The Royal College of Pathologists
Pathology: the science behind the cure

Tissue pathways for bone and soft tissue pathology

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Foreword

The tissue pathways published by The Royal College of Pathologists (RCPath) are guidelines that enable pathologists to deal with routine surgical specimens in a consistent manner and to a high standard. This ensures that accurate diagnostic and prognostic information is available to clinicians for optimal patient care and ensures appropriate management for specific clinical circumstances. 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 be deemed negligent or a failure of duty of care.

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

The following stakeholders were contacted to consult on this document:

- National Musculoskeletal Pathology EQA
- British Orthopaedic Association
- British Society for Rheumatology
- Institute of Biomedical Scientists.

The information used to develop this tissue pathway was obtained by undertaking a systematic search of electronic searches of the medical literature, by reviewing previous recommendations of the RCPath and by reviewing local guidelines in the United Kingdom. Key terms searched included synovial, biopsy, fluid, analysis, soft tissue, tumour, histopathology, dissection, bone in various combinations and dates searched from May 2016 to October 2022. Published evidence was evaluated using modified SIGN guidance (see Appendix A). Consensus of evidence in the guideline was achieved by expert review. Gaps in the evidence were identified by College members via feedback received during consultation.

Such is the nature of histopathology that much of the evidence for the way in which practitioners approach the way they work is based on shared experience of working in specialised fields. As such, much of the evidence base for the processes and procedures described in this tissue pathway reaches GPP (good practice point; see Appendix A). Where this is not the case, the level of evidence is given in the text.

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

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 sub-specialty advisor to the College, to consider whether 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 short notice of change will be incorporated into the pathways and the full revised version (incorporating the changes) will replace the existing version on the publications page of the College.

The pathway has been reviewed by the Professional Guidelines team, Working Group on Cancer Services and Lay Advisory Group and was on the College website for consultation with the membership from 11 January to 8 February 2023. 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 authors of this document have declared that there are no conflicts of interest.

1 Introduction

This document is designed to assist all histopathologists and cytopathologists to achieve best practice in handling samples of bone, joints and other soft tissues sent for pathological assessment. It must be taken in conjunction with the datasets on bone and soft tissue sarcomas in helping the pathologist best assist clinicians in developing the most appropriate management plan for patients with diseases of bones, joints and skeletal soft tissues.

In addition, it touches on areas of specialist pathology, such as the handling of tissue for metabolic bone biopsies, as pathologists in non-specialist units might be required to assist in fixing or processing tissue for despatch to specialist laboratories.

Specimen handling may be modified when pathological tissue is derived from specific sites that fall within the province of other specialist pathologists (e.g. head and neck, oral and maxillofacial pathologists).

1.1 Target users and health benefits of this tissue pathway

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 clinicians who request examination of tissue and cytological samples.

2 Generic issues relating to staffing, workload and facilities

The following recommendations should be met for a general level of acceptable practice:

- the diagnostic laboratory should have sufficient pathologists, biomedical scientists and clerical staff to cover all of its functions. In general, staffing levels should follow the workload guidelines of the College.
- pathologists should:
  - participate in audits
  - participate in the College's continuing professional development (CPD) scheme
  - participate in relevant external quality assessment (EQA) schemes of a general or specialist nature
  - have access to specialist referral opinions on a regional network or national basis.

2.1 Staffing and workload

Samples of tissue from bone, joints and other soft tissues are part of the routine biopsy material received in almost all histopathology laboratories. This guideline is designed to assist pathologists with processing and reporting such specimens. Ideally, there should also be strong links with a local/regional specialist centre, with two or more pathologists specialising in bone and soft tissue pathology, at least one of whom should participate in the National Musculoskeletal Pathology EQA scheme, and with other specialists such as paediatric, dental and neuropathologists with skills in specific areas of bone and joint pathology. As with many areas of histopathology, the nature of musculoskeletal pathology is such that all histopathologists should be comfortable with making common diagnoses and know when they should refer cases to specialists.

The College's *Guidelines for Staffing and Workload in Histopathology and Cytopathology Departments*[1] is a useful benchmark of the time and resources required to undertake this work, but workload will vary with the nature of the material submitted, the type of front-line clinicians supported by the pathology department and the balance between non-tumour and tumour pathology.

For units handling large numbers of bone specimens, biomedical scientist staff with specialist skills in the use of band saws, tissue decalcification and handling of large tissue blocks are essential. These are also skills that are necessary for other subspecialties, including head and neck pathology and haematopathology.

Plastic/resin embedding and microtomy of undecalcified bone tissue has become less commonplace in recent years, with dwindling expertise in the UK. This has caused some difficulty in identifying which laboratories can perform histomorphometry on bone samples for metabolic disease investigation.

