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Tissue pathways for exfoliative cytology and fine needle aspiration cytology

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A Tissue pathways for exfoliative cytology

A1 Staffing and workload

Consultant pathologists reporting exfoliative cytology should have appropriate expertise and participate in relevant continuing professional development (CPD) activities. Sufficient time should be specified in job plans to allow for cytology activities. At least two pathologists should provide the cytology service in a laboratory. Cover must be arranged so that there is always a suitable pathologist available.

Biomedical scientists (BMSs) with sufficient training and experience should be responsible for the preparation of exfoliative cytology specimens. Suitably trained medical laboratory assistants (MLAs) may perform some preparation under BMS supervision. Sufficient staff should be available such that there is no delay in the processing of specimens. BMSs may report negative cytology from some sites, as described in the relevant guidance from the Institute of Biomedical Science and The Royal College of Pathologists. Where BMSs report negative specimens, this should be specified in local, written protocols which designate the training and experience required and the audit procedures for assessing the quality of the service.

BMSs should be encouraged to pre-screen slides and sufficient staffing should be available for this activity.

A2 Specimen submission

Specimens should be collected in the appropriate way as documented in laboratory standard operating procedures (SOPs) and communicated with users by means of a laboratory handbook. Some specimen types may require a BMS to be present at the time the sample is taken, in order to prepare the material appropriately.

Minimum standards for identification of patients and their specimens must be produced by the laboratory. Specimens that do not include sufficient information for certain identification should be rejected.

All relevant clinical information, including previous specimens submitted, previous tumours and any treatment given (such as radiotherapy or chemotherapy) should accompany the specimen on an appropriate request form.

Urine	<p>Freely voided, catheter, ileal conduit specimens or bladder/ureteric washings may be collected. It is essential that the specimen collection method is documented on the request form. Preservative may be used. A maximum of 20 ml of fresh sample is required.</p> <p>The first urine passed in the morning should be avoided. A mid-stream specimen is sub-optimal. For voided urine, an aliquot of the whole voided sample should be submitted.</p> <p>Samples may be taken from the upper tract by clinicians experienced in the technique and should be handled in the same way as urine specimens.</p>
Sputum	<p>This is recognised to be a specimen of limited or no clinical value, and hence should be rarely received. Where patients are unfit for bronchoscopy, three separate sputum samples collected on different days should be sent for cytological examination. Nebulised saline may</p>

	<p>be used to induce sputum production in appropriate clinical circumstances.</p> <p>Guidance should be given to the patient on producing a deep cough sample. A salivary sample is inadequate for cytology. The whole of the expectorated sample should be submitted.</p>
Endoscopic brushings	Endoscopic brushings may be obtained from a variety of sites. Common sites include bronchus and common bile duct. Ideally the material should be placed into transport medium for liquid-based cytology (LBC). The literature indicates that better results are achieved with this approach than with direct smears prepared at the bedside.
Endoscopic washings	Endoscopic washings are commonly obtained from the bronchial tree. The aspirated fluid should be placed in a sterile container. A fixative transport medium may be used.
Serous fluids and peritoneal washings	Collection of the sample may require image guidance. The sample should be removed into a sterile container. 20 ml of fresh sample is required for cytology.
Cerebrospinal fluid (CSF)	Obtained by lumbar puncture. Ideally, the submitting clinician should ensure a sample is submitted to clinical chemistry and microbiology as well, if appropriate. If a central pathology reception is to be responsible for dividing the specimen, this should be done promptly. A 2 ml sample is ideal for cytology, but examination of smaller amounts can be attempted and is often successful.
Synovial fluid	Aspirated fluid should be sent for cytology and microbiology.
Cyst aspirates	Clinically benign breast cysts which aspirate to dryness, where the aspirate is not blood stained, may be discarded. Otherwise up to 20 ml of the specimen should be submitted in a sterile container. Imaging guidance may be required to successfully target some lesions.
Skin and mucosal scrapes Nipple discharge	These should be spread directly onto a slide at the bedside. The slide may be either wet fixed or air-dried, depending on local preference.

