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

Tissue pathways for diagnostic cytopathology

October 2019

Authors: Dr Paul Cross, Gateshead Health NHS Foundation Trust (NHSFT)
Dr Ashish Chandra, Guy's and St Thomas' NHSFT
Dr Anthony Maddox, West Hertfordshire Hospitals NHS Trust
Ms Nadira Narine, Manchester University NHSFT
Dr Thomas Giles, Royal Liverpool and Broadgreen University Hospital NHS Trust

Unique document number	G086
Document name	Tissue pathways for diagnostic cytopathology
Version number	2
Produced by	Dr Paul Cross (MBBS, FRCPath, Consultant Cellular Pathologist), Dr Ashish Chandra (FRCPath DipRCPath [Cytol], Lead Consultant for Cytopathology and Urological Histopathology), Dr Anthony Maddox (MBBS, FRCPath Consultant Cyto/Histopathologist, Lead Clinician for Cellular Pathology), Ms Nadira Narine (MSc, FIBMS, Consultant Biomedical Scientist) and Dr Thomas Giles (MB ChB, FRPath, Consultant Cytopathologist), on behalf of the Working Group on Cancer Services.
Date active	October 2019
Date for full review	October 2024
Comments	This document will replace the 1st edition of *Tissue pathways for exfoliative cytology and fine needle aspiration cytology* published in January 2010. In accordance with the College's pre-publications policy, this document was on The Royal College of Pathologists' website for consultation from 2 May to 30 May 2019. Responses and authors' comments are available to view following final publication of this tissue pathway. **Dr Brian Rous** **Clinical Lead for Guideline Review**

The Royal College of Pathologists
6 Alie Street, London E1 8QT
Tel: 020 7451 6700
Fax: 020 7451 6701
Web: www.rcpath.org

Registered charity in England and Wales, no. 261035

Contents

Foreword 3

1 Introduction 4

2 Generic issues relating to staffing, workload and facilities 4

3 Sample types 5

4 Staining 17

5 Ancillary testing 17

6 Cytopathology reporting 19

7 Cervical cytology 24

8 Quality 24

9 Criteria for audit 24

10 References 26

Appendix A Summary table – explanation of grades of evidence 31

Appendix B AGREE II guideline monitoring sheet 33

Foreword

The tissue pathways published by the Royal College of Pathologists (RCPath) are guidelines that enable pathologists to deal with routine pathological specimens in a consistent manner and to a high standard. This ensures that accurate diagnostic and prognostic information is available to clinicians for optimal patient care and ensures appropriate management for specific clinical circumstances. This guideline has been developed to cover most common circumstances. However, we recognise that guidelines cannot anticipate every pathological specimen type and clinical scenario. Occasional variation from the practice recommended in this guideline may therefore be required to report a specimen in a way that maximises benefit to the patient.

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

The following stakeholders were contacted to consult on this document:

- British Association for Cytopathology
- British Thoracic Society
- United Kingdom Endocrine Pathology Society
- UK Breast Pathology Group

No major organisational changes or cost implications have been identified that would hinder the implementation of the tissue pathway, but some training and backfill may be required to allow cytology staff to fulfill the requirements of this guidance.

The information used to develop this tissue pathway was collected from electronic searches of the medical literature, previous recommendations of the RCPath and local guidelines in the UK. Published evidence was evaluated using modified SIGN guidance (see Appendix A). Consensus of evidence in the tissue pathway was achieved by expert review. The sections of this tissue pathway that indicate compliance with each of the AGREE II standards are indicated in Appendix B.

A formal revision cycle for all tissue pathways takes place on a five-year basis. However, each year the College will ask the author(s) of the tissue pathways, in conjunction with the relevant subspecialty adviser to the College, to consider whether or not the document needs to be updated or revised. A full consultation process will be undertaken if major revisions are required. If minor revisions are required, an abridged consultation process will be undertaken whereby a short note of the proposed changes will be placed on the College website for two weeks for 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. All changes will be documented in the data control section of the relevant pathway.

This tissue pathway was reviewed by the Clinical Effectiveness department, Lay Governance Group and Working Group on Cancer Services and placed on the College website for consultation with the membership from 2 May to 30 May 2019. 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 tissue pathway was developed without external funding to the writing group. The College requires the authors of tissue pathways to provide a list of potential conflicts of interest; these are monitored by the Clinical Effectiveness department and are available on request. The authors have declared no conflicts of interest.

1 Introduction

1.1 Summary of pathways document

The original *Tissue pathways for exfoliative cytology and fine needle aspiration cytology* was issued by the RCPath in January 2010. It was of great value in ensuring consistency of approach and handling of cytopathology samples. Cytopathology can deliver a robust diagnosis based on purely morphological grounds, with a rapid turnaround time, in many situations. However, much has changed in the field of cytopathology since the original document was issued. Advances in medical science have greatly affected the handling and ability of cytological samples to aid not only with clinical diagnosis but also with prognostic and therapeutic information. Developments in the area of reporting and in the roles and remits of staff (both medical and non-medical) have also evolved, and this revised guidance takes all these into consideration. There have also been significant changes within the cervical screening programme (CSP) with the move to primary human papilloma testing with reflex cytology. As such, the historic separation between cervical (gynaecological) cytology and non-gynaecological (diagnostic) cytology is now even more blurred and artificial. These revised guidelines acknowledge this and cover cytology as a whole. They concentrate primarily on non-cervical cytology given the very detailed guidance that already exists in the CSP, but in general the principles are the same.

This updated tissue pathways document reflects these changes and highlight major points. It does not and cannot attempt to be exhaustive or replace standard textbooks and references for diagnostic criteria. Advances are frequent, and hence this document will be updated regularly.

There are many methods available to process cytological specimens and the preference of laboratories will vary. In general terms, no one method is known to be better than any other. Many laboratories use liquid-based cytopathology preparation methods currently (personal communication, UK National External Quality Assurance Services [NEQAS]). It is important for any laboratory to ensure compliance with the manufacturer's guidance, and for the technique used to be appropriate to the sample and requirements for diagnosis/ancillary testing. All laboratories should participate in an appropriate technical and interpretative external quality assurance (EQA) scheme, such as the EQA schemes within the CSP and UK NEQAS Cellular Pathology Technique (CPT) diagnostic cytopathology EQA scheme, and should address any issues of low scores and/or poor performance.

1.2 Target users and health benefits of this guideline

The target primary users of this document are those handling and reporting on cytopathology samples, e.g. consultant cellular pathologists, consultant biomedical scientists, laboratory technical staff and trainee laboratory staff (medical and technical), and clinical users of a cytopathology service. The secondary users are the suppliers of products to laboratories, such as IT, laboratory equipment and consumable suppliers.

2 Generic issues relating to staffing, workload and facilities

In considering any cytopathology service delivery, a laboratory must take into consideration relevant guidance relating to staffing, workload and facilities (laboratory, offices, equipment, IT, etc.). The RCPath has issued relevant guidance that is applicable to medical pathologists and trainees,[1–4] but other guidance (e.g. CSP) may also be applicable, depending on the cytopathology workforce and delivery model.

There must be sufficient pathologists, biomedical scientists, cytoscreeners, clerical and other relevant staff to deliver the service. This must allow for periods of leave/absence, and in general terms should follow the relevant RCPath or other guidance as applicable.

Cytopathologists and consultant biomedical scientists must:

- follow national and local guidance
- participate in audits
- participate in appraisals and continuing professional development (CPD) schemes
- participate in relevant internal and EQA schemes
- strive to comply with relevant UK Accreditation Service (UKAS) standards.

Workloads in cytology can be monitored, but it is essential that all reporting staff see sufficient material to maintain and develop their cytological skills. This must include trainees. Although service models of delivery must be patient centered, they must also attempt to maximise educational opportunities for all staff.

3 Sample types

Cytopathology samples are generally exfoliative (cells shed naturally or by direct sampling), but they can also be obtained by fine needle aspiration (FNA). Whatever the sample type, the pre-analytical phase is as important as the other phases and includes collection and handling, laboratory triage of specimen, and specimen processing for routine examination and for ancillary testing.[5]

In histopathology, the overwhelming majority of specimens are placed in formalin, processed, embedded, cut and stained. By contrast, specimens for diagnostic cytopathology may be received as many sample types, such as air-dried or fixed slides, an aspirate, a saline wash or a needle wash of a needle aspirate. Some may be in saline, cell culture fluid or an alcohol-based fixative. Any of these sample types should ideally be delivered to cytopathology within minutes of the specimen being taken. If delay is anticipated, appropriate storage should be undertaken according to local practice.

After processing in the cytopathology laboratory, the final output may be Papanicolaou- and/or Romanowsky-stained direct spread slides, cytospin slides or machine-produced slides, together with a haematoxylin and eosin (H&E)-stained cell block material. This list is not exhaustive.

This variation in cytology specimen presentation and processing arises for many reasons, and it is possible to produce excellent cytology preparations across this spectrum. It is important to maximise the quality of samples at all stages, working with both clinical and laboratory colleagues to achieve this.

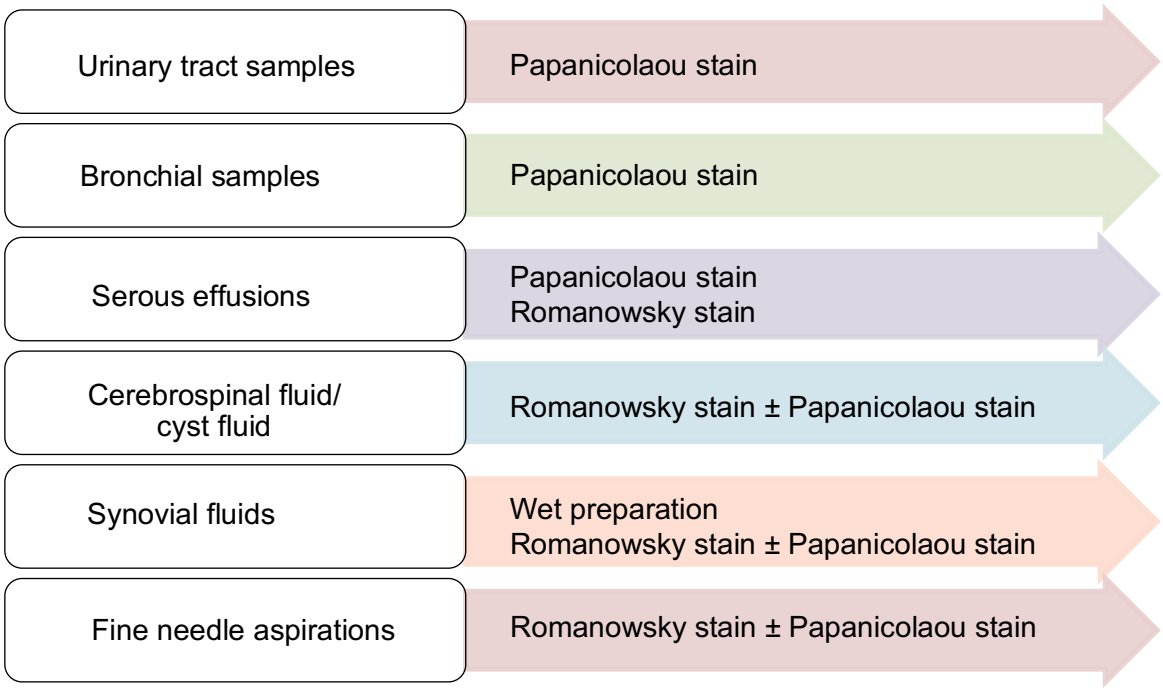
The key principles with any cytological preparation are:

- to enable accurate morphological assessment
- to preserve adequate material for ancillary tests including immunocytochemistry, molecular analysis and others.

