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

Tissue pathways for bone and soft tissue pathology

May 2016

Authors: Professor David C Mangham, Applied Molecular Pathology, University of Manchester
Professor Anthony J Freemont, Osteoarticular Pathology, University of Manchester

Unique document number	G105
Document name	Tissue pathways for bone and soft tissue pathology
Version number	2
Produced by	Professor David C Mangham, Consultant in Osteoarticular Pathology, Professor of Applied Molecular Pathology and Chair of the Orthopaedic EQA Scheme, University of Manchester Professor Anthony J Freemont, Professor of Osteoarticular Pathology, University of Manchester
Date active	May 2016
Date for full review	May 2021
Comments	This document will replace the 1st edition of *Tissue pathways for bone and soft tissue pathology*, published in 2011. In accordance with the College's pre-publications policy, this document will be on The Royal College of Pathologists' website for consultation from 4 April to 2 May 2016. Ten items of feedback were received but no changes were requested. Please email publishing@rcpath.org to see the responses. **Dr Lorna Williamson** **Director of Publishing and Engagement**

The Royal College of Pathologists
4th Floor, 21 Prescot Street, London, E1 8BB
Tel: 020 7451 6700, Fax: 020 7451 6701, Web: www.rcpath.org
Registered charity in England and Wales, no. 261035

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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 stakeholders consulted for this document were members of:

- Orthopaedic EQA,
- International Skeletal Society,
- British Society of Rheumatologists
- British Orthopaedic Research Society

The information used to develop this tissue pathway was collected from electronic searches of the medical literature, previous recommendations of the RCPath, and local guidelines in the United Kingdom. The level of evidence was either grade C or D, or met the GPP/good practice point criteria (see section 1.7 and Appendix A). Consensus of evidence in the tissue pathways was achieved by expert review.

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 five-yearly basis. However, each year, the College will ask the author of the tissue pathways, in conjunction with the relevant sub-specialty advisor to the College, to consider whether or not the document needs to be updated or revised. A full consultation process will be undertaken if major revisions are required. If minor revisions are required, an abridged consultation process will be undertaken whereby a short note of the proposed changes will be placed on the College website for two weeks for members' attention. If members do not object to the changes, the short notice of change will be incorporated into the pathways and the full revised version (incorporating the changes) will replace the existing version on the publications page of the College.

The pathway has been reviewed by the Working Group on Cancer Services and was placed on the College website for consultation with the membership from 4 April to 2 May 2016. 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 Director of Publishing and Engagement.

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

1 General introduction

This document is designed to assist all histopathologists and cytopathologists with achieving 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 cognate 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. The authors believe that busy pathologists are more likely to undertake a task well if they understand the rationale behind it.

1.1 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 guide 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 Orthopaedic 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 osteoarticular pathology is such that all histopathologists should be comfortable with making common diagnoses and know when they should be referring cases to specialists.

The Royal College of Pathologists' *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 science (BMS) staff with specialist skills in the use of band saws, tissue decalcification, handling of large tissue blocks, plastic embedding and sectioning of undecalcified bone are essential. These are, however, skills that are necessary for other subspecialties, including head and neck pathology and haematopathology.

For laboratories handling synovial fluid cytology specimens, BMS staff with skills in a range of cytological techniques such as cell counting and cytocentrifugation are required, as well as an understanding of the health and safety requirements of handling fresh tissues.

More than in many areas of pathology, specialist microscopic techniques such as quantitation (e.g. differential cell counts on cytocentrifuge preparations, bone histomorphometry) and the use of polarising microscopy are regular requirements of bone and soft tissue pathologists if the maximum data are to be extracted from the preparation being examined. These bring their own issues around regulatory accreditation.

1.2 Laboratory facilities

The full range of routine laboratory facilities is essential in specialist centres, including access to immunohistochemistry and electron microscopy. For general bone and soft tissue specimens, the basic laboratory equipment/technology should include:

- appropriate saws (hand, band and/or diamond-coated)

- safe facilities and SOPs 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.