For laboratories handling synovial fluid cytology specimens, biomedical scientist staff with skills in a range of cytological techniques, such as cell counting and cytocentrifugation, are required. Biomedical scientist staff should understand the health and safety requirements of handling unfixed cytology specimens.

Specialist microscopic techniques, such as quantitation (e.g. differential cell counts on cytocentrifuge preparations, bone histomorphometry) and the use of polarising microscopy, may be used by bone and soft tissue pathologists to extract the maximum data from certain specimens.

Synovial fluid analysis should be co-ordinated with cytology laboratory workflows and regulatory accreditation.

2.2 Laboratory facilities

In specialist centres, pathologists should be supported by a fully equipped laboratory to allow the recommended technical procedures to be performed safely. The laboratories should also:

- be accredited by United Kingdom Accreditation Service (UKAS) or equivalent
- participate in the UK national EQA scheme for cellular pathology technique
- participate in the UK national EQA scheme for immunocytochemistry and fluorescence in situ hybridisation (FISH) when these techniques are used in the diagnostic pathway.

In addition to routine laboratory facilities, there should be access to comprehensive immunohistochemistry, molecular pathology diagnostics and, preferably, electron microscopy. Non-specialist laboratories should have formal links to specialist centres for complex musculoskeletal tissue specimens.

For bone and soft tissue specimens, recommended laboratory equipment/technology includes:

- appropriate saws. It is recommended that bone specimens should be cut using machine saws to achieve the best quality bone slices; cutting saws (i.e. butcher's bandsaws) are recommended for most specimens and are essential for larger specimens; grinding/non-cutting saws (i.e. bandsaws with diamond-coated blades) are recommended for small, very hard specimens (e.g. teeth, metalwork-containing bone). The pathologist should decide and lead on the most appropriate macroscopic dissection approach for each individual bone resection specimen.
- specimen x-ray machine for bone cases

- safe facilities and standard operating procedures for:
  - dissecting specimens incorporating bone
  - storage of specimens that, once coarse trimmed, require further fixation or decalcification
  - decalcification
  - non-standard processing (including long- and short-cycle wax embedding and plastic embedding)
  - sectioning large wax embedded blocks and various sizes of resin embedded tissue blocks.

Pathologists reporting connective tissue specimens should have access to polarising microscopy. It is good practice for the polarising microscope to have a quarter wave (interference) plate in the light path, as this is essential for differentiating between the various crystal arthropathies.

Pathologists working on dynamic metabolic bone disease specimens should have access to fluorescence microscopy to detect the tetracycline uptake used as a biomarker of mineralisation. Digital image analysis software is required for bone histomorphometry.

Reports should be held on an electronic database (usually a laboratory information management system [LIMS]) that has facilities to search and retrieve specific data items and that is indexed according to Systematised Nomenclature of Medicine Clinical Terms (SNOMED-CT) or older versions of SNOMED T, M and P codes. It is acknowledged that some of the existing laboratory information systems may not meet this standard; however, the ability to store data in this way should be considered when laboratory systems are replaced or upgraded.

Workload data should be recorded in a format that facilitates the determination of the resources involved and which, if applicable, is suitable for mapping to Healthcare Resource Groups. A number of modern LIMS are capable of storing such data and allow their retrieval for workload assessment.

[Level of evidence – GPP.]

2.3 Tissue receipt and handling

Tissue is usually received in formalin.[2] Large specimens (e.g. amputations) and specimens of bone (e.g. articular surfaces or lengths of bone) fix poorly and need to be dissected, incised or coarse trimmed using a cutting (i.e. butcher's) bandsaw soon after receipt and then left for further fixation prior to generation of tissue blocks. If crystal deposition disease is suspected clinically, samples should be received in absolute alcohol, since monosodium urate crystals are water soluble and are dissolved in formalin. Subsequent processing steps should also be performed using anhydrous solutions. Samples for plastic embedding and sectioning undecalcified should be received in absolute alcohol or a neutral buffered fixative.

An effort should be made, wherever possible, to freeze tissue routinely. This should be recorded. This will allow future molecular genetic studies for diagnostic or research/clinical trials purposes. This is particularly important given the drive from NHS England to offer whole genome sequencing (WGS) routinely. WGS is currently offered as a diagnostic test for bone and soft tissue sarcomas. Importantly, the archiving of frozen diagnostic material does not require additional ethical approval. The use of frozen tissue for research and clinical trials is subject to appropriate ethical, clinical and research governance frameworks.

[Level of evidence – GPP.]