For diagnostic cytopathology, the use of a Papanicolaou-stained and Romanowsky-stained slide is recommended for most samples. This allows the cytological material to be evaluated using both stains and their ability to augment each other for diagnosis. It is evident from experience of samples submitted to the UK NEQAS CPT diagnostic cytopathology technical EQA that this is current practice.[6] In many laboratories, the use of liquid-based cytopathology (LBC) is common for FNA and other sample types. If an LBC medium and preparation technique is used, then a Papanicolaou-stained slide is often adequate.

The figure below summarises the minimum recommendations for sample preparation and staining for the broad categories of diagnostic cytopathology samples received in the laboratory.

Figure 1: Minimum recommendations for sample preparation and staining.



For FNA, one or two passes should be performed per lesion with a pair of slides submitted from each pass. Needle washings are required for ancillary studies (clot, microbiology, flow cytometry, etc.), as indicated by triage. Ideally one or two slides should be prepared from the aspirated material for each stain, which ensures the diagnostic material is easily identified and not diluted by too much blood.[7] Any residual material in the FNA needle can be rinsed into either an LBC or other fluid medium to allow for a cell block sample to be made or other ancillary investigations to be performed.

[Level of evidence – C and GPP.]

3.1 Exfoliative cytopathology

Exfoliative cytopathology samples include, but are not limited to:

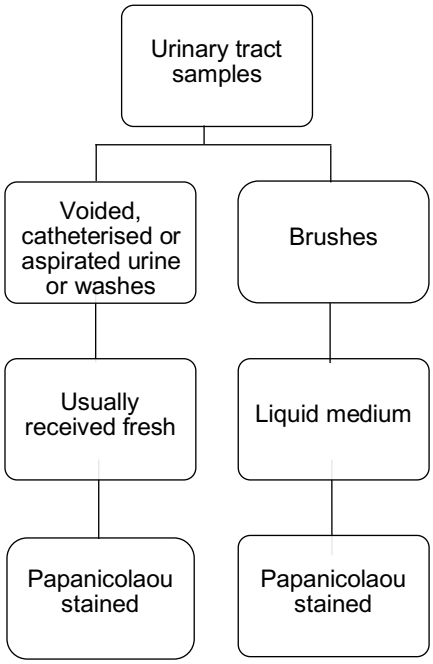
- urinary tract samples – voided, aspirated or catheterised urine, bladder washes and brushes, as well washes and brushes taken from the renal pelvis, ureter or urethra
- bronchial samples – bronchial brushes, traps, aspirates, washes, lavages and bronchoalveolar lavage (BAL)
- serous effusions – pleural, pericardial, peritoneal and ascitic fluids, as well as peritoneal washings
- hepatopancreaticobiliary (HPB) brushes and aspirates including those taken from the common bile duct and hepatic ducts
- synovial fluids
- miscellaneous cysts (ovarian and other cysts) and aspirates/fluids, including cerebrospinal fluid (CSF).

3.1.1 Urinary tract samples

Collection of samples
Voided, aspirated and catheterised urine, as well as washes or brushings from anywhere in the urinary tract, may be collected fresh in a sterile container. For voided urine, the first void of the day is not suitable for cytological analysis because of cellular degeneration overnight. Any subsequent samples of the day can be collected and transported to the laboratory as soon as possible to minimise degeneration. If this is not possible, then the sample can be fixed with an alcohol-based fixative or stored for a few hours at 4°C. Direct spreads from brushes should not be produced as there is usually considerable cell loss and air-drying artefact. Instead, brushes should be completely immersed (or rinsed) in a liquid medium, ideally an alcohol-based fixative. Physiological media such as phosphate-buffered saline (some units use Roswell Park Memorial Institute [RPMI]) may be used if there is an expectation that ancillary tests that preclude alcohol fixation, such as flow cytometry, are needed. In these cases, the specimens must be transported to the laboratory immediately to minimise cell degeneration.

[Level of evidence – GPP.]

Figure 2: Urinary tract samples.



Processing of urinary tract samples
Concentration of all types of urinary tract samples by centrifugation with subsequent preparation by at least one cytospin or a single megafunnel or LBC preparation for Papanicolaou staining is sufficient for cytological reporting (Figure 2).

3.1.2 Bronchial samples

Collection of samples
Exfoliative bronchial samples include bronchial brushes, traps, aspirates, washes and BAL. There is limited clinical value in sputum samples, but these are sometimes submitted to the laboratory if the patient is unfit for invasive investigation.

Brushes should be completely immersed in a liquid medium, ideally an alcohol-based fixative. If ancillary tests that preclude alcohol fixation (such as flow cytometry) are contemplated, it

may be necessary to use a physiological liquid medium and transport the sample to the laboratory quickly (Figure 3).

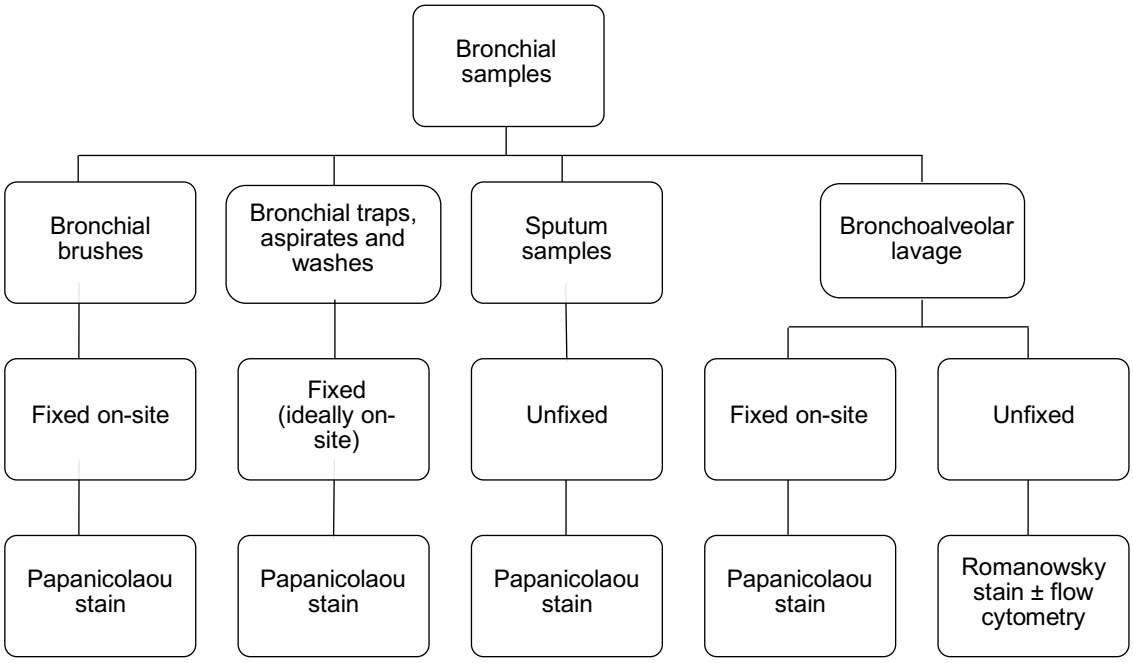
The other bronchial samples all emerge from the bronchoscope as cell suspensions in saline. Within an hour or two at room temperature the cells begin to degenerate, and this is particularly true of granulocytes and bronchial epithelial cells (which shed nuclei of the same size as lymphocytes), rendering accurate cell counts impossible.

All samples should therefore either be transported to the laboratory within the hour, and preferably cooled (particularly in the case of BAL samples requiring a cell count), or preserved on-site by adding an alcohol-based fixative.

Some clinicians require a differential cell count on BAL samples to support the diagnosis and management of patients with interstitial lung disease (ILD). In these instances, the sample may be split with one half fixed in an alcohol-based fixative for cytological analysis and the other half sent unfixed to the laboratory for a differential cell count ± flow cytometry as necessary. Refrigeration of the sample may be of use to prolong specimen integrity. In many laboratories the differential cell count may be undertaken by another pathology section, often haematology, rather than in cytology itself.

[Level of evidence – GPP.]

Figure 3: Respiratory tract samples.



Processing of bronchial samples

Concentration of all types of bronchial samples by centrifugation with subsequent preparation of at least one cytospin or a single megafunnel or LBC preparation for Papanicolaou staining is sufficient for cytological reporting (Figure 3). Direct spreads, although time consuming to screen, are still acceptable for reporting. If the samples are very mucoid, then a mucolytic agent can be used to digest the mucus to make preparation and interpretation easier. For BAL samples requiring a differential cell count, a portion of the unfixed sample can be concentrated to make an air-dried cytospin or direct spread for subsequent Romanowsky staining (Figure 3). The American Thoracic Society Cytology Guidelines[8] (once accessed, click the 'Supplements' tab for a PDF of detailed laboratory procedures) contain useful information on the technique of BAL cell counts. If there is a raised lymphocytic count and ILD is being

investigated, some laboratories will forward the remaining portion of the unfixed BAL sample for flow cytometry, primarily for CD4/CD8 ratios, although this is not mandatory.

Cell blocks may also be produced from bronchial samples to facilitate immunocytochemistry or molecular studies.

[Level of evidence – C.]

