REVIEW ARTICLE DOI:10.1111/j.1365-2303.2009.00679.x

The BSCC Code of Practice – exfoliative cytopathology (excluding gynaecological cytopathology)

A. Chandra*, P. Cross†, K. Denton‡, T. Giles§, D. Hemming†, C. Payne¶, A. Wilson** and P. Wilson††

*Cellular Pathology, Guy's & St Thomas' NHS Foundation Trust, London, UK, †Department of Histopathology, Queen Elizabeth Hospital, Gateshead, UK, ‡Cellular Pathology, Southmead Hospital, Bristol, UK, §Department of Pathology, Royal Liverpool University Hospital, Liverpool, UK, ¶Department of Cytopathology, Royal Gwent Hospital, Newport, UK, **Department of Histopathology, Monklands Hospital, Airdrie, UK and ††Cellular Pathology, St George's Hospital, London, UK

Accepted for publication 16 June 2009

A. Chandra, P. Cross, K. Denton, T. Giles, D. Hemming, C. Payne, A. Wilson and P. Wilson

The BSCC Code of Practice – exfoliative cytopathology (excluding gynaecological cytopathology)

Exfoliative cytopathology (often referred to as non-gynaecological cytology) is an important part of the workload of all diagnostic pathology departments. It clearly has a role in the diagnosis of neoplastic disease but its role in establishing non-neoplastic diagnoses should also be recognised. Ancillary tests may be required to establish a definitive diagnosis. Clinical and scientific teamwork is essential to establish an effective cytology service and staffing levels should be sufficient to support preparation, prescreening, on-site adequacy assessment and reporting of samples as appropriate. Routine clinical audit and histology/cytology correlation should be in place as quality control of a cytology service. Cytology staff should be involved in multidisciplinary meetings and appropriate professional networks. Laboratories should have an effective quality management system conforming to the requirements of a recognised accreditation scheme such as Clinical Pathology Accreditation (UK) Ltd. Consultant pathologists should sign out the majority of exfoliative cytology cases. Where specimens are reported by experienced biomedical scientists (BMS), referred to as cytotechnologists outside the UK, this must only be when adequate training has been given and be defined in agreed written local protocols. An educational basis for formalising the role of the BMS in exfoliative cytopathology is provided by the Diploma of Expert Practice in Non-gynaecological Cytology offered by the Institute of Biomedical Science (IBMS). The reliability of cytological diagnoses is dependent on the quality of the specimen provided and the quality of the preparations produced. The laboratory should provide feedback and written guidance on specimen procurement. Specimen processing should be by appropriately trained, competent staff with appropriate quality control. Microscopic examination of preparations by BMS should be encouraged wherever possible. Specific guidance is provided on the clinical role, specimen procurement, preparation and suitable staining techniques for urine, sputum, semen, serous cavity effusion, cerebrospinal fluid, synovial fluid, cyst aspirates, endoscopic specimens, and skin and mucosal scrapes.

Keywords: exfoliative cytopathology, non-gynaecological cytology, laboratory quality control, pathology accredition, specimen procurement, slide preparation

The Code of Practice (COP) was commissioned by the British Society for Clinical Cytology (BSCC) in its role as a professional society that promotes education and good practice in cytology in the UK. The COP in non-gynaecological cytology includes a document on exfoliative cytology presented here and another on fine needle aspiration (FNA), which will follow in a forthcoming issue of *Cytopathology*. The COP has had the benefit of a lengthy consultation within the BSCC and has been endorsed by the Royal College of Pathologists (RCPath). While some of the organisations, such as Clinical Pathology Accreditation (UK) Ltd (CPA), and terms such as biomedical scientists (BMS), known as cytotechnologists outside the UK, apply to practice within the UK we hope

Correspondence:
Dr A. Chandra, Pathology Department, St Thomas's Hospital, London, SE1 7EH, UK.
Tel.: +44 20 7188 2946; Fax: +44 20 7188 2948;
E-mail: ashish.chandra@gstt.nhs.uk

that the general principles addressed within the documents should be applicable internationally.