A3 Specimen preparation

Trained laboratory staff should record the gross appearance of all specimens. There should be an SOP for processing each specimen type. Appropriate health and safety precautions should be observed when handling specimens. Although it is acceptable for an MLA to be trained to carry out most cytological preparations under supervision, a BMS should supervise the procedures and assess the slides to make sure they are satisfactory.

Urine	<p>Cytocentrifuge or LBC methods are acceptable</p> <p>A single wet fixed slide is sufficient with all preparation methods.</p>
Sputum	<p>Pick and smear technique, Saccomanno technique or LBC are all acceptable.</p> <p>A minimum of one wet fixed slide is prepared.</p>

Endoscopic brushings	Cytocentrifuge or LBC methods are acceptable for specimens submitted in liquid transport medium. At least one fixed slide is required. Two slides may give a greater diagnostic yield with cytocentrifuge methods.
Endoscopic washings	With unfixed specimens, centrifugation and direct smears made from cell deposits are acceptable. Specimens collected or received in the laboratory in fixative transport medium may be prepared by one of the LBC techniques. At least one wet fixed slide should be produced.
Serous fluids and peritoneal washings	Direct smear, cytocentrifugation and LBC methods are acceptable. At least one air-dried and one fixed preparation are required. Any clot in the specimen should be prepared histologically as a cell block and reported. A cell block may be required for further investigation. An aliquot of the specimen should be retained so that this may be prepared.
CSF	Due to the small specimen volume and generally low cellularity, carry over is a particular risk with this specimen. Technique must be meticulous to minimise the risks of contamination occurring. Where sufficient material is submitted, one air-dried and one fixed cytospin preparation should be prepared. If only a small volume of material is available, preference should be given to the air-dried preparation.
Synovial fluid	The minimum recommended is one wet preparation, one wet fixed slide and one spare slide. An air dried slide may also be helpful. A wet unstained specimen should be examined with polarised light.
Cyst aspirates	Centrifugation with direct smearing of the cell button, cytocentrifugation and LBC methods are all accepted methods. One wet fixed and one air-dried slide are recommended as a minimum. Air-dried slides cannot be produced with LBC methods.
Skin and mucosal scrapes Nipple discharge	Minimal preparation is required in the laboratory as prepared slides should be received.

A4 Staining

Urine	Papanicolaou stain.
Sputum	Papanicolaou stain. Additional appropriate special stains on request by the pathologist.
Endoscopic brushings	Papanicolaou stain. At least one wet fixed slide is required.
Endoscopic washings	Papanicolaou stain. At least one wet fixed slide is required. Diagnostic yield may be increased with cytospin methods by examining two slides. Additional appropriate special stains on request by the pathologist.

Serous fluids	Both Papanicolaou and Romanowsky stains should be prepared when possible. If LBC methodology is used, the sample should be split to allow preparation of air-dried material. Cell blocks should be stained with H&E with appropriate special stains on request by the pathologist.
CSF	Romanowsky and Papanicolaou stains should be prepared, depending on the quantity of material received. Additional appropriate special stains on request by the pathologist.
Synovial fluid	Papanicolaou stain and direct examination of a wet preparation with polarised light. Additional appropriate special stains on request by the pathologist.
Cyst aspirates	One Papanicolaou and one Romanowsky stained slide are recommended as a minimum.
Skin and mucosal scrapes Nipple discharge	All of the submitted material should be stained using either Papanicolaou or Romanowsky stain, depending on local preference and fixation.

A5 Further investigations

A negative result of an exfoliative cytology sample is not sufficient evidence to exclude significant disease. Discussion of cases at multi-disciplinary team meetings and submission of further samples, if deemed clinically appropriate, should thus be a routine part of the diagnostic pathway. Cytology results should be correlated with histology findings. Audit against final outcomes (which may be clinical) should be performed.

Special stains (such as PAS and Grocott) to highlight infective causes of disease should be used when appropriate.

Immunocytochemistry should be used to investigate possible malignant disease and to establish differentiation as appropriate. In general, it is particularly important to distinguish lymphoma, melanoma, carcinoma, mesothelioma and sarcoma as this has significant implications for management. Immunocytochemistry for various tissue specific antigens and cytokeratins may be used to suggest or support a primary site in metastatic disease.

Flow cytometry is the method of choice for confirming and typing lymphoma in fluid samples. The appropriate transport medium should be agreed locally. Rapid transit is required.