2.4 Decalcification

There are many factors that need to be considered when optimising decalcification.[2] The pathologist must decide which specimens require decalcification and, if decalcification is to be undertaken, at what stage in the process of trimming. In particular, the pathologist must decide whether to cut the tissue to final block size before or after decalcification. The former requires the use of a cutting bandsaw (or a diamond, grinding saw if the specimen is very hard, i.e. contains teeth or metal). Smaller bone specimens may be decalcified intact, if it is determined that prior adequate tissue fixation can be achieved and then subsequently trimmed. Laboratory practice should follow the bone pathologist's guidance in this respect.

If tumour is suspected or identified during trimming, tumour tissue (fresh, if possible, or fixed) should be obtained prior to decalcification, particularly if immunohistochemistry or in situ hybridisation are to be undertaken, as assessment may be impaired on acid decalcified tissue (e.g. HER2 assessment may be impaired, particularly for in situ hybridisation). This may require blunt scraping or chipping if necessary; tiny fragments of calcified bone within a specimen do not prevent it being sectioned. Appropriate provision for sampling of unfixed tissue is important for tumours where next generation sequencing or other molecular pathology studies require fresh tissue. Specialist centres regularly receiving tumour specimens should endeavour to put pathways in place to allow this.

Choice of decalcification agent should be geared to the type of specimen and the clinical question/pathology being investigated by the clinician or pathologist. Generally, proprietary decalcifying agents should be avoided unless their effects on antigens and the cell walls of infective organisms are fully understood and completely reproducible. Generic decalcifying agents include chelating agents (e.g. ethylenediaminetetraacetic acid (EDTA)) and 5–10% formic acid. Strong acids, e.g. 5–10% nitric acid or hydrochloric acid, should not be used as they cause significant tissue damage.[3] For larger specimens of bone, to achieve decalcification in an acceptable time period, a slab should be cut using a butcher's bandsaw and then subjected to formic acid decalcification.

Chelating agents take longer to decalcify bone specimens and the agents require regular refreshing. Such agents preserve tissue well for immunohistochemistry, tinctoral histochemistry for organisms and in situ hybridisation techniques.

For most circumstances, the agent of choice is formic acid as this provides a useful compromise between chelating agents and nitric acid. The concentration can vary from 5–10% in sodium citrate buffer. Uniform and more rapid decalcification can be achieved if the specimen is continuously agitated (e.g. by being placed on a roller bed).

Surface decalcification of the tissue block may be required if tissue decalcification is incomplete (this is often focal within the block) or small amounts of unsuspected calcified material are present within the tissue (this is not uncommon in synovial biopsies). This can be achieved by placing gauze soaked in 10% formic acid on the sample or dipping the surface of the wax block in 10% formic acid for about 10 minutes.

Assessing when the decalcification process is complete can be achieved in a number of ways. The best, but most time-consuming, way is radiography, for which purpose a specialist small laboratory x-ray unit is required. This unit has other uses such as assessment of fracture, localising areas of pathological bone sclerosis and/or loss and identifying soft tissue calcification in tissues submitted to the laboratory. Other methods of assessing completion of decalcification include palpation, trial incision with a scalpel, and/or ammonium hydroxide precipitation test.

2.5 Frozen sections

Difficulties in the diagnosis of long-standing infection of prostheses has led to the use of intra-operative frozen sections to look for neutrophils as a diagnostic technique.[4] When considering offering this as a service, it must be recognised that identifying small numbers of neutrophils in frozen sections, particularly in synovium containing prosthetic debris, can be difficult. This is the only regular use of frozen sections in the diagnosis of non-tumour pathology in this field.

Frozen sections may also be employed, by some units, for tumour diagnosis.

[Level of evidence – Grade D and GPP.]

2.6 Target users of this guideline

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 senior, specialist biomedical scientist staff, as indicated above.

3 Synovial biopsy

3.1 Specimen dissection

Most of these specimens are obtained arthroscopically and, in consequence, are often very small. These should be blocked together without further orientation. As with all very small specimens, care should be taken not to lose any tissue (the use of filter paper or small mesh bags should be considered).

Less frequently, larger pieces of synovium are received. It is usually obvious which aspect is the synovial surface as it has a frond-like/non-smooth appearance. These samples should be orientated to include the full thickness of the synovium and underlying tissue. These specimens should be measured in three dimensions and the colour of such specimens should be recorded, particularly if nodular synovitis, ochronosis or haemarthrosis is suspected.

