

CSF needs to be adequately re-suspended before processed for Cytospin to yield a standardised volume with high white blood cell counts. The speed, time and acceleration of the machine has to be adjusted so that an optimum yield of morphologically preserved cells in a monolayer can be obtained. Additional slides may be prepared for subsequent immunohistochemical tests, for example to confirm the epithelial nature of atypical cells. The number of additional slides can be adapted to the cell count, which should be done prior to the CSF Cytospin.

- i. Giemsa preparation on one slide. Prepare an additional two or more slides and methanol-fix, when fluid volume is sufficient.
- ii. Giemsa preparation on one slide. If clinical differential diagnosis includes neoplastic spread in the CNS, prepare several spare slides if possible to enable subsequent immunohistochemical stainings to characterise atypical cells.
- iii. High red blood cell counts are usually an indication of blood contamination.

### **8.3 Additional investigations**

In some cases, further special stains (e.g. gram, PAS, Grocott and Ziehl Neelsen) may be required for investigation of infectious diseases. Perl's stain should be done where the clinical suspicion is superficial siderosis. Mucicarmine or PAS or other adequate stains should be done 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**

To include:

- clinical information received with the cytology fluid
- cell counts (total red blood cells and white blood cells); description of the cytological findings
- interpretation of the findings amounting to specific or differential diagnosis. The reports include a descriptive report of the cells present, whether extraneous cells or contaminants are present (e.g. chondrocytes in lumbar puncture specimens or glial

tissue in external ventricular drainage (EVD) specimens) and if any atypical cells are seen. If no atypical cells are visualised, this is also recorded

- 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 is also indicated on the report to alert the clinician. In addition, in cases where there has been any delay in processing (for example if the specimen has been misdirected) with deterioration of specimen, a note on the quality of the specimen is included for the clinician and the possibility of an additional specimen may be suggested
- comparison with concurrent or previous biopsy or CSF examination
- conclusion
- SNOMED codes.

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

## **9 Bone and soft tissue from skull or axial skeleton**

See The Royal College of Pathologists' *Tissue pathways for bone and soft tissue pathology* (2011).

## **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 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.

### **10.1 Skin and subcutaneous tissues**

See The Royal College of Pathologists' *Tissue pathways for inflammatory and non-neoplastic dermatoses and non-neoplastic lesions* (2008) for general diagnostic approaches.

Samples for investigation of suspected:

- neuronal ceroid lipofuscinosis (including Batten's disease, Kuf's disease) fixation in glutaraldehyde with epoxy resin embedding for electron microscopy. Skin formalin fixation, wax embedding and IHC for subunit C of mitochondrial ATP synthase and saposin D (sphingolipid activating protein D)
- Lafora body disease: skin bearing apocrine glands (preferable) or eccrine glands formalin fixation wax embedding with staining to detect inclusions, PAS plus and minus diastase and/or Lugol's iodine. Fixation in glutaraldehyde with epoxy resin embedding for electron microscopy. Photography for documentation recommended as the Lugol stain may fade rapidly
- Niemann-Pick disease type C: fixation in glutaraldehyde followed by epoxy resin embedding for electron microscopy. Frozen tissue for fibroblast culture (mandatory). Formalin fixation and paraffin embedding for light microscopy (to look for characteristic but non-specific foam cells) only if plenty of tissue<sup>10</sup>

- CADASIL: skin formalin fixation and paraffin wax embedding. Fixation in glutaraldehyde with epoxy resin embedding for electron microscopy to look for granular osmiophilic material. Stain paraffin embedded tissue for PAS to look for granular deposits in the media of small arteries and arterioles. Immunostaining with antibody to the extracellular domain of NOTCH3 is possible
- rabies: nuchal skin biopsy, immunostaining for rabies virus. Skin biopsy specimen of 5–6 mm in diameter, taken from the back of the neck at the hairline, and contain at least 10 hair follicles ([www.cdc.gov/rabies/specific\\_groups/doctors/ante\\_mortem.html](http://www.cdc.gov/rabies/specific_groups/doctors/ante_mortem.html)). 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 because of its neurotropic nature. For more information on rabies in the UK see <https://www.gov.uk/government/collections/rabies-risk-assessment-post-exposure-treatment-management>.

## **10.2 Peripheral blood**

### **10.2.1 Clinical indication and specimen collection**

The examination of blood films is only offered in a small number of specialist centres but provides a very rapid and, in several diseases, very specific test. The clinical context will usually be in the investigation of a suspected storage disorders, principally lysosomal storage disorders (LSD), for example Batten's disease (neuronal ceroid lipofuscinosis), Salla disease, I cell disease,  $\beta$  galactosidase deficiency (GM1 gangliosidosis), mucopolysaccharidoses, Niemann-Pick disease, fucosidosis, mannosidosis, Wolman's disease, and certain glycogenoses. For example, it can provide a specific diagnosis in Wolman's disease and some neuronal ceroid lipofuscinoses (NCL) or can provide a diagnosis where no biochemical test is available (some forms of NCL).