A6 Report content

The final report should include a gross description of the specimen and a text report commenting on adequacy where appropriate and the nature of any morphological changes, providing at least a differential diagnosis of their causes. Where special stains or immunocytochemistry are performed, the findings should be included in the body of the report.

The report should include the diagnosis of negative sample, non-neoplastic abnormality or neoplasm. The degree of certainty should be described. Where a neoplasm is diagnosed,

this should be classified in as much detail as possible. Where infections are suggested, an indication of the likely causative organism should be made if possible.

Peritoneal washings are part of the formal staging of gynaecological malignancies. The report should state categorically whether it is positive or negative for staging purposes.

A coded classification similar to the C1–C5 category used for the Breast Screening Programme may be applied for audit purposes, but this should not replace a formal written report. Management should always be planned against the text report.

The report should cross-reference any other cytology or histology specimen reported at the same time.

A7 References

Denton K, on behalf of the Cytopathology Sub-Committee. *Extended Role for Biomedical Scientists in the Reporting of Diagnostic Cytopathology Specimens*. London: The Royal College of Pathologists, 2007. www.rcpath.org/index.asp?PageID=1305

Working Party on Histopathology and Cytopathology of Limited or No Clinical Value. *Histopathology and Cytopathology of Limited or No Clinical Value (2nd edition)*. London: The Royal College of Pathologists, 2005. www.rcpath.org/resources/pdf/HOLNCV-2ndEdition.pdf

Chandra A, Cross P, Denton K, Giles T, Hemming D, Payne C, *et al*. The BSCC codes of practice – exfoliative cytology (excluding gynaecological cytopathology). *Cytopathology* 2009;20:211–23.

B Tissue pathways for fine needle aspiration cytology

B1 General aspects

B1.1 Staffing and workload

A comprehensive fine needle aspiration (FNA) cytology service should be under the supervision of a named lead pathologist, usually the head of cytology services. All cytopathologists reporting fine needle aspirates should be suitably trained and experienced, and should undergo appropriate CPD activities. Biomedical scientist (BMS) staff may be trained to assess adequacy and should be encouraged to examine FNA samples, but may not issue final reports.

FNA is unusual amongst pathology specimens in that the sample taker may well be the cytopathologist. Assessment of workload must include consideration of the pathologist's time spent attending FNAs at remote sites and within radiology, performing FNA clinics, travelling time and reporting FNA material, whether taken themselves or by other individuals.

Pathologists performing FNA clinics must be adequately supported by BMS staff and, where appropriate, by nursing, healthcare assistant and clerical staff.

B1.2 Specimen submission

Pathologists participating in FNA clinics should follow guidance on the administration and protocols of such clinics defined in British Society of Clinical Cytology (BSCC) codes of practice.

Specimens will also be submitted by clinicians and radiologists. Satisfactory methods of preparing and transporting the specimens must be agreed with the head of FNA service (see specific specimen guidelines).

B1.3 Specimen preparation

Specimen preparation may occur in a number of settings:

- by BMSs or medical laboratory assistants (MLAs) under BMS supervision, in the laboratory
- by BMSs or pathologists in a remote clinical setting
- by clinicians.

Staff making the preparations must have been suitably trained, receive feedback on their technique, pay attention to the health and safety issues of handling potentially infective material, and have adequate time allowed for the activity. Care should be taken to ensure that air-dried preparations are dried rapidly and that fixed preparations are fixed promptly (see BSCC codes of practice).

B1.4 Staining

See specimen-specific guidelines. Generally, it is ideal to have both air-dried Romanowsky stained material and wet fixed Papanicolaou stained material. Haematoxylin and eosin (H&E) staining offers no benefits over Papanicolaou staining and should not be used.

B1.5 Further investigations

Methods must be in place to allow immunocytochemistry, histochemical stains and occasionally other investigations such as flow cytometry. See specimen-specific guidelines.

B1.6 Report content

The report must include an indication of adequacy, description of material present and ideally a final conclusion/summary diagnosis. Where nationally recognised, coded categories of diagnosis are agreed, these must be used in addition to the text.