It is increasingly common for fragments of synovium to be received from patients undergoing joint replacement revision. It is important to ensure that the synovial surface is included in the block (to assess for the presence of a polymorph infiltrate, the indicator of bacterial infection) and to ensure that large pieces of debris are removed as they interfere with microtomy. If necessary, the pieces of debris can be blocked separately or removed from the surrounding tissue and then submitted for histological analysis.

Aseptic lymphocyte-dominated vasculitis-associated lesions are an allergic reaction to metal debris that has been described in patients with metal-on-metal hip resurfacing implants.[5] Orientation of the synovium/periprosthetic tissue can assist in making the histological diagnosis.

If white chalky material is seen, suggestive of crystal deposition, some can be removed from the tissue with a needle and smeared onto a slide for examination under polarised light once dry.

[Level of evidence – Grade D and GPP.]

3.2 Sectioning

Routinely, a single section is usually sufficient for diagnostic purposes but, when the specimen is small, it is appropriate to have more than one level on each slide.

3.3 Staining

Haematoxylin and eosin (H&E) stained sections are required in all cases. Stains for organisms and amyloid may, on occasion, be required.

3.4 Report content

The report must record the presence and degree, or absence, of synovial hyperplasia as well as the distribution and nature of any inflammatory cell infiltrate. The presence/absence of haemosiderin, crystals, bone or cartilage fragments, foreign material or granulomatous disease should be recorded. Any infective agents or features suggestive of infection (e.g. a heavy polymorph infiltrate) should be recorded and commented upon; it is good practice to state that there is no evidence of infection when reviewing material from revision arthroplasties.

[Level of evidence – Grade D and GPP.]

4 Suspected benign soft tissue neoplasms

4.1 Specimen dissection

It may be difficult to define, with accuracy, whether some soft tissue swellings are benign, malignant or even neoplastic on radiographic imaging. Histological assessment of suspected neoplasms may be undertaken by performing needle biopsy, open biopsy or excision biopsy of the lesion. Fine needle aspiration is not commonly applied as many diagnoses rely on architectural as well as cytological appearance. In each of these specimen types, the tissue pieces should be counted and measured. For needle biopsies, the length of the core(s) is the key measurement. Open and excisional biopsies are measured in three dimensions.

Needle biopsies are processed in their entirety, preferably dividing the tissue cores into multiple cassettes to maximise tissue availability for ancillary studies.

Open biopsies are also submitted in their entirety. Where possible, it is good practice to divide these into multiple cassettes for the same reason as for needle biopsies.

Until histologically confirmed as benign, excision biopsies are best treated as potentially representing a malignant tumour and the procedures for handling and block selection should follow those described for sarcomas.[6] If the specimen has been orientated by the surgeon, this should be recorded in the report.

There is a debate around inking the surface of excision specimens, as ink may permeate through the first millimetre or so of connective tissue, giving false positive margins. While many regard it as good practice, this should be at the pathologist's discretion/preference.

It is best practice to take one block per centimetre length of longest axis of homogenous appearing swellings; heterogenous lesions should be more extensively sampled to obtain representative areas. It is essential to sample resection margins for excision specimens as benignity cannot necessarily be known in advance.

It is beyond the scope of this document to give a classification of all benign soft tissue neoplasms; the reader is directed to the appropriate literature.[7]

4.2 Sectioning

A single section per block is usually sufficient for diagnostic purposes and, to maximise tissue availability for ancillary studies, it is also prudent to take a single, full-face section of core

biopsies for initial assessment. Depending on the initial findings, sufficient tissue is then available for further levels and/or immunohistochemical/molecular studies.

4.3 Staining

H&E-stained sections are required in all cases. Immunohistochemical stains may be required to assess histogenetic phenotype and identify overexpression of transcription factors and tumour drivers.

4.4 Report content

The report contents depend in part on the type of specimen assessed. Reports on needle core biopsies and open biopsies of benign neoplasms should give the histological diagnosis and details of any immunohistochemical stains or other ancillary studies performed. For excisional biopsies, the histological location of the tumour and the nature of the surrounding tissues should be included, if possible, dependent on the specimen. In addition, completeness of excision should be assessed. The distance of tumour from the nearest resection margin should be reported as a minimum.

Should the clinically suspected benign tumour turn out to be malignant, the report should follow the guidelines set out in the dataset for reporting of soft tissue sarcomas.[6]

[Level of evidence – D.]

5 Other non-neoplastic soft tissue specimens

5.1 Specimen dissection

The approach to these specimens follows the general guidelines for histopathology specimens; they need to be described and measured, and representative areas sampled for tissue blocks. Specimens tend to have components of fat or dense fibrous tissue, requiring thorough fixation and processing if they are to section well.