3.1.3 Serous effusions

Collection of serous effusions
Serous effusions include pleural, pericardial, peritoneal and ascitic fluids as well as peritoneal washings and may be collected fresh in a sterile container. It is essential that they are transported as soon as possible to the laboratory to minimise degeneration. There is recent evidence in the literature that a minimal volume range of 50–75 ml should be adopted in order to diminish potential false negatives and optimise the test sensitivity for pleural fluid samples.[9] The minimal volume has shown to be 60 ml for pericardial fluid[10] and may be different for ascitic fluid samples. In order to perform morphological analyses as well as ancillary testing, clinicians should submit the volumes above to the laboratory as there is little increase in diagnostic yield above this volume. In addition, some laboratories do not have the facilities to process large volumes of fluid.[9,11]

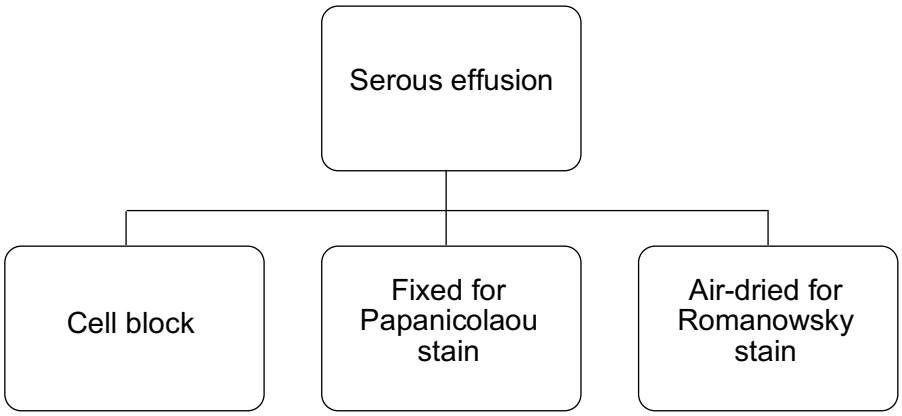
[Level of evidence – C.]

Processing of serous effusion samples
Any clots formed in a serous effusion should be removed and processed for reporting as part of the cytological sample (Figure 4). The fluid portion should be processed such that Papanicolaou and Romanowsky stains are produced and the remaining material is preserved for ancillary tests, if needed.

One way to facilitate this is to split the specimen, with half fixed in an alcohol-based fixative for preparation and Papanicolaou staining and half prepared as air-dried cytospins or direct spreads for Romanowsky staining (Figure 4). If there is no natural clot and a cell block is required, this can be prepared from the residual material. Other laboratories will produce stained slides and process the whole of the remaining specimen to cell block.

[Level of evidence – GPP.]

Figure 4: Serous fluid samples.



3.1.4 HPB brushes and biliary fluid aspirate/brush washings

The majority of HPB samples are bile and hepatic duct brushes. In some instances, brushing from elsewhere in the HPB tract as well as cyst fluids are sent for cytological analysis. It is essential that HPB brushes are fixed on-site to prevent degeneration and allow for comprehensive morphological interpretation. In instances of metastatic disease, residual material can be used for ancillary studies, while on the occasions when there is a suspicion of malignancy, molecular analysis can be performed on this residual material. Biliary cyst fluids can be submitted in a dry, sterile container.

[Level of evidence – GPP.]

Processing of HPB samples

Similar to bronchial brushes, concentration of HPB brushes by centrifugation with subsequent preparation of at least one cytospin or a single megafunnel or LBC preparation for Papanicolaou staining is sufficient for cytological reporting. Fixed direct spreads are acceptable for reporting.

Any submitted cyst fluid samples can be prepared as per serous effusions (sections 3.1.3 and 3.1.5).

[Level of evidence – GPP.]

3.1.5 Miscellaneous cysts/cyst fluid samples

Collection of miscellaneous cysts and aspirates

Miscellaneous cysts and cyst fluid samples, including ovarian and other cysts, can be submitted from a variety of departments and should be received in a sterile container. Quite often, only small volumes are received and the challenge for the laboratory is to prepare and give as comprehensive a report as possible to aid in diagnosis and patient management.

[Level of evidence – GPP.]

Processing of miscellaneous cysts and aspirates

Any submitted cysts or aspirates samples can be prepared as per serous effusions (section 3.1.3).

[Level of evidence – GPP.]

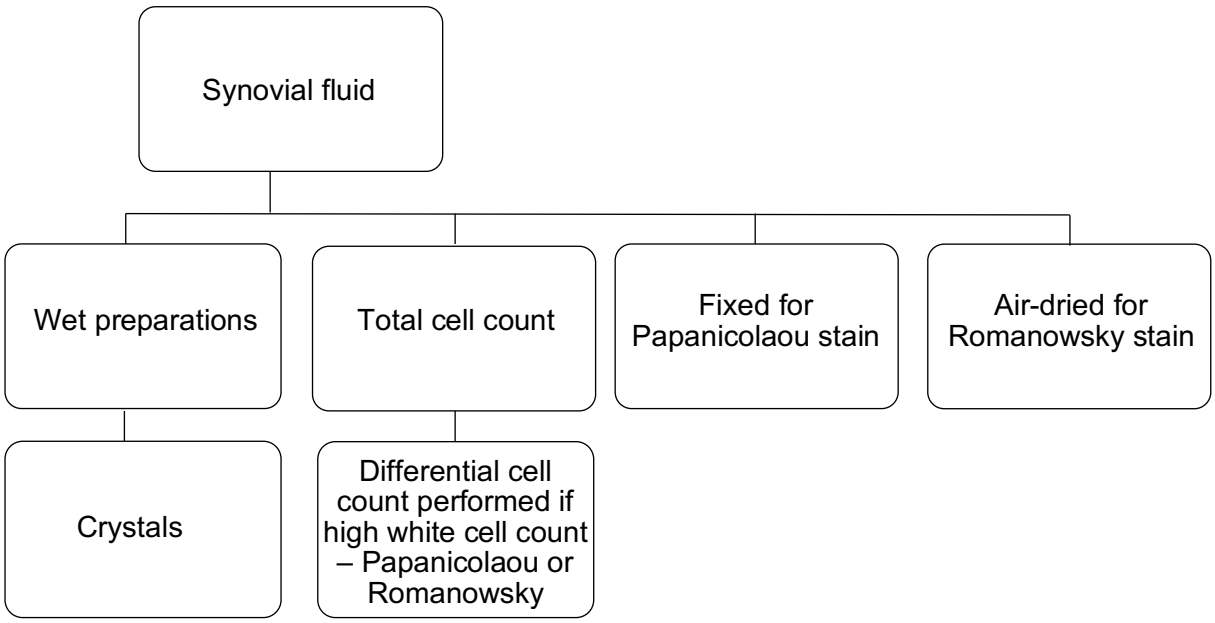
3.1.6 Synovial fluids

Collection of synovial fluids

Synovial fluid analysis is of greatest value in distinguishing inflammatory from non-inflammatory arthropathies and in defining specific disorders within these two groups. It is also important in the diagnosis of early inflammatory disease, when it might be possible on the basis of cytopathology to identify a specific arthropathy before the clinical syndrome develops. In these cases, accurate early diagnosis often allows the institution of specific therapy before irreversible joint damage has occurred. Finally, it permits the very rapid diagnosis of joint disease, particularly in disorders such as septic arthritis, in which the prognosis is inversely related to delay in diagnosis. At least 300 µl of sample is required for full analysis and some laboratories provide paediatric lithium heparin tubes to prevent samples from clotting. As with all unfixed samples, delay in sample receipt by the laboratory will result in degeneration and limited analysis.

[Level of evidence – GPP.]

Figure 5: Synovial fluids.



Processing of synovial fluids
As a minimum, synovial fluids should be assessed for crystals and a cell count performed (Figure 5).[12] Several cell counting techniques exist and whichever is used will require internal validation. Ragocyte assessment can be done on either this wet preparation or a second.

[Level of evidence – C and GPP.]

3.2 FNA cytopathology

FNA cytopathology is a minimally invasive, relatively inexpensive and diagnostically accurate procedure that involves a needle being used to harvest cells from almost anywhere in the body for diagnoses of a number of diseases and/or infections. It may be performed with or without radiological guidance and, in more recent times, using endoscopic or endobronchial guidance. Aspirators, whatever their clinical specialty, must be trained to undertake FNAs and perform regular FNAs to maintain skills and competency. The number of aspirators should be sufficient to offer the clinical service, but there should not be so many that skills deteriorate due to lack of exposure. Aspirators will benefit from regular feedback on inadequacy rates.

Superficial lesions may be aspirated freehand or with ultrasound guidance while computed tomography (CT), ultrasound, or endoscopic or endobronchial guidance may be used for deeper seated lesions. Although this document does not attempt to identify and recommend procedures for performing and preparing FNA samples from all body sites, it does attempt to provide guidance for FNA from some of the more common areas from which FNA is performed. These include:

- head and neck FNA and other superficial lesions, such as breast, axilla and groin, usually performed freehand or with ultrasound guidance
- FNA of lung and other deep-seated organs, such as the liver, performed with radiological guidance (ultrasound and CT)
- endoscopic ultrasound (EUS) FNA most often used to sample the HPB tract and surrounding lymph nodes

- endobronchial ultrasound (EBUS) FNA used to sample lesions of the lung and lymph nodes in the diagnosis and staging of lung cancers and investigation of mediastinal lymphadenopathy
- breast FNA under ultrasound or stereotactic guidance or sometimes performed freehand.

The use of Rapid On Site Evaluation (ROSE) is recommended for all sites if resources permit and if it is felt that there would be a benefit in doing so. The major advantage is the ability to control and manage the specimen to ensure the best possible specimen quality as well as appropriately triaging for ancillary tests.[13–16] There is also the benefit of immediate feedback for the aspirator and potential provisional diagnosis. ROSE also enables the production of slides to be limited in that, once diagnostic material is seen on the rapid stains, the remainder of the passes may be wholly directed to ancillary investigations such as cell block. In addition, ROSE may have different benefits at different sites. For example, in the head and neck, there is good evidence that ROSE improves the adequacy of cytology samples taken,[17] while, in the mediastinum, it reduces the number of sites that need to be sampled when investigating possible malignant disease.[18,19]

The provision of ROSE, however, is limited in some centres by the shortage of experienced cyto/histopathologists, clinical commitments, off-site location of FNA clinics and the financial costs incurred by having clinicians at FNA centres for extended periods. Thus, in many hospitals and outpatient centres in the UK, biomedical scientists provide assistance at FNA clinics not only to prepare the spreads but also to assess samples on-site for specimen adequacy to ensure there are satisfactory numbers of cells and/or material that are representative of the lesion and tissue aspirated. In fact, a ROSE service, including specimen triage, may be entirely provided by appropriately competency-assessed biomedical scientists.[20–22]

When managing specimens in the clinic, it is important to bear in mind the need for diagnostic material on rapidly stained slides and liquid-based material for ancillary studies. The balance between these two requirements will vary depending on site and clinical need.