All pathologists reporting non-tumour connective tissue specimens must have access to polarising microscopy, as many diagnoses cannot be made unless this facility is available. 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 metabolic bone disease must have fluorescent microscopy to detect the tetracycline uptake used as a biomarker of mineralisation and access to a computerised image analysis system.

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

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

1.3 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’ soon after receipt and then left for further fixation prior to production of blocks. Samples for plastic embedding and sectioning undecalcified should be received in a fixative for which there is no risk of free acid being generated; absolute alcohol is such a fixative.

1.4 Decalcification

The pathologist must decide which specimens require decalcification and if decalcification is to be undertaken at what stage in the process of trimming. In addition, he/she must decide whether to cut the tissue to final block size before or after decalcification. The former requires only one intervention by the pathologist, but may generate bone dust (which could be carefully removed with a brush) or fragment the specimen, both of which can obscure histological detail. The latter makes accurate block sizing and orientation easier, but with the risk of poorer fixation.

If tumour is suspected or identified during trimming, it is worth attempting to obtain some for examination without decalcification, particularly if immunohistochemistry or *in-situ* hybridisation are to be undertaken (for instance, HER2 assessment cannot be undertaken on decalcified tissue). This may require blunt scraping or chipping if necessary; tiny fragments of bone within a specimen do not prevent it being sectioned without decalcification. In most

cases, it should be possible to get at least some tumour without disrupting the entire specimen.

Choice of decalcification agent should be geared to the type of specimen and the question/pathology being investigated by the clinician or pathologist. As a general rule, proprietary decalcifying agents should be avoided unless their effects on antigens and the cell walls of infective organisms is fully understood and completely reproducible. Generic decalcifying agent include chelating agents, 5–15% formic acid and 5–10% nitric acid.[3]

Chelating agents take a very long time to decalcify bone specimens and the agent requires changing to prevent exhaustion. However, such agents preserve tissue well for immunohistochemistry, tinctoral histochemistry for organisms and *in-situ* hybridisation techniques.

Nitric acid is a very rapid decalcifying agent and over-decalcification with tissue denaturation or removal of nucleic acids and histones can result unless great care is taken. Over-decalcification leads to poor morphology and loss of nuclear straining. However, nitric acid is sometimes used for fast decalcification of specimens containing significant amounts of cortical bone.

The agent of choice is formic acid as this strikes 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 assured 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 easily the most expensive in terms of reagents and labour, is radiology, for which purpose a specialist small unit (e.g. Faxitron)[4] 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.

1.5 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.[5] 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.

1.6 Target users of this guideline

The target primary users of the tissue pathway are trainee and consultant cellular pathologists and BMS staff, as indicated above.

1.7 Levels of evidence

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.

2 Synovial biopsy

2.1 Specimen dissection

Most of these specimens are obtained arthroscopically and in consequence are small, consisting of the tips of synovial villi and attached fibrin. These should be blocked together without further orientation – care being taken, as with all tiny specimens, not to lose any tissue.

Less frequently sheets of synovium are received. It is usually obvious which is the synovial surface, as it has a frond-like or roughened 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 becoming 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 which is the harbinger of infection) and to ensure that large pieces of debris are avoided because they interfere with sectioning. If necessary, the pieces of debris can be blocked separately or removed from the surrounding tissue and then submitted for histological analysis.

A new disorder named ALVAL (aseptic lymphocyte dominated vasculitis-associated lesions – a poor name, as there is no vasculitic component) has recently been described in patients with metal-on-metal hip resurfacing implants.[6] Orientation of the synovium is particularly important in these cases, as without it there can be difficulties in making the 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.

2.2 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.

2.3 Staining

Haematoxylin and eosin (H&E) stained sections are required for all cases. Stains for organisms and immunohistochemical analysis may be required, as may stains for amyloid.