5.2 Sectioning

Routinely a single section per block is adequate for reporting.

5.3 Staining

Sections are routinely stained with H&E.

5.4 Further investigations

Many of these tissues consist of organised collagenous fibrous tissue. The high level of collagen fibre orientation may be altered, for instance by trauma or vascular ingrowth. The pattern of collagen fibres and defects in the expected alignment of the fibres detected by polarising microscopy may give clues to potential disease processes.

5.5 Report content

In addition to describing native and infiltrating cells, it is important to document changes in the matrix and the vasculature, which are both altered in many of the disorders of connective tissues, including those caused by chronic or acute trauma.

[Level of evidence – GPP.]

6 Articular and intervertebral disc cartilage

6.1 Specimen dissection

It is rare for these tissues to be biopsied for diagnostic purposes. Much more commonly they are removed as part of a surgical procedure. Exceptions include the biopsy of sites seeded with autologous chondrocytes for repair of defects where biopsy is used to assess bio-integration and biopsy of the intervertebral disc for the diagnosis of discitis. Most specimens can be treated in the same way as soft tissue specimens; it is important to document the presence of crystals.

6.2 Sectioning

A single section per block is usually sufficient for diagnostic purposes.

6.3 Staining

H&E staining is usually sufficient.

6.4 Further investigations

Significant information can be gained from assessing changes in matrix molecules, particularly proteoglycans. For this, metachromatic stains may be helpful, e.g. toluidine blue.

Other stains of matrix molecules, e.g. safranin O and van Gieson, are often employed to further identify the nature and distribution of matrix molecules.[2]

6.5 Report content

The report should detail changes in both the cells and matrix. Of particular note is the formation of chondrocyte clusters and fissures within the matrix. In cartilage, it is important to describe any disruption in the collagen network and the loss (if any) of proteoglycans, a hallmark of degenerative joint disease. In addition to these features, in biopsies of intervertebral disc, the presence of inflammatory cells, particularly polymorphs, indicating bacterial infection, and vessel/nerve ingrowth, which may explain back pain, should be noted.

[Level of evidence – GPP.]

7 Needle biopsies of bone

7.1 Specimen dissection

These biopsies are usually received as multiple fragmented cores and it is important to include all the fragments in the tissue block. These specimens may be plastic embedded but, in most laboratories, they are processed for paraffin embedding after decalcification in formic acid or, less commonly, a chelating agent – see section 2.4.

7.2 Sectioning

A single section per block is usually sufficient for diagnostic purposes and, to maximise tissue availability for ancillary studies, it is also prudent to take a single, full-face section of core biopsies for initial assessment. Depending on the initial findings, sufficient tissue is then available for further levels and/or immunohistochemical/molecular studies.

7.3 Staining

H&E-stained sections are required in all cases.

7.4 Further investigations

Immunohistochemistry is often required if neoplasia (usually haematological neoplasm or metastatic carcinoma) is suspected. Generally, immunohistochemical stains are reliable in formic acid decalcified tissue. However, each laboratory should be careful to validate the repertoire of primary antibodies employed and perform appropriate tissue controls.

Stains for organisms (most commonly Gram stain, Ziehl–Neelsen (ZN) or periodic acid–Schiff) if infection is suspected.

7.5 Report content

The report should contain the histological diagnosis or differential diagnoses. Depending on the size of the biopsy, the report should indicate whether there is evidence of bone lysis or sclerosis and comment on the nature of any new bone formation. Features specific to any underlying condition should be described; for example inflammation (e.g. acute and chronic osteomyelitis), organisms, neoplastic cells, fibrosis (e.g. chronic inflammation, osteomyelosclerosis, Paget's disease) and enlarged frequent osteoclasts (e.g. Paget's disease) and zonal empty osteocyte lacunae (e.g. avascular bone necrosis, fracture necrosis). The presence of exclusively normal marrow often excludes adjacent disease.

[Level of evidence – GPP.]

8 Large pieces of bone other than suspected bone tumours

8.1 Specimen dissection

The use of a butcher's bandsaw to cut large, non-decalcified specimens may be necessary, at the pathologist's discretion. Depending on size, the amount of bone present and the fragility of the specimen, the sample may be trimmed prior to decalcification or after (see decalcification above – section 2.4). Where orientation is desirable, oversize cassettes and oversize slides (megablocks and megaslides) may sometimes be employed in soft tissue and bone pathology.

8.2 Sectioning

Sectioning is standard, except for large blocks when conventional rotary microtomes may be unsuitable; powered or manual horizontal bed microtomes may be required.