The nature of the liquid medium used to collect/transfer the cytology sample may vary with local practice provided that it facilitates high-quality cell block production and other analyses. The processing of material that is not used to make slides differs according to the ROSE service offered by the clinic and may include:

- placing all material in an alcohol-based fixative
- placing solid material directly into formalin and liquid material into saline
- placing all material in buffered saline or RPMI.

Microbiological and flow cytometric analyses may also be needed (see sections 5.2 and 5.3).

[Level of evidence – A.]

3.2.1 FNA of head and neck, superficial cutaneous lesions and breast

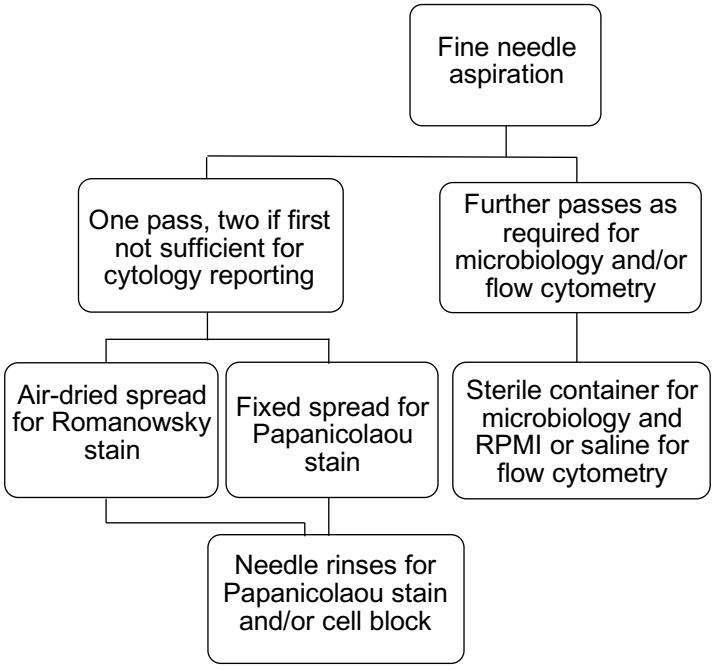
Head and neck lesions include those from the thyroid, lymph nodes and salivary glands and it is recommended that they be aspirated under ultrasound guidance so as to ensure the needle is in the lesion when the sample is being aspirated. Other superficial lesions include those of the axilla and groin as well as other palpable/visible lesions.

One of the recommendations of the National Cancer Peer Review programme (2011)[23] states that head and neck clinics should “offer a service whereby an FNA sample may be taken from the patient, and the clinic informed on the same day by the lab that the sample is adequate or not, giving time for the sample to be repeated on the same day.”[22]

The most cost-efficient method of providing such a laboratory service to head and neck clinics with no compromise to quality is to have trained biomedical scientists attend these clinics to provide on-site assistance and specimen adequacy assessment (ROSE). Trained biomedical scientists should be available to support clinicians and radiologists[24] and ensure direct spreads (air-dried and alcohol-fixed slides) are evenly prepared for staining and reporting. Biomedical scientists can also ensure the optimum triage of residual material from the needle so that cytological preparations and ancillary and molecular tests may be performed as required. The use of ROSE on any FNA sample can potentially be of use in reducing inadequate rates if these are high, such as in thyroid FNAs. This gives immediate feedback to the aspirating clinicians as to sample adequacy and enables repeat aspirations to be taken if required. For each site aspirated, it is recommended one pass be performed, and if sufficient for cytology reporting the procedure may either be concluded or further material may be collected for other studies. If not, further passes may be required. One air-dried ± one fixed preparation should be made for each pass, with the remainder of material triaged appropriately (Figure 6). If tuberculosis or lymphoma is suspected, separate passes must be made for samples for microbiological or flow cytometry, respectively.

[Level of evidence – C.]

Figure 6: Superficial FNA sample pathway.



Processing of FNA of head and neck and superficial lesions

The air-dried preparation(s) may be stained with a rapid Romanowsky stain by the attending biomedical scientist to assess for adequacy to ensure enough viable cells of the lesion being investigated are present. If prepared, the fixed spread may be stained by a Papanicolaou stain and the needle rinses from all the passes can be made into a Papanicolaou-stained preparation and/or cell block as required (Figure 6).

[Level of evidence – GPP.]

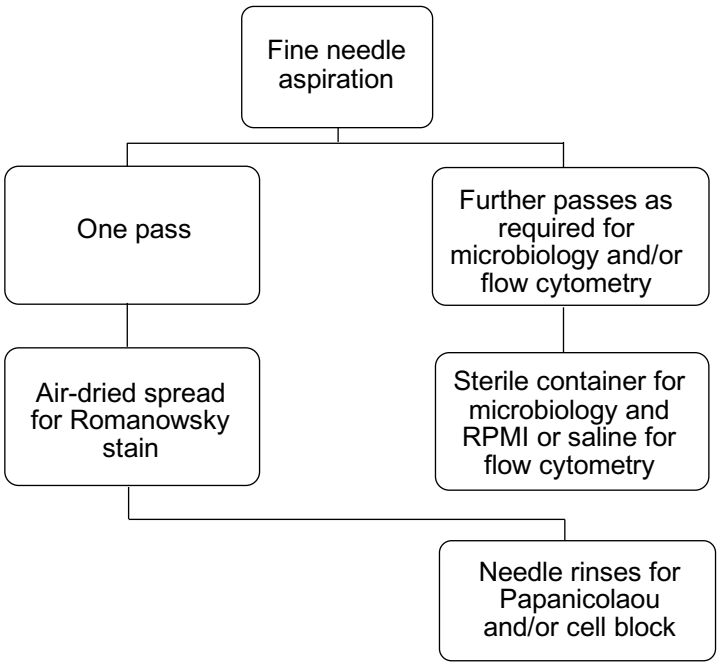
3.2.2 FNA of lung and other deep-seated lesions performed under radiological guidance

Deep-seated lesions include, among others, lung, stomach and pancreas, and are usually aspirated under CT or ultrasound guidance. When performing FNA of the lung, where pneumothorax is a frequent complication, only one to two passes may be possible. Therefore, to preserve material for ancillary and molecular tests, only one air-dried preparation per pass

is recommended with the needle then rinsed in a liquid medium (Figure 7). However, for other sites as many as three to four passes may be possible and both air-dried and fixed spreads can be prepared.

[Level of evidence – GPP.]

Figure 7: FNA of deep-seated lesions.



Processing of deep-seated lesions

For samples such as FNA of the lung, one method of preserving precious material would be to have the air-dried preparation stained with Romanowsky stain and screened for malignancy. If this is confirmed, all the needle rinses in the liquid medium can be used for a cell block preparation and ancillary testing. A Papanicolaou-stained preparation will only be necessary if no definite malignant cells are present on the Romanowsky-stained preparation. For other samples, where adequate numbers of passes are possible, the pathway for preparation for superficial FNA samples may be followed.

[Level of evidence – GPP.]

3.2.3 EUS FNA samples

EUS FNA samples include those from the pancreas, stomach, bile duct, liver, adrenal glands, lymph nodes or retroperitoneum, and these lesions may be solid as well as cystic. Different gauge needles may be used depending on site and aspirator preference. One pass may suffice, but several may be required to obtain sufficient material. If more than one site is aspirated (e.g. head of pancreas and peripancreatic lymph node), a different needle should be used for each site aspirated and material prepared as separate samples. If a gastrointestinal stromal tumour is suspected, adequate numbers of passes should be taken so that a cell block can be prepared for immunohistochemistry. It is very important that any cyst fluid samples aspirated are submitted for cytopathology, biochemistry and/or microbiology for full analyses depending on clinical and radiological findings. An aliquot of the sample should be sent in a dry, sterile container to each department with separate request forms including full clinical details (Figure 8).

[Level of evidence – GPP.]

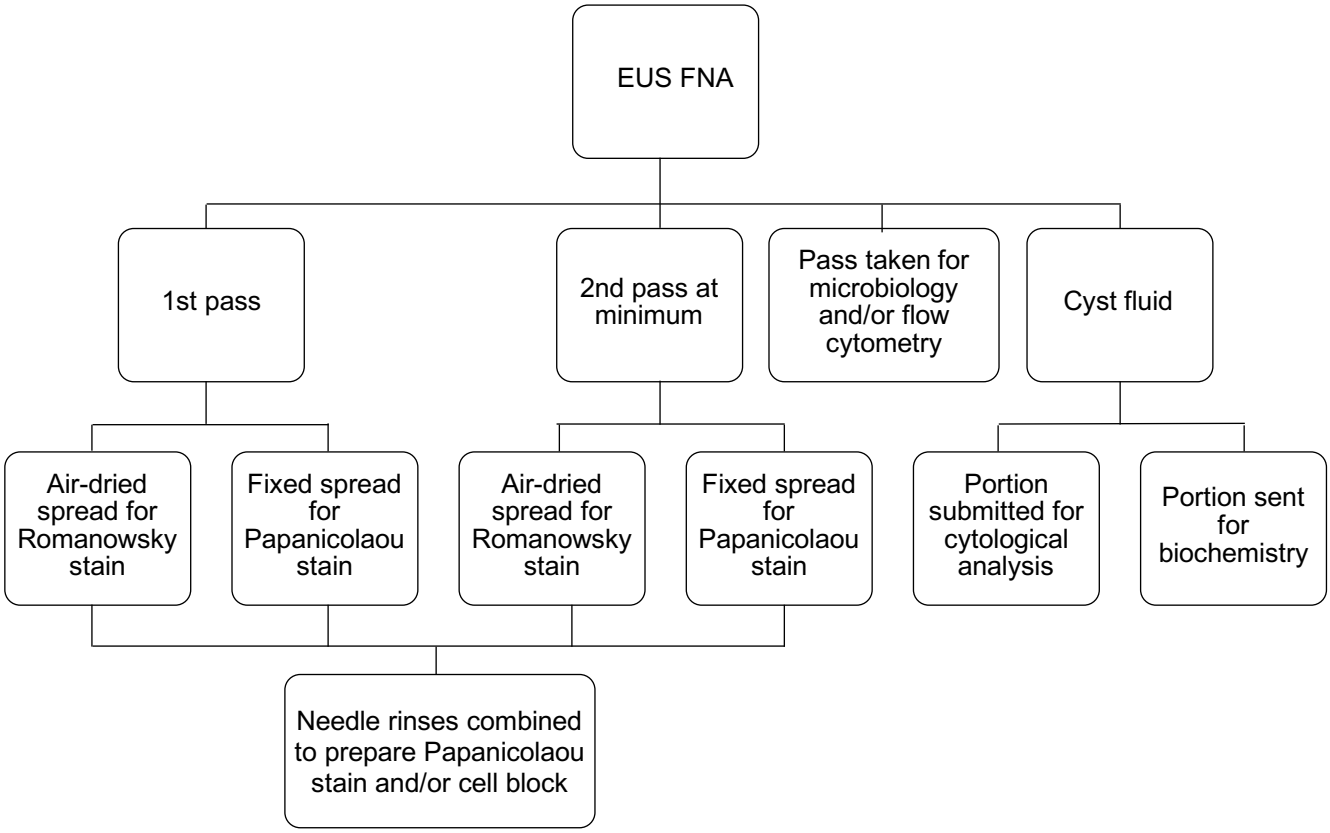
Processing of EUS FNA samples

The air-dried preparations can be Romanowsky stained while the fixed spreads can be Papanicolaou stained. Any solid material/clots from the needle rinses must be made into a cell block for H&E staining and should be reported as part of the cytological sample since cell blocks are used to complement direct spreads and should not be used as a substitute.[25] The needle rinses can be concentrated and a Papanicolaou slide or cell block can be prepared from the pellet.