### **10.2.2 Specimen handling**

A sample of EDTA preserved whole blood should be received by the laboratory. Heparinised whole blood is also acceptable. Check for infection-risk label and also assess risk from 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.

### **10.2.3 Routine examination**

The blood film should be examined at 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 regard to the detection and morphological characteristics of the lymphocytes. The optimal number of lymphocytes to be examined is uncertain but if less than 20 are seen in the first film, a second film should be examined.<sup>15</sup>

### **10.2.4 Ultrastructural examination**

A buffy coat should be prepared for electron microscopy 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 electron microscopy). Ultrastructural examination of inclusions in lymphocytes may allow a specific diagnosis and has particularly aided the identification of variant subtypes of NCL. At least 100 lymphocytes should be examined, as in some variants of NCL, only a minority contain diagnostic material.<sup>16</sup>

## 10.2.5 Report content

To include:

- clinical information received with the sample
- 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.
- conclusion
- SNOMED codes.

*[Level of evidence B; see section 3.6. and references below].*

## 10.3 Bone marrow

Bone marrow aspirate is helpful in the diagnosis of some lysosomal storage disease, e.g. where 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

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

## 10.4 Hair

### 10.4.1 Clinical indication and specimen collection

The examination of hair samples is a very specialised test but may help in a wide range of paediatric disorders. Abnormalities of hair shafts may be seen in conditions such as Menkes, Netherton's, trichothiodystrophy, Chediak-Higashi, Griscelli, or uncombable hair syndrome.<sup>17</sup>

Most abnormalities of the shaft are best diagnosed in cut samples of hair as plucking may cause unwanted breakages at the sites of trichorrhexis 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 less than 10 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.<sup>17</sup>

### 10.4.2 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; Hybaid, Basingstoke, Hampshire, UK) 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.<sup>17</sup>

If Chediak-Higashi or Griscelli syndrome is suspected, a mountant (DPX) is used to give a clearer view of the specimen.<sup>11</sup>

#### **10.4.3 Routine examination**

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.

#### **10.4.4 Ultrastructural examination**

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

#### **10.4.5 Report content**

To include:

- clinical information received with the sample
- 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 the light of appropriate clinical information
- any recommendation for further investigation. In all patients, a diagnostic test should be undertaken to confirm a specific diagnosis
- conclusion
- SNOMED codes.

*[Level of evidence B; see section 3.6. and references below].*

### **10.5 Urine**

#### **10.5.1 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.

#### **10.5.2 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.

#### **10.5.3 Routine examination**

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

### **10.6 Other samples**

Brain biopsies are rarely undertaken in the investigation of genetic and metabolic disorders in modern practice but are occasionally encountered in paediatric patients with undiagnosed encephalopathic illnesses. The samples should be treated as above but with an emphasis on preserving material for electron microscopy 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 above).

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

## 11 Criteria for audit of the tissue pathway

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

In addition, audits should be performed against The Royal College of Pathologists' key performance indicators (KPIs) (see *Key Performance Indicators – Proposals for implementation* [July 2013] on [www.rcpath.org/clinical-effectiveness/kpi/KPI](http://www.rcpath.org/clinical-effectiveness/kpi/KPI)):

- 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 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 |
|----------------------------------------------------------------------------------------------------------|---------------------------|
| <b>SCOPE AND PURPOSE</b>                                                                                 |                           |
| 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.                      | Foreword                  |
| <b>STAKEHOLDER INVOLVEMENT</b>                                                                           |                           |
| 4. The guideline development group includes individuals from all 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.                                                    | 1                         |
| <b>RIGOUR OF DEVELOPMENT</b>                                                                             |                           |
| 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.                   | 12                        |
| 13. The guideline has been externally reviewed by experts prior to its publication.                      | Foreword                  |
| 14. A procedure for updating the guideline is provided.                                                  | Foreword                  |
| <b>CLARITY OF PRESENTATION</b>                                                                           |                           |
| 15. The recommendations are specific and unambiguous.                                                    | 4–11                      |
| 16. The different options for management of the condition are clearly presented.                         | 4–11                      |
| 17. Key recommendations are easily identifiable.                                                         | 4–11                      |
| 18. The guideline is supported with tools for application.                                               | 2–10                      |
| <b>APPLICABILITY</b>                                                                                     |                           |
| 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.                | 1                         |
| 21. The guideline presents key review criteria for monitoring and/audit purposes.                        | 11                        |
| <b>EDITORIAL INDEPENDENCE</b>                                                                            |                           |
| 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 the Director of Communications that there is no reason to consult directly with patients or the public regarding this document 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.

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