8.3 Staining

Conventional H&E staining is the first employed stain and, depending on the presence of certain pathologies (e.g. infection, certain tumours), special histochemical stains or immunohistochemistry may be considered.

8.4 Further investigations

Immunohistochemistry may be required if neoplasia is unexpectedly encountered. The use of weaker acids as decalcifying agents (e.g. formic acid) is encouraged because of circumstances such as this. Generally speaking, chelating agents (e.g. EDTA) decalcify too slowly for use on large pieces of bone.

Special stains for organisms may be required should infection be considered a possibility. It is possible to perform (static) bone histomorphometry on wax-embedded decalcified tissue if, for instance, an osteoporotic fracture is suspected, but disorders of abnormal mineralisation, such as osteomalacia, preferably require undecalcified tissue (see below). When considering histomorphometry in any site, it is important to have well-sourced control data; this is not generally available for tissues other than the iliac crest, femoral head and ribs.[8]

8.5 Report content

The content of the report is similar to that for needle biopsies of bone. Detected fractures should be described and, if relevant, an attempt made to age them.[9]

[Level of evidence – D.]

9 Suspected benign bone neoplasms

9.1 Specimen dissection

With the exception of small, acral, curettaged cartilaginous tumours and exostoses, primary benign bone neoplasms are uncommon in general histopathology departments. Patients with bone neoplasms are referred to specialist bone tumour treatment centres for the biopsy, so that the surgeon/radiologist can plan the approach path of the diagnostic biopsy appropriately.

The pathologist should regard all biopsied and resected bone lesions as potentially malignant neoplasms and dissect and process the tissue to give the optimal structural and cytological information in accordance with the bone sarcoma dataset.[10]

Curettage and resected benign bone tumour (e.g. osteochondroma) tissue may require decalcification in formic acid prior to trimming; for details on decalcification processes, see section 2.4. Sometimes these specimens require larger blocks than conventional specimens for optimal histological examination.

It is beyond the scope of this document to give a classification of all benign bone neoplasms and the reader is directed to the appropriate literature.[7]

[Level of evidence – D and GPP.]

9.2 Sectioning

Sectioning is by standard microtomy, except for large blocks where conventional rotary microtomes may be unsuitable and powered or manual horizontal bed microtomes are required.

9.3 Staining

Conventional H&E staining is usually sufficient.

9.4 Further investigations

Immunohistochemistry may be required to assess histogenetic phenotype and identify overexpression of transcription factors and tumour drivers.

9.5 Report content

As well as the intrinsic features of the tumour, growth pattern, evidence of fracture, presence/absence of extra-osseous extension and, where possible, completeness of resection

and nature of the resection margins should be recorded. Other changes that may be present, e.g. bone lysis or sclerosis, inflammation, organisms, fibrosis, bone remodelling activity, appearance of bone marrow, should be recorded.

10 Arthroplasty excision specimens

10.1 Specimen dissection

These specimens are usually removed at the time of joint replacement. Fractures through or below articular surfaces should always be examined to exclude/identify an underlying cause (pathological fracture).

If the articular surface has been taken at joint replacement for osteoarthritis this should be confirmed histologically and the severity of disease graded as mild, moderate or severe. Comment should be made on the presence/absence of a primary inflammatory or crystal arthropathy or avascular bone necrosis. Both the synovium and the cartilage/bone should be sampled. Sampled synovium should be processed as for a synovial biopsy (see section 3 above). The articular surface and underlying bone should be processed as for large pieces of bone other than suspected bone tumours – section 8 above.

[Level of evidence – GPP.]

10.2 Sectioning

For blocks of bone, synovium and articular surface tissue a single section is usually sufficient.

10.3 Staining

Synovium

H&E-stained sections are required in all cases. Stains for organisms and amyloid may, on occasion, be required.

Bone and articular surfaces

H&E-stained sections are required in all cases. Significant information may be gained from assessing changes in matrix molecules, particularly proteoglycans. For these metachromatic stains may be helpful, e.g. toluidine blue. Other stains of matrix molecules, e.g. safranin O and van Gieson, are often employed to further identify the nature and distribution of matrix molecules.[2]

10.4 Report content

Synovium

The report must record the details of synovial tissue abnormalities, as outlined in section 3.4.

Articular surfaces

The report should detail changes in both the cells and articular matrix – see section 6.5.

Bone

The report should include bone changes related to articular disease as well as the presence and features of any intrinsic bone disease – see sections 7.5, 8.5 and 9.5.

[Level of evidence – Grade D.]