Any fluid samples can be prepared as described for serous effusions (section 3.1.3). There is, as yet, no formal role for molecular analysis of solid pancreatic tumours. There is some use for molecular analysis in pancreatic cystic lesions[26] and this may be a topic of discussion in local protocols.

[Level of evidence – GPP.]

Figure 8: EUS FNA samples.



3.2.4 EBUS FNA samples

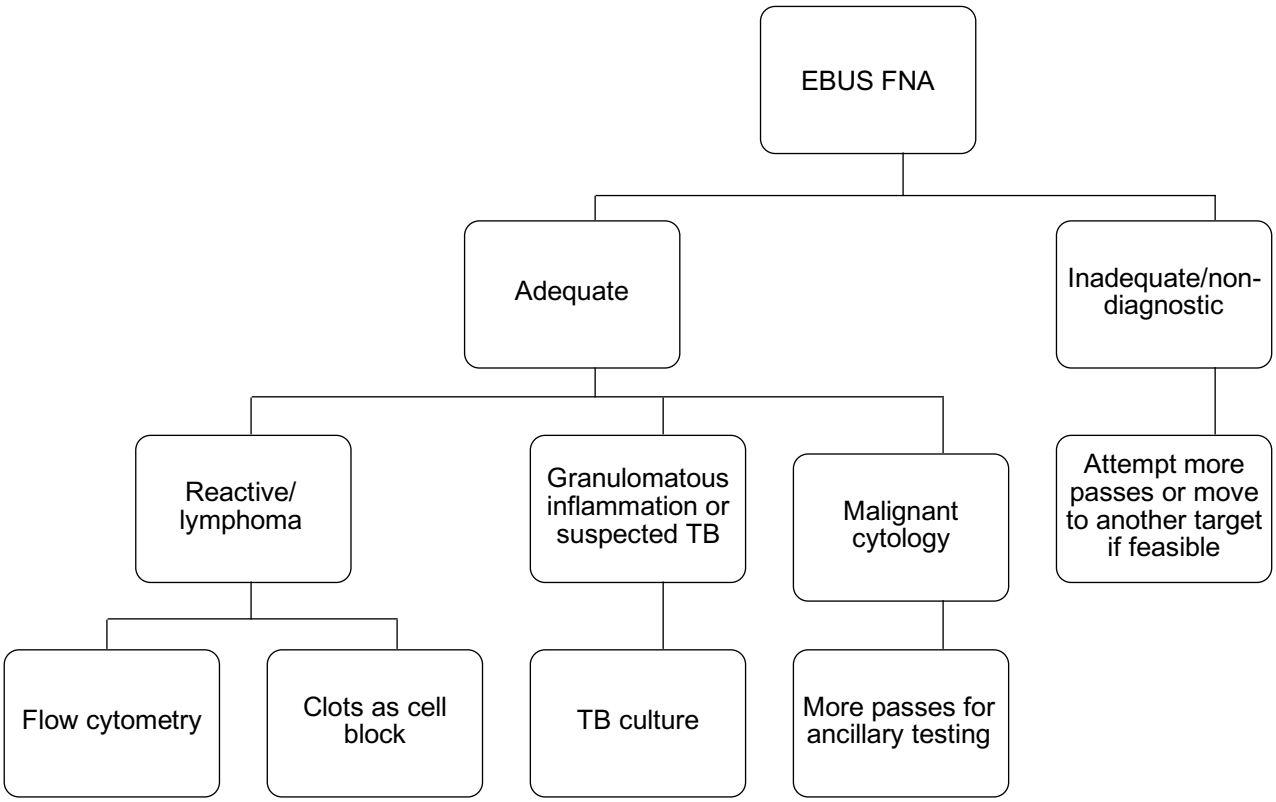
Since NICE issued guidance[27] on the use of EBUS FNA to investigate mediastinal masses, predominantly in the context of lung cancer staging, it has been adopted throughout the UK. EBUS FNA is also used to investigate other conditions associated with mediastinal lymphadenopathy, including cancer of other organs, atypical infections and sarcoidosis, and in the investigation of hilar lymphadenopathy. It is vitally important that the material is triaged to allow all necessary investigation to be performed on these limited samples (see also section 5.4).

Ideally, if resources allow and there is a need to do so, the attendance of a biomedical scientist to assist in sample preparation is recommended. Best practice would entail the presence of a cytopathology team to provide sample adequacy (ROSE) at the very least. This allows the material to be quality controlled appropriately. In some laboratories appropriately qualified staff can offer a preliminary diagnosis (Figure 9), enabling the material to be triaged appropriately.

There is good evidence that ROSE reduces the number of mediastinal sites sampled in the context of malignant disease, allowing shorter procedures and more efficient use of resources.[18,19]

[Level of evidence – C.]

Figure 9: EBUS FNA ROSE pathway.



3.2.5 Andrology

Andrology, or semen analysis, is a service used in two discrete settings: an assessment of male fertility and an assessment of the effectiveness of a male sterilisation procedure (post-vasectomy).

The service has been provided, historically, by a variety of departments within and outside pathology. However, it is ideally suited to those trained in cytomorphology and may provide an opportunity, now and in the future, for screening staff from the UK CSP to continue to use these valuable skills.

It is not appropriate to consider specimen pathways and requirements in detail here, but it is noted that the service must be clinically led by an appropriately competent consultant or equivalent, established procedures and guidelines should be followed[28–32] and accreditation provided, as in other branches of pathology, by UKAS under ISO15189.

[Level of evidence – C.]

3.2.6 Cerebrospinal fluid

CSF samples are often small in volume and need to be split between cytopathology, microbiology and biochemistry. The volume available for cytopathology often means only one cytological preparation can be prepared for examination.[33]

[Level of evidence – GPP.]

3.2.7 Touch imprint cytopathology

Touch imprint cytopathology is a useful cytopathology technique and is only performed in certain situations (e.g. brain smears, frozen section, tumour margins, core imprints). Samples are often used for intraoperative reporting to help guide surgery. Two to three such touch prints may be required for examination. Neuropathology smears is a specialist area and relevant guidance should be followed.[34]

[Level of evidence – GPP.]

4 Staining

Cytological diagnosis rests upon interpretation of the cells present on the basis of their cytological and architectural features. The criteria for this are well detailed in standard cytology textbooks. The appearances of cells can vary depending on how the sample was obtained and the method of preparation. Again, the reader should be familiar with the types of sample and preparations used in their own setting. Historically, cytopathology has used two main stains – Papanicolaou[35] and Romanowsky.[36] These are used in all standard textbooks and reflect not only the evolution of cytopathology but also the differences between sample handling – air-dried versus alcohol-fixed samples. As such, it is imperative that laboratories achieve the maximum technical quality when processing cytopathology samples. The use of standard stains and participation in relevant technical EQA schemes will assist with this.[6]

Some laboratories, invariably at the behest of pathologists, use H&E staining in place of or in addition to the above two stains on cytological samples. The H&E stain is not designed to show fine nuclear detail, assist with cytoplasmic interpretation or be used on non-formalin-fixed samples. The RCPath endorses the statement made in the original tissue pathways document that H&E staining should not be used for primary cytological interpretation.

[Level of evidence – C and GPP.]

5 Ancillary testing

Cytopathology samples are increasingly being used for reasons other than pure cytomorphological interpretation. This can vary with body/sample site and anticipated diagnosis. As such, consideration prior to taking any sample may be needed to ensure that the sample taken is suitable for such ancillary testing.

5.1 Cytopathology samples: clots/cell blocks and cell scraping of previously prepared slides

Cytopathology samples may come with, or lend themselves to the making of, clots/cell blocks.[37]In addition to aiding cytomorphological interpretation, cell blocks can be used in ancillary testing, including molecular analysis.[38]

Naturally formed clots from fluids and FNA samples may be extracted, fixed in formalin and processed for reporting as part of the overall cytopathology sample (Figure 10).

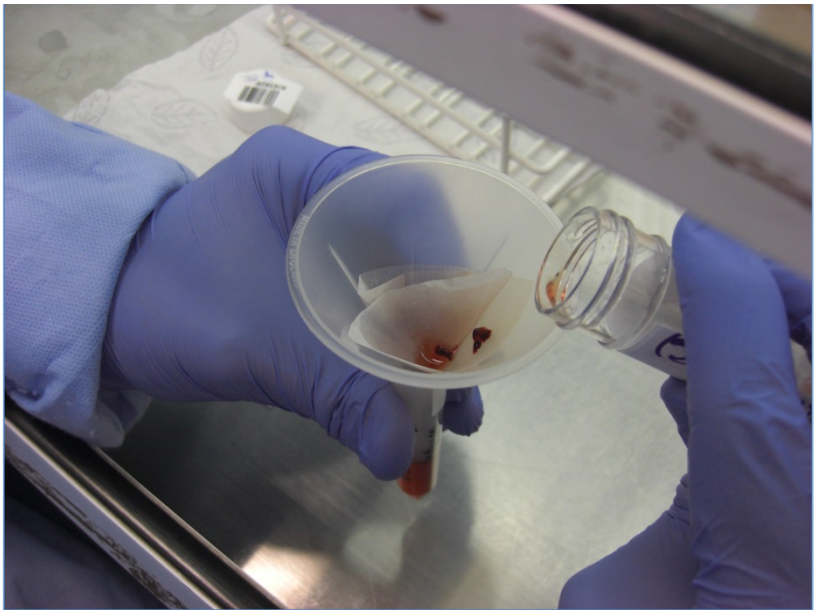
Cells suspended in a fluid medium may be prepared into a cell block by a variety of methods. A review of these methods is beyond the scope of this document, however, readers are advised to choose and validate the most appropriate method to suit their department's scope of practice. It is recommended that whatever method is chosen, it is compatible with the immunohistochemistry and molecular techniques employed.[39,40]

Scrapings from previously prepared slides may also be used for molecular analysis in this era of personalised medicine. This allows molecular studies to be performed on an enriched

population of cells. This can be done on samples used for morphological diagnosis or from extra prepared slides. If morphological diagnostic slides are used for cell dissection, then selected areas should be photographed or, if possible, whole slides should be scanned and stored in accordance with RCPath guidance.[41]

[Level of evidence – C and GPP.]