The COP is a collective piece of work to which members of the BSCC and its Council have contributed. It has been a complex task, particularly in areas where there is little published evidence for good practice. This has been drawn from the experience of the authors and contributors of this document. There are few guidelines for best practice in exfoliative cytology and little in the way of technical external quality assurance (TEQA), reporting or pre-screening external quality assurance (EQA) or guidance for clinical audit. The COP aims to fill that gap by referring, where possible, to evidence-based literature about existing standards in specimen preparation and reporting. We aim to describe what we regard as best practice while understanding that resources and facilities vary from laboratory to laboratory (and from country to country).

We would have liked to provide workload-staff ratios for non-gynaecological cytology but have found this impossible as exfoliative cytology is so closely integrated with FNA, gynaecological cytology and histopathology practice in most laboratories. Also, guidelines on workload-staff ratios are provided by the RCPath and Institute of Biomedical Science (IBMS) and are available on their websites. We aim to explain what may safely be carried out by laboratory assistants under supervision, career cytotechnologists and cytopathologists respectively and how their roles link up. Laboratory managers may then work with individual departments to consider the equipment and manpower implications of using liquid-based cytology (LBC), immediate assessment of slides to decide ancillary tests and availability of those tests.

Principles of microscopic examination

Many different types of specimens from all major organ systems are included in the category of exfoliative non-gynaecological cytopathology. The samples vary considerably in cell content but all include cells that naturally, or induced by instrumentation, exfoliate from mucosal and other internal and external body surfaces. Some specimens are passed naturally and are freely available for collection (urine, sputum, semen), some require active drainage of body fluids (serous fluids, CSF, cyst aspirates) while others require scraping, brushing or washing of body surfaces (skin scrapings, endoscopic brushings and washings).

Whilst cytological examination of exfoliated material clearly has a role in the diagnosis of neoplasia, the importance of diagnosing non-neoplastic conditions should also be recognised. In many cases, the cytopathological diagnosis forms the basis for clinical management. Others require the results of additional investigations to be factored into the clinical assessment before definitive treatment is given. Reliable identification of a disease process requires a multidisciplinary team approach, taking into account the results of all investigations and clinical findings and this is particularly relevant to cytopathology. Ancillary tests such as special stains, immunocytochemistry, microbiological investigations and molecular analysis are often necessary as an adjunct to morphological interpretation and the cytopathology laboratory should develop or have access to these ancillary tests.

Clinical and scientific teamwork is central to providing, maintaining and developing an efficient cytopathology service. It is essential that cytopathology services are maintained at the highest achievable standard and are subject to routine clinical audit.[1] Both laboratory staff and users of the service must have a clear understanding of the benefits and limitations of cytopathology in the diagnostic arena. There should also be frequent direct communication between users and providers of service.

Reliable identification of a disease process requires a multidisciplinary team approach, taking into account the results of all investigations and clinical findings and is particularly relevant to cytopathology.

Health and safety

Many exfoliative cytopathology specimens are received fresh (unfixed). Every effort should be made to minimise risk to laboratory staff and to those responsible for collecting and transporting samples to the laboratory. Samples must be submitted to the laboratory in keeping with Health and Safety regulations[2] and CPA standards.[3] The laboratory should carry out a risk assessment by a trained officer to establish that these regulations are being followed. As far as possible, samples should be prepared and rendered safe for examination in a safety cabinet to minimise the risk associated with aerosols. If specimen preparation occurs at a remote site, then risk assessments must be in place and the procedures carried out in such a way as to minimise risk.