2.4 Report content

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

3 Potential benign soft tissue neoplasms

3.1 Specimen dissection

It is notoriously difficult to define with accuracy whether some soft tissue swellings are benign, malignant or even neoplastic on imaging, which is the mainstay of screening prior to surgical resection or referral to a specialist multi-disciplinary team (MDT). Needle biopsy is a frequent diagnostic tool (but fine needle aspiration is not commonly applied as many diagnoses rest on structural as well as cytological information), as are open biopsy and excision biopsy.

Needle core biopsies do not usually require dissection and open biopsies, because of their size, rarely require more than bisection, but it is good practice in both cases to process all the tissue.

The heterogeneity of benign lesions and the possibility that they may turn out to be malignant mean that excision biopsies of soft tissue swellings should be treated as if they were malignant.[7] The classification of soft tissue tumours is too extensive to be covered here, but is well described in the literature, including standard pathology texts.

There is a debate about inking the surface of such specimens as the ink may permeate through the first millimetre or so of connective tissue biopsies giving false margins, but we consider it good practice. It is best practice to take one block per centimetre length of longest axis of homogenous appearing swellings and heterogenous lesions should be widely sampled to obtain representative areas. It is essential to include resection margins.

[Level of evidence – D]

3.2 Sectioning

Routinely a single section per block 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 for all cases. Immunohistochemical stains may be required.

3.4 Report content

It is important to specify the location of the swelling as assessed macroscopically and by microscopic examination. It is also important to specify whether lesional tissue extends to the resection margins, and best practice to specify the distance of a tumour from selected resection margins and the nature of the intervening tissue in all other respects. Sample description and report content are similar to other tissue specimens.

[Level of evidence – D]

4 Other soft tissues

4.1 Specimen dissection

These specimens are little different from most received in the pathology laboratory. They need to be described, measured and representative areas converted into tissue blocks. Samples tend to have components of fat or dense fibrous tissue requiring thorough fixation and processing if they are to section well.

4.2 Sectioning

Routinely a single section per block is adequate for reporting.

4.3 Staining

Sections are routinely stained with H&E.

4.4 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.

4.5 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.

5 Cartilage and intervertebral disc (IVD)

5.1 Specimen dissection

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

5.2 Sectioning

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

5.3 Staining

Significant information can be gained from assessing changes in matrix molecules, particularly proteoglycans. For this, H&E staining is usually sufficient.

5.4 Further investigations

Other stains of matrix molecules, particularly stains such as safranin O, van Gieson and toluidine blue, are often employed to identify the nature and distribution of matrix molecules of different types.

5.5 Report content

The report should detail changes in both the cells and matrix. Of particular note is the formation of chondrocyte clusters and slits within the matrix. In cartilage, it is particularly important to describe any disruption in the collagen network (or in the case of biopsies taken from sites of autologous chondrocyte implantation [ACI], whether there is integration of collagen fibres across the ACI/native cartilage interface) and the loss (if any) of aggrecan. In addition to these features, in biopsies of IVD the presence of inflammatory cells, particularly

polymorphs, which indicate infection, and vessel/nerve ingrowth, which may explain back pain.

6 Needle biopsies of bone

6.1 Specimen dissection

These biopsies are usually received as multiple fragmented cores together with what appears to be blood clot. This may be marrow 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 'wax' embedding following decalcification in formic acid or, less commonly, a chelating agent.

6.2 Sectioning

Sectioning is standard.

6.3 Staining

H&E staining is standard.

6.4 Further investigations

Immunohistochemistry is often required if neoplasia (usually haematological neoplasm [including plasmacytoma] or metastatic carcinoma) is suspected and stains for organisms (most commonly Gram stain, ZN or PAS) if infection is queried. Some immunostains (importantly HER2) become unreliable in acid decalcified tissue.