B2 FNA of breast

B2.1 Staffing and workload

Palpable breast masses may be aspirated by a cytopathologist; however, many FNAs will be undertaken by radiologists or surgeons. Units may require immediate reporting of aspirates in a rapid diagnosis clinic, in which case the time spent in sites remote from the laboratory should be recognised. Cytology staff can either be present at these clinics, or samples can be taken to the laboratory for very urgent reporting. In either case, dedicated pathologist time and BMS support must be adequate. It is essential that clinical, radiological and pathological findings are correlated before deciding on a definitive diagnosis; access to clinical and radiological assessment must be available in the one-stop setting.

B2.2 Specimen submission

These may be direct smears (either air-dried or fixed) or collected in saline or other liquid media for LBC. Cyst fluid is submitted if it is haemorrhagic, although clear fluid may be discarded at clinical discretion.

If ancillary studies for prognostic markers are required, a liquid sample should be submitted.

B2.3 Specimen preparation

Direct smears are generally the method of choice, particularly if immediate reporting in a rapid diagnosis setting is required. Rapid air drying of directly prepared slides is important. The most effective method is using a hairdryer on a cool setting. If fixed direct smears are submitted, fixation should be prompt. Cytospins are generally suitable for cyst fluid and needle washings. Appropriate LBC techniques may be employed. Cell blocks may be prepared for immunohistochemistry where appropriate.

B2.4 Staining

Romanowsky and Papanicolaou stains are performed on air-dried and fixed slides respectively. Commercial LBC preparations can be stained with Papanicolaou only. H&E staining offers no benefits over Papanicolaou staining and should not be used.

B2.5 Further investigations

Assessment of oestrogen and progesterone hormone receptors by immunocytochemistry and HER-2 status by immunocytochemistry or FISH is required for all invasive breast carcinomas. These assessments may be performed on either cytological or histological material, providing sufficient is available. If semi-quantitative techniques are performed on cytological material, the process must be fully validated on cytology material. It should not be assumed that protocols used for histological material can be implemented with no modification.

Immunocytochemistry may also be required when unusual diagnoses are suspected, for instance neuro-endocrine carcinomas, lymphoma or metastatic tumours.

Whilst these investigations will often be easier to perform on a core biopsy, there are clinical settings where this is not in the best interests of the patient. In these instances, laboratories should be prepared to undertake these investigations on cytology specimens.

B2.6 Report content

This must include a text report. The C1–C5 categorisation may be employed as an additional feature, and is mandatory for cases taken as part of the NHS Breast Screening Programme.

The final diagnosis and management must be determined by an appropriate multi-disciplinary team.

B2.7 References

Pathology Reporting of Breast Disease. NHSBSP publication no 58. 2005. NHS Cancer Screening Programmes. www.cancerscreening.nhs.uk.

Kocjan G, Chandra A, Cross P, Denton K, Giles T, Herbert A, *et al*. BSCC Codes of Practice – fine needle aspiration cytology. *Cytopathology* 2009;20:283–96.

B3 FNA of thyroid

B3.1 Staffing and workload

Thyroid aspirates may be taken by physicians, surgeons, radiologists or pathologists. Palpable masses may be aspirated without image guidance. Many thyroid aspirations will need to be taken under ultrasound control. The results obtained are improved by immediate assessment of adequacy, either by a cytopathologist or suitably trained BMS. In this case, the time spent in sites remote from the laboratory should be recognised.

B3.2 Specimen submission

Specimens may be submitted either as directly prepared slides or in a liquid medium.

B3.3 Specimen preparation

Samples received in liquid should be prepared by the cytopspin method, or by techniques appropriate for LBC.

Where immediate assessment of adequacy is required, direct air-dried smears should be prepared and stained with a Romanowsky method.

B3.4 Staining

For directly prepared slides, all the material should be stained. For liquid specimens, representative samples may be used. Ideally, both Papanicolaou and Romanowsky methods should be employed. H&E staining offers no benefits over Papanicolaou staining and should not be used.

B3.5 Further investigations

These are only rarely required but may include immunocytochemistry or flow cytometry. Where immediate assessment is performed, appropriate material for further investigations should be collected based on initial microscopic interpretation.

B3.6 Report content

This must include a text report. In addition, classification according to the RCPATH guidance on reporting of thyroid cytology should be included. Final diagnosis and management should be determined by an appropriate multi-disciplinary team.