Workload and establishment

To run an effective diagnostic cytopathology service, adequate resourcing is essential. It should be recognised that preparation, prescreening and reporting of samples are all appropriate in order to provide an effective service. The preparation process may be complex and time-consuming depending on the type of sample, methods of preparation, refinement techniques and ancillary testing involved. Compared to cervical cytology,[4] there has been little in the way of explicit guidance on workload[5] and establishment for non-gynaecological cytopathology services. Well-trained, experienced members of staff are central to delivering and developing high quality services. The departmental and individual workload should take into account the cervical and non-gynaecological diagnostic cytology workload and accurately reflect the time taken to prepare, examine and report different categories of samples. As there are no firm guidelines, laboratories should carry out their own assessments of time taken for these procedures as part of local business planning and annual appraisal. Furthermore, time taken to prepare specimens and assessing their adequacy in clinical areas such as bronchoscopy should be included in the overall costing of the procedures.

The laboratory activities of gynaecological and non-gynaecological cytology are usually carried out by the same staff, often on rotation, and require similar skills, training, supervision and quality assurance. Managers should take account of the diverse location of clinical areas in which samples are collected so that non-gynaecological services may be coordinated with those of cervical cytology, which may be more centralised.

We hope hat further guidance establishing calculation of workload and staffing ratios will be forthcoming in the future. This would allow standardization of methods for estimating the time taken for different activities so that appropriate number of staff is employed, ultimately enhancing quality.

Managers should take account of the diverse location of clinical areas in which samples are collected so that non-gynaecological services may be coordinated with those of cervical cytology, which may be more centralised.

Specimen procurement

Appropriate specimen collection and submission to the laboratory is essential, otherwise the quality of the sample and the ultimate diagnosis may be compromised. The laboratory should play an advisory role and provide written guidance to assist in the procurement of samples. This should be available in the form of a laboratory handbook. For specimens that require tests in more than one laboratory, clinicians should be advised that, ideally, they should send separate samples to each laboratory. For microbiology, the sample should be collected using aseptic techniques. Fetal calf serum may be added to part of the specimen if flow cytometry is required and if there is likely to be a delay of more than two hours before processing, bearing in mind that this would render it unsuitable for microbiology.

Receipt and identification of specimens

All exfoliative cytopathology specimens must be entered in the laboratory database/daybook and assigned an accession number. In keeping with CPA requirements, the test requisition (manual or electronic) should include the patient's full name, identifiers such as national health service (NHS) and/or hospital/clinic number, date of birth, date and time of specimen collection and type of specimen.[6] The identity of the clinician requesting the test, the ward/clinic location to which result is to be forwarded and relevant clinical history should be provided on the request form. Laboratories should have a standard operating procedure (SOP) specifying criteria for matching details on the label of the specimen container with those on the request form. Specimens that meet these criteria are given a laboratory accession number. The laboratory must follow an SOP covering specimens that do not meet the requirements for accession.[7] This should include documentation of attempts to contact the requesting clinician.

BMS staff should accurately record the gross appearances (volume, consistency, colour etc) of each specimen before processing and this should be included in the final report. There should be an SOP for processing each specimen type.

Best practice

Laboratories should have a standard operating procedure (SOP) specifying criteria for matching details on the label of the specimen container with those on the request form.

Specimen preparation

Specimen preparation is critical to its subsequent interpretation and reporting. It is of utmost importance to ensure that suitably trained laboratory personnel handle specimen processing. Although it is acceptable for a medical laboratory assistant (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 for reporting. Criteria for unsatisfactory slides should be stated in the SOP.

BMS should take part in the cytological preparation rotas to maintain their own expertise. They should pay particular attention to standards of preparations of direct smears and cytocentrifuge preparations, which require considerable technical expertise. All slides should be assessed microscopically by BMS as part of quality control of specimen preparation. Even if the consultants do not require exfoliative cytopathology specimens to be prescreened, BMS should review slides with pathologists or senior BMS staff on a regular basis so that they have the experience to assess slides for adequacy of preparation, which requires recognition of the main diagnostic criteria. This is essential if they are to be expected to assess sample adequacy in a clinical setting such as a bronchoscopy suite.

> Acceptable standard
>
> Although it is acceptable for an MLA to be trained to carry out most cytological preparations, a BMS should supervise the procedures and assess the slides to make sure that they are satisfactory for reporting.