Figure 10: Clots from an EBUS sample being filtered and pre-cassetted.



5.2 Flow cytometry

An adequate flow cytometry sample is complementary to the morphological analysis of slide and cell block material for the diagnosis of low-grade lymphoma. Cytopathology specimens (particularly sufficiently cellular FNAs as well as serous effusions) are potentially suitable for the diagnosis of lymphoma in the majority of cases provided suitable and sufficient material is made available for flow cytometry, immunocytochemistry and cytogenetics (the last two generally utilise cell blocks).[42,43]

It is certainly acknowledged that an accurate diagnosis and subtyping of lymphoma is not possible on all cytological specimens, but it is also true that the omission of flow cytometry markedly reduces the sensitivity and diagnostic yield.

Flow cytometry transport media and arrangements should be discussed with the local immunophenotyping laboratory and typically involve some form of saline suspension and transport to the laboratory within one to two days of the specimen being taken.

Tests for circulating tumour cells are usually undertaken in a haematology department using flow cytometry or molecular techniques.

[Level of evidence – C and GPP.]

5.3 Infectious conditions

If an infectious condition is clinically suspected at the outset, then any sample taken may need to be sent for microbiology/virology testing. Such samples must be taken in line with the requirements of this testing (often fresh for microbiology and/or in some suitable virological medium).

[Level of evidence – GPP.]

5.4 Molecular

Cytopathology specimens are ideally suited for molecular analysis.[40,44] Most material undergoing molecular testing has been formalin fixed and paraffin embedded (FFPE), however, formalin is known to cause DNA artefacts. Air-dried or alcohol-fixed cytopathology material does not have this issue. Even in the case of FFPE cytopathology cell blocks, it is generally possible to optimise (i.e. minimise) fixation time by coordinating production of the block with subsequent processing.

Different tumour sites have different molecular requirements. Examples include:

- lung non-small-cell, non-squamous carcinomas require analysis of *EGFR* mutations and *ALK* and *ROS-1* translocations
- metastatic melanomas needing *BRAF* analysis
- metastatic colorectal carcinomas requiring *KRAS* mutation assessment.

Many tumour sites do not currently have mandated molecular analysis, but the field is growing rapidly. It is worth being aware of potential, imminent developments, such as the use of molecular tests in cytologically indeterminate thyroid nodules,[45] as well any technical issues relating to sample types and these tests.

The most recent guidelines for molecular analysis in lung cancer[46] specifically state that it is possible to obtain excellent results from material microdissected from cytopathology slides as well as well-processed cell blocks. This flexibility should be borne in mind when submitting material for molecular analysis. In particular, one of the most important parameters for the suitability of a sample for mutational analysis is the number of tumour cells as a proportion of all nucleated cells. If a particular slide has an area rich in tumour cells, it is more likely to yield success than sections cut from a cell block with a high background population of inflammatory cells. Generally, at least 5% of the cells in the sample should be tumour cells but 20% or greater is preferred.

Although not strictly a molecular test, it may be appropriate to state here that there is a current technical issue with some of the antibodies used for PD-L1 staining in the context of lung and other cancers in that they are not strictly validated for alcohol-fixed material. However, preliminary reports suggest that there may not be a significant problem.[47] Laboratories may have to examine local protocols accordingly.

If the material is to be sent to another laboratory for molecular analysis, it may be possible to choose a particular platform (single-gene tests or next-generation sequencing), taking into account local requirements, recent developments and cost. It is beyond the scope of this document to review these options in detail but, whatever the choice, there should be a clear discussion with the referral laboratory as to specimen requirements. The outcomes should be audited to ensure there is an acceptable success rate.

[Level of evidence – C.]

6 Cytopathology reporting

6.1 Report structure

The reporting of cytopathology samples must include the following information:

- sample site
- sample type

- clinical details as provided by the sample taker
- sample received (slides, fluid, volume, number of samples, etc.)
- cytological description and interpretation
- use of ancillary tests (if performed)
- cytological diagnosis in text form and classification coding (if appropriate to sample type)
- names of reporter(s)
- date of receipt and date of report
- SNOMED coding (ideally SNOMED CT if laboratory systems allow).

It is imperative that clinical details are provided to allow the reporting cytologist to adequately interpret the cytopathology sample submitted in the correct clinical context. No, or a lack of sufficient, details should be fed back to clinical users to help improve information for future samples.

6.2 Reporting systems

Historically, diagnostic cytopathology reporting was largely descriptive and often lacked consistency in reporting criteria and terminology. This has been problematic not only for cytologists but also for clinical users receiving cytopathology reports. In some body sites, a standardised reporting terminology has been developed, for example in thyroid cytopathology. International groups have developed several systems that aim to standardise reporting, terminology and classification. The adoption and use of current international systems are supported and endorsed by RCPath. Some are relatively new, but international evidence on the utility and advantages of such an approach is growing. Evidence in the UK setting is either lacking or very scanty in general terms, but the adoption of such internationally used systems will allow the production of UK data and comparison with the worldwide literature. It seems illogical to attempt to amend or reinvent such systems just for UK use.

RCPath therefore endorses the adoption and use of the following cytopathology reporting systems:

- urine cytopathology – Paris system for reporting urinary tract cytopathology[48]
- respiratory cytopathology – RCPath *Dataset for histopathological reporting of lung cancer*[49]
- pancreas and biliary cytopathology[26] – Papanicolaou Society system for reporting pancreaticobiliary cytopathology
- salivary cytopathology – Milan system for reporting salivary gland cytopathology[50]
- thyroid cytopathology – RCPath *Guidance on the reporting of thyroid cytology specimens*[51]
- Breast Screening Programme (BSP) classification for breast FNA.[52]

Their use in the UK setting will allow for production of UK data that can be shared and compared with others, and such data can help influence further amendments to these systems. Their adoption will require education and familiarity not only by cytologists but also by clinical users to ensure no ambiguity or misunderstanding of their use. This approach may require site/user education, possibly in the setting of a multidisciplinary team (MDT). It may be necessary to use both the current system and new system concurrently to aid transition. Regular audit of the use of the systems adopted will also aid in assessing their use, application and outcome. The introduction of the systems outlined briefly below will require a thorough

reading of the relevant guidance documents and discussion with clinical users to ensure everyone understands the approach being used.

Body sites without an existing agreed international reporting/terminology approach should be reported in line with the general principles outlined in section 6.1. If, and when, other systems develop and are agreed internationally, then the UK should follow suit. Any decision not to do so would require sound reasoning and mapping to other systems to allow for comparison. The RCPath thyroid cytopathology system[51] is now well established and easily equates to other systems. Its continued use is recommended.

[Level of evidence – B and C.]

6.2.1 Reporting of urinary cytopathology

The Paris urinary cytopathology reporting system is based on international consensus, with a structured approach to diagnostic criteria and reporting.[48] It recognises that urological cytopathology is primarily for diagnosing high-grade urothelial lesions and is less effective at diagnosing lower grade lesions. The adoption of its diagnostic criteria and reporting format will aid cytological:histological correlation and make urological cytopathology reporting more consistent.

Table 1: Outline of the Paris system for reporting urinary tract cytopathology.

I	Non-diagnostic or unsatisfactory
II	Negative for high grade urothelial carcinoma
III	Atypia
IV	Suspicious for high grade urothelial carcinoma
V	Low grade urothelial neoplasia (LGUN)
VI	High grade urothelial carcinoma (HGUC)
VII	Other malignancies, primary and metastatic

6.2.2 Reporting of lung cytopathology

For the reporting of lung samples, the RCPath recommends that an unequivocally malignant case be reported according to the small biopsies and cytopathology format (Appendix E) in the RCPath *Dataset for histopathological reporting of lung cancer*,[49] in turn derived from the 2015 WHO classification.

The Papanicolaou Society system for reporting respiratory cytopathology[53] is comprehensive, but it is not as yet widely adopted and as such is not advocated for general use.

6.2.3 Reporting of pancreatobiliary cytopathology

The Papanicolaou Society system for reporting pancreatobiliary cytopathology is advocated to allow for consistency of approach and reporting. This system is not fully internationally accepted but offers the best current integrated approach to reporting samples from this site.[26,54]

Table 2: Outline of the Papanicolaou Society approach to reporting pancreatobiliary cytopathology.

I	Non-diagnostic
II	Negative (for malignancy)
III	Atypical
IV	Neoplastic: benign or other
V	Suspicious (for malignancy)
VI	Positive (malignant)

6.2.4 Reporting of salivary cytopathology

The Milan salivary cytopathology reporting system is relatively new but builds on international consensus for reporting salivary lesions and aids clinical management and reporting consistency.[50,55]

Table 3: Outline of the Milan system for reporting salivary gland cytopathology.

1	Non-diagnostic
2	Non-neoplastic
3	Atypia of undetermined significance (AUS)
4 – a – b	Neoplastic: Benign Uncertain malignant potential (SUMP)
5	Suspicious for malignancy
6	Malignant

6.2.5 Reporting of thyroid cytopathology

The reporting of thyroid cytopathology has several international systems, and in the UK it is recommended that the RCPath reporting system is used.[51] This system is now well accepted in the UK and correlates well with histological outcomes and MDT decision-making. It maps well to other international systems, especially the Bethesda thyroid cytopathology system.[51]

6.2.6 Reporting of breast cytopathology

Breast FNAs are relatively infrequent within the BSP, their use in general having been superseded by the use of histological core biopsies. However, they are still performed in some screening cases and in symptomatic women. The established BSP classification (C1–C5) has been widely adopted and accepted for reporting of breast FNA material.[52]

For lymph node FNAs performed in the context of breast pathology, the established LN1-LN5 categories are recommended for the same reasons. The role of nipple cytology in the diagnosis and management of spontaneous nipple discharge is debated, but it would be logical to report along similar lines to breast FNA cytology.

Table 4: Outline of breast FNA reporting categories.

C1	Inadequate for cytological diagnosis
C2	Benign breast tissue
C3	Atypia, probably benign
C4	Suspicious, probably malignant
C5	Malignant

6.3 Reporting staff

Medical pathologists who have finished and completed their training (Stage D)[56] are qualified to report diagnostic cytopathology. This includes in-service specialty doctors and staff grade doctors. Such training may not have covered all body sites and sample/preparation types and, in such circumstances, training and a period of double reporting and competency assessment is required to ensure they have the necessary skills for reporting. This may require a period spent at another laboratory to ensure familiarity.