B3.7 References

Guidance on Reporting of Thyroid Cytology Specimens. London: Royal College of Pathologists, 2009. www.rcpath.org/index.asp?PageID=38

Kocjan G, Chandra A, Cross P, Denton K, Giles T, Herbert A, *et al*. BSCC Codes of Practice – fine needle aspiration cytology. *Cytopathology* 2009;20:283–96.

B4 FNA of lung

B4.1 Staffing and workload

These samples are always taken under radiological control. It is advisable to have at least a suitably trained biomedical scientist present at the examination to advise on adequacy and to assess the need for material for further investigations. It may be necessary for a consultant to attend to give an immediate diagnosis. These requirements must be included in workload calculations.

B4.2 Specimen submission

Where immediate assessment is performed, some air-dried slides will be stained with a Romanowsky stain. Further material including directly spread slides and a sample in a suitable fluid for immunocytochemistry should also be submitted. It is preferable to have both air-dried and fixed material submitted.

B4.3 Specimen preparation

Appropriate health and safety precautions should be in place if unfixed material is handled in the laboratory. Submission of part of the sample for microbiological examination may be appropriate.

B4.4 Staining

Ideally both Romanowsky and Papanicolaou stained material should be examined.

B4.5 Further investigations

Material must be available if required for immunocytochemistry, either on a liquid based sample or a cell block.

B4.6 Report content

The report should be primarily text based. There is no formal coding system for respiratory cytology.

B4.7 References

Kocjan G, Chandra A, Cross P, Denton K, Giles T, Herbert A, *et al.* BSCC Codes of Practice – fine needle aspiration cytology. *Cytopathology* 2009;20:283–96.

B5 FNA of head and neck

B5.1 Staffing and workload

Rapid-result (one-stop) clinics are a requirement of cancer guidelines for the management of head and neck malignancy. Cytology staff may be present at these clinics and may perform FNA in the clinic, or samples may be taken to the laboratory for very urgent reporting. In either case, staffing must be adequate. It must be recognised that a proportion of cases assessed in a rapid-result clinic will require additional work and further consideration in the laboratory at a later date.

B5.2 Specimen submission

Directly prepared slides are submitted. If unusual or unknown pathology is suspected, a liquid sample should also be sent.

B5.3 Specimen preparation

Directly prepared slides. Cytospin or LBC preparations may be prepared in addition. Liquid samples should be prepared for immunocytochemistry if required, either as cytospins, LBC samples or cell blocks. FNA for suspected lymphoma should be managed separately (see below).

B5.4 Staining

Papanicolaou and Romanowsky stains should ideally both be used.

B5.5 Further investigations

No further investigations are usually required for metastatic squamous carcinoma, but more unusual lesions may require immunocytochemistry.

B5.6 Report content

A text-based report is always required. There is no formal coding system. Metastatic squamous carcinoma is the most common diagnosis and usually presents few difficulties. However, in some head and neck tumours, especially lesions of salivary glands, a definitive diagnosis can be very difficult on FNA and, if in doubt, a differential diagnosis can be given.

B5.7 References

Kocjan G, Chandra A, Cross P, Denton K, Giles T, Herbert A, *et al.* BSCC Codes of Practice – fine needle aspiration cytology. *Cytopathology* 2009;20:283–96.

Service Guidance on Improving Outcomes in Head and Neck Cancers. National Institute of Clinical Excellence. <http://www.nice.org.uk/guidance/csghn>

B6 FNA of pancreas

B6.1 Staffing and workload

Aspiration of pancreatic masses requires appropriate image guidance. An effective team including the radiology and endoscopy units is thus essential. The most common guidance modalities are computerised tomography (CT) or endoscopic ultrasound. In either case, the effectiveness of the technique is improved by immediate on-site assessment of adequacy, which may be performed either by a suitably trained BMS or pathologist. Staff time spent on this activity must be included in job planning.

B6.2 Specimen submission

Direct air-dried smears are preferable. Material may also be collected in saline or other liquid media for LBC or cell block preparation. Cystic lesions should be aspirated and the fluid submitted.