6.5 Report content

Depending on the size of the biopsy, the report should indicate whether there is evidence of bone lysis or sclerosis, particularly 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) or empty osteocyte lacunae (e.g. avascular bone necrosis, fracture more than 2 hours old). The presence of normal marrow often excludes adjacent disease and can be used as evidence of the need for further biopsies.

7 Large pieces of bone other than suspected bone tumours

7.1 Specimen dissection

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). Sometimes these specimens require larger blocks than conventional specimens for optimal histological examination. Deep and oversize cassettes and oversize slides are readily available, and are a necessity for bone and soft tissue pathology.

7.2 Sectioning

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

7.3 Staining

Conventional H&E staining is standard.

7.4 Further investigations

Immunohistochemistry may be required if neoplasia is suspected, as may special stains for organisms, 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 require undecalcified tissue (see below). However, when considering histomorphometry in any site, it is essential to have well-sourced control data, which are not generally available for tissues other than the iliac crest, femoral head and ribs.

[Level of evidence – C]

7.5 Report content

The content of the report is similar to that for needle biopsies of bone. If a fracture is present, every attempt should be made to age it.[8]

8 Suspected primary bone neoplasms

8.1 Specimen dissection

Primary bone neoplasms are rarely seen in diagnostic practice outside treatment centres. This is because of a fear that if the neoplasm is malignant and biopsy site soft tissues become seeded with tumour, optimal definitive surgery may be compromised. As a consequence, patients with bone neoplasms are usually referred to specialist treatment centres for biopsy, so that the surgeon who would be undertaking definitive therapeutic surgery can take the diagnostic biopsy.

That said, neoplasms diagnosed as benign on imaging are removed in general orthopaedic, head and neck and thoracic surgical settings. Unfortunately, as for soft tissue neoplasms, it is notoriously difficult to define with accuracy radiologically whether some bone lesions are primary neoplasms and, if they are, whether they are benign or malignant, and malignant neoplasms are inadvertently resected or biopsied. The pathologist should therefore treat all bone lesions as potential malignant neoplasms[9] and dissect and process lesional tissue to give the optimum amount of structural and cytological information possible.

Depending on the nature of the biopsy (benign bone tumours or suspected tumours are often removed by curettage and cannot be reconstructed), it is best practice to keep the anatomy of the sample as intact as possible. This may require decalcifying the tissue in formic acid prior to trimming. 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 bone neoplasms. The reader is directed to the appropriate cancer datasets[9] and specialist laboratory textbooks.

8.2 Sectioning

Sectioning is standard, except for large blocks when conventional rotary microtomes may be unsuitable and powered or manual horizontal bed microtomes are required. Sometimes multiple levels are required to exclude permeative tumour growth, which is a hallmark of malignancy.

8.3 Staining

Conventional H&E staining is standard.

8.4 Further investigations

Immunohistochemistry may be required, as may special stains for matrix components.

8.5 Report content

Tumour matrix and growth pattern and, where possible, completeness of resection and the nature of the resection margins must be recorded, as must evidence of fracture and extra-osseous extension.

9 Articular surfaces

9.1 Specimen dissection

These specimens are usually removed at the time of joint replacement. There is some debate as to whether there is any need to examine these specimens histologically. 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 and osteoarthritic change is obvious and no other pathology is seen, then a case can be made for not processing the tissue for microscopy. Where resources permit, it is best practice to examine all tissue samples microscopically, as only in this way is it possible to exclude an underlying unsuspected (or at least undiagnosed) primary inflammatory or crystal arthropathy or avascular bone necrosis. It also allows the histological spectrum of osteoarthritis to be experienced by the pathologist, who will hopefully prevent significant diagnostic errors such as mistaking an osteoarthritic geode for a myxoid neoplasm or longstanding osteoarthritis for rheumatoid disease. To this end, the articular surface should be treated as two specimens, i.e. the synovium and the cartilage/bone of the surface itself. Every effort should be made to identify synovium, remove a sample and process it like any synovial biopsy (Section 2 above). The articular surface itself and underlying bone should be processed as for a large bone biopsy (Section 7 above). Wherever possible, the continuity of the specimen should be retained, where necessary by using oversized cassettes and slides.