Trainee pathologists should be allowed to see and provisionally report diagnostic cytopathology in line with recommended training guidance under supervision.[56]

In many laboratories, biomedical scientists often pre-screen diagnostic cytopathology samples prior to reporting by pathologists. Such an approach is seen as good practice, as it allows for double reporting. The adoption of ROSE by trained biomedical scientist staff is also advocated where appropriate (see section 3).

Biomedical scientists may also report diagnostic cytopathology after suitable training.[57] Biomedical scientists holding the Diploma of Expert Practice (DEP) in non-gynaecological cytopathology may sign out negative cytopathology samples from urine, serous fluid and respiratory tract (excluding FNA material).[24] Biomedical scientists holding the Advanced Specialist Diploma (ASD) in non-gynaecological cytopathology may also report positive and negative samples from the same sites (excluding lung FNA material). It is envisaged that holders of the DEP and ASD in non-gynaecological cytopathology will be able to extend their reporting range by education and suitable training and competency assessment (guidance in development by the Conjoint Board for Cytopathology).

All staff in the diagnostic cytopathology reporting team must have regular exposure to all the specimen types in their department. This is to maintain skills and competency and to ensure workflow and service is maintained during periods of reduced staffing (e.g. leave). Trainees must also have similar exposure, and this should not be to the detriment of other staff in attaining the above.

[Level of evidence – GPP.]

6.4 Professional and clinical leadership

Professional and clinical leadership requires a good mix of clinical and scientific knowledge. The RCPath advocates that this should be from a pathologist, whose broad training in medicine and pathology allows them to be best placed to marry these two areas together.[58]

[Level of evidence – GPP.]

7 Cervical cytology

The requirements for handling, processing and reporting cervical cytology, which is mostly derived from the CSP, are outlined in guidance issued by the four countries of the UK. Pathologists and consultant biomedical scientists should follow the appropriate guidance document according to their location.[59–63] While historically the reporting of such material was regarded as a screening test, the material is now considered a diagnostic cytology sample owing to the move to primary human papilloma virus (HPV) cervical screening with reflex cytology. As such, any pathologist or consultant biomedical scientist with cytomorphological skills, training and relevant qualifications should be able to assess and report these samples. However, the various UK CSPs have guidance on the requirements for all aspects of the handling, processing and reporting of such samples, and staff and laboratories should follow these.

8 Quality

All laboratories reporting cytopathology should aspire to holding the relevant, current ISO standard. Attainment of this should ensure the laboratory as a whole has a commitment to a quality approach in all aspects of its work.

All laboratories should ensure all staff are suitably trained and qualified to undertake the procedures they perform. This includes staff at all levels. All laboratories should undertake a regular audit of their service and reporting/outcomes, correlating with histology wherever possible. The use of consistent terminology and reporting categories will significantly aid in this respect.

All laboratories should participate in technical EQA schemes relevant to cytopathology, such as that run by UK NEQAS CPT.[6,64] All laboratory reporting staff should participate in any relevant interpretative EQA schemes as and when they are developed. Currently, no such national scheme exists in the UK, but one is under active development.[6]

[Level of evidence – C and GPP.]

9 Criteria for audit

As recommended by the RCPath as key performance indicators (see *Key Performance Indicators – Proposals for implementation,* July 2013, www.rcpath.org/profession/guidelines/kpis-for-laboratory-services.html):

- cytopathology cases are reported and authorised within seven to ten calendar days of the procedure
  - standard: 80% of cases must be reported within seven calendar days and 90% within ten calendar days.

Specific cytopathology audit suggestions and criteria include the following:

- use of appropriate recommended cytopathology reporting system (>95% of cases)
- report format to follow recommendations in this guidance (>95%)
- correlation with histological outcomes (where available) for all cases classed as suspicious of malignancy or malignant (>90% of those identified with histology)
- correlation with histological outcomes (where available) for all other cases (>90%)

- reasons for supplementary reports if reports are amended
- quality of samples – inadequate rates, suspicious rates, etc.
- value of ROSE on diagnostic/inadequate rate.

The above list is not exhaustive and will depend on local service and service needs.

10 References

1. Thorpe A, Al-Jafari M, Allen D, Carr R, Helliwell T, Sanders S. *Guidelines on staffing and workload for histopathology and cytopathology departments* (*4th edition)*. London, UK: The Royal College of Pathologists, 2015. Available at: www.rcpath.org/profession/guidelines/specialty-specific-publications.html

2. Furness P. *Clarification of the use of the College publication 'Guidelines on staffing and workload for histopathology and cytopathology departments' in limiting the workload of pathologists*. London, UK: The Royal College of Pathologists, 2018. Available at: www.rcpath.org/profession/guidelines/specialty-specific-publications.html

3. Lowe J. *Workload management in laboratory medicine: patient safety and professional practices* (*3rd edition)*. London, UK: The Royal College of Pathologists, 2014. Available at: www.rcpath.org/profession/guidelines/cross-specialty-publications.html

4. Thorpe A. *Recommendations for work space for consultant and associate specialist cellular pathologists.* London, UK: The Royal College of Pathologists, 2016. Available at: www.rcpath.org/profession/guidelines/specialty-specific-publications.html

5. Michael CW, Davidson B. Pre-analytical issues in effusion cytology. *Pleura Peritoneum* 2016;1:45–56.

6. UK NEQAS. *UK NEQAS Cellular Pathology Technique.* Accessed October 2018. Available at: www.ukneqascpt.org

7. Petrone MC, Arcidiacono PG. Basic technique in endoscopic ultrasound-guided fine needle aspiration for solid lesions: How many passes? *Endosc Ultrasound* 2014;3:22–27.

8. Meyer KC, Raghu G, Baughman RP, Brown KK, Costabel U, du Bois RM *et al.* An official American Thoracic Society clinical practice guideline: the clinical utility of bronchoalveolar lavage cellular analysis in interstitial lung disease. *Am J Respir Crit Care Med* 2012;185:1004–1014.

9. Rooper LM, Ali SZ, Olson MT. A minimum fluid volume of 75 mL is needed to ensure adequacy in a pleural effusion: a retrospective analysis of 2540 cases. *Cancer Cytopathol* 2014;122:657–665.

10. Rooper LM, Ali SZ, Olson MT. A minimum volume of more than 60 ml is necessary for adequate cytologic diagnosis of malignant pericardial effusions. *Am J Clin Pathol* 2016;145:101–106.

11. Thomas SC, Davidson LR, McKean ME. An investigation of adequate volume for the diagnosis of malignancy in pleural fluids. *Cytopathology* 2011;22:179–183.

12. Freemont AJ, Denton J. *Atlas of Synovial Fluid Cytopathology*. Dordrecht, The Netherlands: Springer Netherlands, 1991.

13. Schmidt RL, Witt BL, Lopez-Calderon LE, Layfield LJ. The influence of rapid onsite evaluation on the adequacy rate of fine-needle aspiration cytology: a systematic review and meta-analysis. *Am J Clin Pathol* 2013;139:300–308.

14. Sung S, Crapanzano JP, DiBardino D, Swinarski D, Bulman WA, Saqi A. Molecular testing on endobronchial ultrasound (EBUS) fine needle aspirates (FNA): Impact of triage. *Diagn Cytopathol* 2018;46:122–130.

15. Trisolini R, Cancellieri A, Tinelli C, de Biase D, Valentini I, Casadei G *et al.* Randomized trial of endobronchial ultrasound-guided transbronchial needle aspiration with and without rapid on-site evaluation for lung cancer genotyping. *Chest* 2015;148:1430–1437.

16. Jain D, Allen TC, Aisner DL, Beasley MB, Cagle PT, Capelozzi VL *et al.* Rapid on-site evaluation of endobronchial ultrasound-guided transbronchial needle aspirations for the diagnosis of lung cancer: a perspective from members of the pulmonary pathology society. *Arch Pathol Lab Med* 2018;142:253–262.

17. Moberly AC, Vural E, Nahas B, Bergeson TR, Kokoska MS. Ultrasound-guided needle aspiration: impact of immediate cytologic review. *Laryngoscope* 2010;120:1979–1984.

18. Collins BT, Chen AC, Wang JF, Bernadt CT, Sanati S. Improved laboratory resource utilization and patient care with the use of rapid on-site evaluation for endobronchial ultrasound fine-needle aspiration biopsy. *Cancer Cytopathol* 2013;121:544–551.

19. Oki M, Saka H, Kitagawa C, Kogure Y, Murata N, Adachi T *et al.* Rapid on-site cytologic evaluation during endobronchial ultrasound-guided transbronchial needle aspiration for diagnosing lung cancer: a randomized study. *Respiration* 2013;85:486–492.

20. Stevenson T, Powari M, Bowles C. Evolution of a rapid onsite evaluation (ROSE) service for endobronchial ultrasound guided (EBUS) fine needle aspiration (FNA) cytology in a UK Hospital: A 7 year audit. *Diagn Cytopathol* 2018;doi: 10.1002/dc.23967 (Epub ahead of print).

21. Schacht MJ, Toustrup CB, Madsen LB, Martiny MS, Larsen BB, Simonsen JT. Endobronchial ultrasound-guided transbronchial needle aspiration: performance of biomedical scientists on rapid on-site evaluation and preliminary diagnosis. *Cytopathology* 2016;27:344–350.

22. Thiryayi SA, Rana DN, Narine N, Najib M, Bailey S. Establishment of an endobronchial ultrasound-guided transbronchial fine needle aspiration service with rapid on-site evaluation: 2 years experience of a single UK centre. *Cytopathology* 2016;27:335–343.

23. National Cancer Peer Review Programme. *National Cancer Peer Review Programme Report 2010−11*. London, UK: Department of Health and Social Care, 2011. Accessed October 2019. Available at: www.gov.uk/government/publications/national-cancer-peer-review-programme-report-2010-11

24. British Association for Cytopathology. *Role of Biomedical Scientists within the provision of a non-gynaecological cytology service*. Accessed October 2018. Available at: www.britishcytology.org.uk/resources/role_of_biomedical_scientists_in_provision_of_non-gynae_cytology_service.pdf

25. Weston BR, Bhutani MS. Optimizing Diagnostic Yield for EUS-Guided Sampling of Solid Pancreatic Lesions: A Technical Review. *Gastroenterol Hepatol (N Y)* 2013;9:352–363.

26. Pitman MB, Layfield LJ. *The Papanicolaou Society of Cytopathology System for Reporting Pancreaticobiliary Cytology: Definitions, Criteria and Explanatory Notes*. Cham, Switzerland: Springer International Publishing Switzerland, 2015.