11 Bone biopsies for metabolic bone disease

11.1 Specimen dissection

Interpretation of bone biopsies for the purpose of diagnosing those generalised diseases of the skeleton known as metabolic bone diseases is a very specialised area of pathology, requiring specific skills of biomedical scientist staff, clinicians and pathologists, and non-standard equipment for processing, sectioning, staining and analysing tissue sections. These biopsies are best handled in a specialist centre. Currently there are only two centres in the UK that undertake metabolic bone biopsies – Cambridge and Sheffield.

A recent paper suggests that the proper management of patients with renal bone disease requires every patient to undergo bone biopsy, indicating considerable potential for enhancing the service in the UK.[11] The biopsies that give most information are 8–10 mm core biopsies, taken down through the iliac crest or across the iliac bone (trans-iliac). The biopsies do not usually require dissection and should be orientated so that cortical and trabecular bone are incorporated in each section. These specimens must be processed undecalcified, which usually requires embedding in a plastic resin.

[Level of evidence – D and GPP.]

11.2 Pre-preparation

So-called static bone histomorphometry can be applied to any undecalcified tissue section of bone. However, to generate the maximum amount of clinical data, the patient should be given two doses of the fluorochrome tetracycline, separated by a known period of time. Typically, the patient is given 70 mg/kg of tetracycline in divided doses over 24 hours on day 1, repeated on day 10 and the biopsy taken on day 14. Tetracycline is taken up at sites of active mineralisation.

The distribution of tetracycline and the distance between bands of tetracycline assessed histomorphometrically takes bone histomorphometry to a new value level, allowing the behaviour of bone cells to be defined. This is important as all therapeutic agents target osteoblasts or osteoclasts or their stem cells.

11.3 Sectioning

Following receipt in absolute ethanol, the tissue is permeated by plastic monomer, which is then polymerised. The plastic embedded tissue is usually sectioned with a powered microtome. It is possible to get disposable blades for a powered microtome, but the alternative is to use tungsten-tipped microtome knives. These require professional sharpening.

11.4 Staining

Traditionally sections are stained using either Masson Goldner or toluidine blue stains. If the patient's bone has been pre-labelled with tetracycline, an unstained section produced for examination in transmitted or incident ultraviolet light is also required.

In patients with renal osteodystrophy, detection of deposited aluminium and iron within bone matrix should be considered.[12]

11.5 Further investigations

For osteoporosis, the bone biopsy may be reported subjectively by an experienced pathologist. The best objective data comes from biopsies that have been double tetracycline labelled where extrapolation using accepted algorithms allows an insight into the dynamic aspects of bone formation, mineralisation and removal. There are two essentials before metabolic bone

biopsies can be interrogated and interpreted: a method for making measurements and a control data set against which changes in the bone can be assessed.

There are many commercially available automated image analysis programmes that can be adapted for bone histomorphometry. The main parameters being measured are length, thickness, depth, linear separation and area which are fundamental measurements for most mensurating software packages. Once derived, the primary data are uploaded into a reporting algorithm that generates the full spectrum of data required for patient management. Interpretation requires comparison with a normal dataset.[8]

Bone histomorphometry changes with age and sex so there is not a single normal template. Furthermore, studies that report reference data are mostly from specialist centres, such as paediatric renal units, who have derived their own controls. However, these reference data can be extrapolated to other settings.

11.6 Report content

As with all biopsies, the report should detail the pathologist's findings in the biopsy; in this case, it should also include a full histomorphometric analysis.

[Level of evidence – D and GPP.]

12 Synovial fluid

12.1 Specimen examination

This is a form of cytological examination.[13] The sample should be received in the laboratory in a non-crystalline anticoagulant, e.g. lithium heparin. The synovial fluid requires the normal level of care when handling unfixed cytological preparations. The sample should be analysed within 12 hours of aspiration but, if refrigerated, the interval between aspiration and analysis can be up to 48 hours without significant reduction in the quality of the results.

The number of nucleated cells should be measured manually or by cell counter. An unstained sample of fluid is examined for crystals under polarised light, foreign material and to identify a cell known as the ragocyte, a marker of rheumatoid disease and septic arthritis. Finally, the fluid is diluted to about 400 cells/mm and a cytocentrifuge preparation is made and stained with Jenner Giemsa stain.[3] The nature of the cells in the sample is recorded. With some cells (e.g. neutrophils, lymphocytes and macrophages) the proportion as a percentage of all nucleated cells should be quantified, as these are key diagnostic features.

[Level of evidence – D and GPP.]

12.2 Further investigations

Gram and ZN stains are frequently used to identify organisms in cytocentrifuge preparations. Occasionally immunohistochemistry is required, but this is unusual.