9.2 Sectioning

Synovium and articular surface: routinely a single section is usually sufficient for diagnostic purposes.

9.3 Staining

Synovium: H&E-stained sections are required for all cases. Stains for organisms and immunohistochemical analysis may be required, as may stains for amyloid.

Articular surfaces: significant information can be gained from assessing changes in matrix molecules, particularly proteoglycans. For this, H&E staining is usually sufficient but matrix stains such as toluidine blue or van Gieson stain may be required.

9.4 Report content

Synovium: The report must record the state of the synoviocyte layer, the distribution and nature of any inflammatory cell infiltrate, and record the presence of haemosiderin, crystals, bone or cartilage fragments and foreign material. Any infective agents or features suggestive

of infection should be recorded and commented on. It is good practice to state that there is no evidence of infection when reviewing material from revision arthroplasties if this is the case.

Articular surfaces: the report should detail changes in both the cells and matrix.

10 Bone biopsies for metabolic bone disease

10.1 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 both BMS staff and pathologists, and non-standard equipment for processing, sectioning, staining and analysing tissue sections. These biopsies are best handled in a specialist centre. The biopsies that give most information are 8–10 mm core biopsies, taken down into 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.

10.2 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.

10.3 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.

10.4 Further investigations

The biopsy can be reported subjectively, but further information can be gained by quantitative analysis of tissue sections using bone histomorphometry. Histomorphometry requires the technology for generating data, nowadays usually computerised analysis, and a dataset of age- and sex-matched normal measurements for comparison.[10] So-called static bone histomorphometry can be applied to any undecalcified tissue section of bone. However, to generate the maximum amount of useful data, the patient should have been 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 absorption can be assessed by analysis of a sample of urine taken at the end of days 1 and 10. Tetracycline is taken up at sites of active mineralisation. Uptake can be assessed by identifying an unstained section of bone in ultraviolet light. Full histomorphometric analysis measures the proportion of bone surfaces bearing any tetracycline label, the proportion of bone surfaces bearing two labels and the distance between those labels.

In patients with renal osteodystrophy, there is still a requirement to identify deposition of aluminium and iron within bone matrix.[11]

10.5 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 – B]

11 Synovial fluid

11.1 Specimen examination

This is a form of cytological examination.[12] The sample should be received in the laboratory in a non-crystalline anticoagulant, e.g. lithium heparin. The synovial fluid is 'live' and 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, foreign material and to identify a cell known as the ragocyte, which is a marker of rheumatoid disease and septic arthritis. Finally, the fluid is diluted to about 400 cells/mm^3 and a cytocentrifuge preparation is made and stained with Jenner Giemsa stain. 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.

11.2 Further investigations

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

11.3 Report content

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

[Level of evidence – D]

12 Criteria for audit

The following are recommended by the RCPath as key performance indicators (see *Key Performance Indicators – Proposals for implementation*, July 2013, https://www.rcpath.org/profession/clinical-effectiveness/key-performance-indicators-kpi.html).

- Cancer resections must 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 are required to implement the structured recording of core pathology data in the COSD by January 2016.

  Standard: 95% of reports must contain structured data.

- Histopathology cases that are reported, confirmed and authorised within 7–10 calendar days of the procedure.

  Standard: 80% of cases must be reported within seven calendar days and 90% within 10 calendar days.

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Appendix A Summary table – Explanation of grades of evidence

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

Grade (level) of evidence	Nature of evidence
Grade A	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 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	Appendices
20 The potential resource implications of applying the recommendations have been considered	Foreword
21 The guideline presents monitoring and/or auditing criteria	12
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