27. National Institute for Health and Clinical Excellence. Endobronchial ultrasound-guided transbronchial needle aspiration for mediastinal masses. London, UK: NICE, 2008. Accessed October 2019. Available at: www.nice.org.uk/guidance/ipg254

28. World Health Organization Department of Reproductive Health and Research. *WHO laboratory manual for the examination and processing of human semen* (*5th edition)*. Geneva, Switzerland: World Health Organization Press, 2010.

29. Hancock P, Woodward BJ, Muneer A, Kirkman-Brown JC. 2016 Laboratory guidelines for postvasectomy semen analysis: Association of Biomedical Andrologists, the British Andrology Society and the British Association of Urological Surgeons. *J Clin Pathol* 2016;69:655–660.

30. Tomlinson MJ, Harbottle SJ, Woodward BJ, Lindsay KS, Association of Biomedical Andrologists. Association of Biomedical Andrologists – Laboratory Andrology Guidelines for Good Practice version 3 – 2012. *Hum Fertil (Camb)* 2012;15:156–173.

31. Long S, Woodward B, Tomlinson M. Sperm toxicity testing: UK best practice guideline from the Association of Biomedical Andrologists. *Br J Biomed Sci* 2018;75:53–60.

32. Sanders D, Fensome-Rimmer S, Woodward B. Uncertainty of measurement in andrology: UK best practice guideline from the Association of Biomedical Andrologists. *Br J Biomed Sci* 2017;74:157–162.

33. Brandner S, Thom M, Holton J, Jacques T, Phadke R, Anderson G *et al. Tissue pathways for non-neoplastic neuropathology specimens*. London, UK: The Royal College of Pathologists, 2018. Available at: www.rcpath.org/profession/guidelines/cancer-datasets-and-tissue-pathways.html

34. Wharton SB, Hilton D, Ironside JW, Grant R, Collins VP, Brandner S *et al. Dataset for tumours of the central nervous system, including the pituitary gland*. London, UK: The Royal College of Pathologists, 2016. Available at: www.rcpath.org/profession/guidelines/cancer-datasets-and-tissue-pathways.html

35. Chantziantoniou N, Donnelly AD, Mukherjee M, Boon ME, Austin RM. Inception and Development of the Papanicolaou Stain Method. *Acta Cytol* 2017;61:266–280.

36. Piaton E, Fabre M, Goubin-Versini I, Bretz-Grenier MF, Courtade-Saidi M, Vincent S *et al.* Guidelines for May-Grunwald-Giemsa staining in haematology and non-gynaecological cytopathology: recommendations of the French Society of Clinical Cytology (SFCC) and of the French Association for Quality Assurance in Anatomic and Cytologic Pathology (AFAQAP). *Cytopathology* 2016;27:359–368.

37. Jain D, Mathur SR, Iyer VK. Cell blocks in cytopathology: a review of preparative methods, utility in diagnosis and role in ancillary studies. *Cytopathology* 2014;25:356–371.

38. Bellevicine C, Malapelle U, Vigliar E, Pisapia P, Vita G, Troncone G. How to prepare cytological samples for molecular testing. *J Clin Pathol* 2017;70:819–826.

39. Rollins SD, Russell DK. Cytopathology in focus: Cell blocks: Getting the most from the least invasive method. *CAP Today Online,* 2017. Accessed October 2019. Available at: www.captodayonline.com/cytopathology-cell-blocks-getting-least-invasive-method

40. da Cunha Santos G, Saieg MA, Troncone G, Zeppa P. Cytological preparations for molecular analysis: A review of technical procedures, advantages and limitations for referring samples for testing. *Cytopathology* 2018;29:125–132.

41. Cross S, Furness P, Igali L, Snead D, Treanor D. *Best practice recommendations for implementing digital pathology.* London, UK: The Royal College of Pathologists, 2018. Available at: www.rcpath.org/profession/guidelines/specialty-specific-publications.html

42. Grosu HB, Iliesiu M, Caraway NP, Medeiros LJ, Lei X, Jimenez CA *et al.* Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration for the Diagnosis and Subtyping of Lymphoma. *Ann Am Thorac Soc* 2015;12:1336–1344.

43. Moonim MT, Breen R, Fields PA, Santis G. Diagnosis and subtyping of de novo and relapsed mediastinal lymphomas by endobronchial ultrasound needle aspiration. *Am J Respir Crit Care Med* 2013;188:1216–1223.

44. Salto-Tellez M. More Than a Decade of Molecular Diagnostic Cytopathology Leading Diagnostic and Therapeutic Decision-Making. *Arch Pathol Lab Med* 2018;142:443–445.

45. Nishino M, Nikiforova M. Update on molecular testing for cytologically indeterminate thyroid nodules. *Arch Pathol Lab Med* 2018;142:446–457.

46. Lindeman NI, Cagle PT, Aisner DL, Arcila ME, Beasley MB, Bernicker EH *et al.* Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors: Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. *Arch Pathol Lab Med* 2018;142:321–346.

47. Tsao MS, Kerr KM, Dacic S, Yatabe Y, Hirsch F. *IASLC atlas of PD-L1 immunohistochemistry testing in lung cancer*. Aurora, CO, USA: International Association for the Study of Lung Cancer, 2017.

48. Rosenthal DL, Wojcik EM, Kurtycz DF. *The Paris system for reporting urinary cytology*. Cham, Switzerland: Springer International Publishing, 2016.

49. Nicholson AG, Kerr K, Gosney J. *Dataset for histopathological reporting of lung cancer* (*6th edition)*. London, UK: The Royal College of Pathologists, 2018. Available at: www.rcpath.org/profession/guidelines/cancer-datasets-and-tissue-pathways.html

50. Faquin WC, Rossi ED, Baloch Z, Barkan GA, Foschini MP, Kurtycz DF *et al. The Milan System for reporting salivary gland cytopathology*. Cham, Switzerland: Springer International Publishing, 2018.

51. Cross P, Chandra A, Giles T, Johnson S, Kocjan G, Poller D *et al. Guidance on the reporting of thyroid cytology specimens* (*2nd edition)*. London, UK: The Royal College of Pathologists, 2016. Available at: www.rcpath.org/profession/guidelines/specialty-specific-publications.html

52. Lee AH, Carder P, Deb R, Howe M, Knox F, Shrimankar J *et al. Guidelines for non-operative diagnostic procedures and reporting in breast cancer screening*. London, UK: The Royal College of Pathologists, 2016. Available at: www.rcpath.org/profession/guidelines/cancer-datasets-and-tissue-pathways.html

53. Layfield LJ, Baloch Z, Elsheikh T, Litzky L, Rekhtman N, Travis WD *et al.* Standardized terminology and nomenclature for respiratory cytology: the Papanicolaou Society of Cytopathology guidelines. *Diagn Cytopathol* 2016;44:399–409.

54. Wright PK, Shelton DA, Holbrook MR, Thiryayi SA, Narine N, Slater D *et al.* Outcomes of endoscopic ultrasound-guided pancreatic FNAC diagnosis for solid and cystic lesions at Manchester Royal Infirmary based upon the Papanicolaou Society of Cytopathology pancreaticobiliary terminology classification scheme. *Cytopathology* 2018;29:71–79.

55. Thiryayi S, Low Y, Shelton D, Narine N, Slater D, Rana D. A retrospective 3-year study of salivary gland FNAC with categorisation using the Milan reporting system. *Cytopathology* 2018;29:343–348.

56. The Royal College of Pathologists. *Histopathology Curriculum.* Available at: www.rcpath.org/trainees/training/training-by-specialty/histopathology.html

57. The Institute of Biomedical Scientists. *Education*. Accessed October 2018. Available at: www.ibms.org/education

58. Cross PA. BPR Clinical responsibility for cytology services. London, UK. The Royal College of Pathologists, 2019. Available at: www.rcpath.org/profession/guidelines/specialty-specific-publications.html

59. NHS England, Public Health England. *NHS cervical screening (CSP) programme*. Accessed January 2019. Available at: www.gov.uk/topic/population-screening-programmes/cervical

60. NHS Wales. *Cervical Screening Wales*. Accessed October 2018. Available at: www.cervicalscreeningwales.wales.nhs.uk/home

51. National Services Division. *Scottish Cervical Screening Programme*. Accessed October 2018. Available at: www.nsd.scot.nhs.uk/services/screening/cervicalscreening/index.html

52. NI Quality Assurance Reference Centre (QARC). *Cervical Screening.* Accessed October 2018. Available at: www.cancerscreening.hscni.net/1827.html

63. British Association for Cytopathology. *Recommended code of practice for cytology laboratories participating in the UK cervical screening programmes*. London, UK: British Association for Cytopathology, 2017.

64. Cross PA, Hodgson C, Crossley J, Crossley B. Development of a technical external quality assurance scheme in non-gynaecological cytology in UK. *Cytopathology* 2015;26:71–74.

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 population 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 population.
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 population 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 population 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.

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 population 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 population.
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 population 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 population 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 guideline monitoring sheet

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

AGREE standard	Section of guideline
Scope and purpose	
1 The overall objective(s) of the guideline is (are) specifically described	Introduction
2 The health question(s) covered by the guideline is (are) specifically described	Introduction
3 The population (patients, public, etc.) to whom the guideline is meant to apply is specifically described	Foreword
Stakeholder involvement	
4 The guideline development group includes individuals from all the relevant professional groups	Foreword
5 The views and preferences of the target population (patients, public, etc.) have been sought	Foreword
6 The target users of the guideline are clearly defined	Introduction
Rigour of development	
7 Systematic methods were used to search for evidence	Foreword
8 The criteria for selecting the evidence are clearly described	Foreword
9 The strengths and limitations of the body of evidence are clearly described	Foreword
10 The methods for formulating the recommendations are clearly described	Foreword
11 The health benefits, side effects and risks have been considered in formulating the recommendations	Foreword and Introduction
12 There is an explicit link between the recommendations and the supporting evidence	2–8
13 The guideline has been externally reviewed by experts prior to its publication	Foreword
14 A procedure for updating the guideline is provided	Foreword
Clarity of presentation	
15 The recommendations are specific and unambiguous	2–8
16 The different options for management of the condition or health issue are clearly presented	2–8
17 Key recommendations are easily identifiable	2–8
Applicability	
18 The guideline describes facilitators and barriers to its application	Foreword
19 The guideline provides advice and/or tools on how the recommendations can be put into practice	2–8
20 The potential resource implications of applying the recommendations have been considered	Foreword
21 The guideline presents monitoring and/or auditing criteria	9
Editorial independence	
22 The views of the funding body have not influenced the content of the guideline	Foreword
23 Competing interests of guideline development group members have been recorded and addressed	Foreword