12.3 Report content

The report should include a description of the cells present (and the proportion of ragocytes, neutrophils, lymphocytes, macrophages and synoviocytes), crystals and infective organisms, as well as an interpretation and, where possible, a definitive diagnosis.

[Level of evidence – D.]

13 Criteria for audit

The following are recommended by the RCPath as key assurance indicators (see Key assurance indicators for pathology services, November 2019) and key performance indicators (see Key Performance Indicators – Proposals for implementation, July 2013):

- cancer resections should be reported using a template or proforma, including items listed in the English COSD, which are, by definition, core data items in RCPath cancer datasets. English trusts were required to implement the structured recording of core pathology data in the COSD
  - standard: 95% of reports must contain structured data
- histopathology cases must be 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.

Further suggested audit standard:

- decalcification turnaround times should be recorded and reviewed at regular intervals and weighed against clinical need.

14 References

1. The Royal College of Pathologists. *Guidelines on Staffing and Workload for Histopathology and Cytopathology Departments (4th edition).* London, UK: The Royal College of Pathologists, 2015. https://www.rcpath.org/profession/publications/specialty-specific-publications.html

2. Suvarna KS, Layton C, Bancroft JD (eds). *Bancroft's Theory and Practice of Histological Techniques (8th edition) Expert Consult: Online and Print.* London, UK: Elsevier, 2018.

3. Yuehuei AH, Kylie ML (eds). *Handbook of Histology Methods for Bone and Cartilage (1st edition).* New Jersey, USA: Humana Press, 2003.

4. Athanasou NA, Pandey R, de Steiger R, Crook D, Smith PM. Diagnosis of infection by frozen section during revision arthroplasty. *J Bone Joint Surg Br* 1995;77:28–33.

5. Campbell P, Ebramzadeh E, Nelson S, Takamura K, De Smet K, Amstutz HC. Histological features of pseudotumor-like tissues from metal-on-metal hips. *Clin Orthop Relat Res* 2010;468:2321–2327.

6. Fisher C, Thway K. The Royal College of Pathologists. *Dataset for Histopathological Reporting of Soft Tissue Sarcomas*. London, UK: The Royal College of Pathologists, 2022. Accessed May 2022. Available at: https://www.rcpath.org/uploads/assets/369456b0-f7b4-4631-a0c6d161176f5782/Dataset-for-histopathological-reporting-of-soft-tissue-sarcoma.pdf

7. WHO Classification of Tumours Editorial Board. *Soft Tissue and Bone Tumours (5th edition, Vol. 3).* Lyon, France: International Agency for Research on Cancer, 2020.

8. Rehman MT, Hoyland JA, Denton J, Freemont AJ. Age related histomorphometric changes in bone in normal British men and women. *J Clin Pathol* 1994;47:529–534.

9. Rutty GN. *Essentials of Autopsy Practice: New Advances, Trends and Developments (1st edition).* London, UK: Springer, 2008.

10. Flanagan AM, Tirabosco R. *Dataset for Histopathological Reporting of Primary Bone Tumours.* London, UK: The Royal College of Pathologists, 2021. Accessed September 2021. Available at: www.rcpath.org/profession/guidelines/cancer-datasets-and-tissue-pathways.html

11. Fusaro M, Re Sartò GV, Gallieni M, Cosmai L, Messa P, Rossini M *et al.* Time for revival of bone biopsy with histomorphometric analysis in chronic kidney disease (CKD): Moving from skepticism to pragmatism. *Nutrients* 2022;14:1–15.

12. Freemont AJ, Denton J. Demonstration of aluminium on bone using different staining techniques and spectrophotometry. *J Clin Pathol* 1989;42:671–672.

13. Freemont AJ, Denton J. *Atlas of Synovial Fluid Cytopathology (volume 18).* New York, USA: Springer, 2012.

Recommended standard texts

- Bullough PG. *Orthopaedic Pathology 5th ed.* Maryland Heights, Missouri: Mosby Elsevier, 2010.

- Klein MJ, Bonar FS, Freemont T, Vinh TN, Lopez-Ben R, Siegel HJ *et al*. *Non-neoplastic Diseases of Bones and Joints, Atlas of Nontumor Pathology, First series, Fascicle 9.* Washington, DC: American Registry of Pathology, 2011.

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

Appendix B AGREE II compliance 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	N/A
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, Introduction
12 There is an explicit link between the recommendations and the supporting evidence	2–11
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	Throughout
16 The different options for management of the condition or health issue are clearly presented	Throughout
17 Key recommendations are easily identifiable	Throughout
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	Throughout
20 The potential resource implications of applying the recommendations have been considered	Foreword
21 The guideline presents monitoring and/or auditing criteria	13
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