B6.3 Specimen preparation

Direct smears are generally the method of choice. Rapid air drying of directly prepared slides is important. The most effective method is using a hairdryer on a cool setting. Cytospins are generally suitable for cyst fluid.

Because of the length of the needle, aspirated material from endoscopically obtained specimens may be plentiful. It may be useful to limit the spread of material to 4–6 direct smears and use the remainder for a cell block preparation if required.

Cell blocks may be prepared for histology, special stains or immunochemistry where appropriate.

Cyst fluid should be prepared as for other sites (see B2.3)

B6.4 Staining

Romanowsky and Papanicolaou stains are performed on air-dried and fixed slides respectively. LBC preparations can be stained with Papanicolaou only. H&E staining offers no benefits over Papanicolaou staining and should not be used.

B6.5 Further investigations

Recognition of neuro-endocrine differentiation is important; material should be made available for immunocytochemistry and histochemistry.

B6.6 Report content

This must include a text report, which should indicate the pathological diagnosis and degree of certainty. Any ancillary studies performed to support the diagnosis should be recorded. There is no formal coding system for pancreatic cytology.

B6.7 References

Kocjan G, Chandra A, Cross P, Denton K, Giles T, Herbert A, *et al.* BSCC Codes of Practice – fine needle aspiration cytology. *Cytopathology* 2009;20:283–96.

B7 FNA of lymph node

B7.1 Staffing and workload

FNA of palpable lymph nodes may be taken by a variety of clinicians, or by cytopathologists working in a clinic setting. In this instance, medical and BMS staffing calculations must take this into account. FNA of deep nodes, e.g. para-aortic nodes, will usually be taken under radiological control. In this instance, it is advantageous for a BMS to attend to prepare the slides and to provide an immediate assessment of adequacy.

B7.2 Specimen submission

Where immediate assessment is performed, some air-dried slides will be stained with a Romanowsky stain. Further material including directly spread slides and a sample in a suitable fluid for immunocytochemistry should also be submitted. In some instances, samples should also be submitted in suitable media for cytogenetics and flow cytometry (see below). If clinically appropriate, a sample may be submitted for microbiology.

B7.3 Specimen preparation

Directly prepared slides.

Liquid samples should be prepared for immunocytochemistry if required, either as cytopins, LBC samples or cell blocks.

Samples for cytogenetics and flow cytometry should be placed in appropriate media and transported promptly for testing.

B7.4 Staining

Romanowsky staining is critical and should always be performed. Papanicolaou staining may be performed in addition. H&E offers no benefit over Papanicolaou and should not be used.

B7.5 Further investigations

Immunocytochemistry may be performed on LBC samples, cytopins or cell blocks. Cell blocks are probably the preferred method because of the ability to make multiple slides easily.

Stains for infective organisms eg ZN, may sometimes be useful

B7.6 Report content

There has been debate about the utility of FNA in diagnosing lymphoma. However, in many cases the diagnosis will not be apparent clinically and cytology is a very accurate means of making a preliminary diagnosis. High-grade non-Hodgkin's lymphoma (NHL) and Hodgkin's disease can usually be reliably diagnosed. A firm diagnosis of low grade NHL will be difficult if no ancillary studies are available. Most cases of lymphoma diagnosed on cytology will require a biopsy for further typing but in selected cases, if local protocols including mandatory flow cytometry are in place, a full diagnosis sufficient to commence treatment may be made on cytology material alone.

FNA also has an important role to play in the diagnosis of metastatic carcinoma, recurrent lymphoma and transformation to high-grade disease.

In all the above situations, the report should detail if a specimen is diagnostic, and if not the differential diagnosis and degree of certainty.

Further investigations, including those performed outside the originating department, should be appended to the cytology report.

A number of non-malignant lymphoid lesions may also be confidently diagnosed on cytology specimens.

All lymphoma and suspected lymphoma cases should be discussed at an appropriate multi-disciplinary team meeting.

B7.7 References

Kocjan G, Chandra A, Cross P, Denton K, Giles T, Herbert A, *et al.* BSCC Codes of Practice – fine needle aspiration cytology. *Cytopathology* 2009;20:283–96.

Kocjan G. Best practice no 185. Cytological and molecular diagnosis of lymphoma. *J Clin Pathol* 2005;58:561–567.