Prescreening and reporting

Exfoliative cytopathology slides may be examined by suitably qualified and competent BMS and by trainee medical staff. BMS should be encouraged to prescreen slides and sufficient numbers of staff should be available for that activity. Quality control is improved by more than one person examining the slides and time spent by pathologists in screening slides may be reduced this way. Consultant pathologists should sign out the majority of exfoliative cytopathology cases. However, in some laboratories, experienced BMS in the UK currently sign out selected types of samples at the discretion of and under the responsibility of the lead cytopathologist in line with agreed protocols.

The Diploma of Expert Practice in Non-gynaecological Cytology is already offered by the IBMS and begins to provide an educational basis for formalising the role of the BMS in exfoliative cytopathology. There is currently no equivalent in exfoliative cytopathology to the Advanced Specialist Diploma that exists in in the UK for cervical cytology. The role of the BMS in exfoliative cytopathology has been reviewed by a joint committee represented by the Royal College of Pathologists (RCPath) and the IBMS. The recommendation is for BMS to sign out negative reports on a number of specimen types such as urine, serous effusions and specimens from the respiratory tract. Consultant and trainee pathologists should see adequate numbers of negative cases to maintain their own experience and education.

Although no minimum data sets currently exist for reporting exfoliative cytology, synoptic reports and templates are used in many departments. These should be agreed upon by the reporting team and may be used for the sake of improving consistency and turnaround times of reporting.

> Best practice
>
> BMS should be encouraged to pre-screen slides and sufficient numbers of staff should be available for this activity.

Audit, quality control and quality assurance

Cytopathological diagnoses should be discussed alongside histology at multidisciplinary meetings (MDM) and may either provide the main clinical diagnosis or strengthen or weaken an equivocal histological diagnosis. Revised or modified cytological diagnoses should only be made by pathologists with experience in that field of cytopathology. The revised diagnosis should be recorded in the MDM minutes and on a supplementary cytology report if the change is clinically significant.

Cytological diagnoses and MDM decisions should be audited against outcome, which may be clinical rather than histological. Audit may be helped by cytopathological diagnoses including a final assessment on the lines of the NHS breast screening programme (NHSBSP) reporting guidelines[8] for FNA specimens (C1- inadequate; C2- benign; C3- atypia, probably

benign; C4- suspicious for malignancy; C5- malignant) or by using equivalent SNOMED codes.[9] The use of a C4 category avoids poor quality samples being overcalled while C3 provides a mechanism for follow-up, clinical discussion and repeat biopsy in equivocal cases. Such cases should be reviewed in a multidisciplinary setting in order to minimise the chances of a false positive result. The C3 and C4 categories should be used sparingly and their outcome monitored. It must be emphasised here that the text report rather than the C1–C5 reporting categories should be used for making management decisions. The reporting categories are a convenient way of recording data for audit purposes but not sufficient grounds for deciding clinical management.

There should be competency assessments at all stages of preparation and reporting of specimens and guidelines should be in place for this. In order to eliminate bias, the same consultant should not report the cytology and the corresponding histological sample. Alternatively, arrangements for regular peer review should be in place. Similarly, quality control (QC) should be performed by a BMS other than the one preparing the specimen. Poorly prepared samples should be discussed with the BMS or MLA who prepared the sample. Where possible, BMS should perform microscopic examination on specimens they have prepared themselves; this provides a first-hand assessment of the quality of the preparation. Where MLAs prepare slides, the supervising BMS should routinely check the adequacy of preparations before they are passed on to a pathologist or senior BMS. Quality control should aim to minimise the proportion of unsatisfactory preparations, which may cause difficulty in morphological interpretation. Guidelines for quality criteria at each stage of the process should outlined in the SOPs.

There are presently no national quality assurance schemes (apart from the Andrology EQA referred to below) specific to exfoliative cytopathology although a few regional EQA schemes are running successfully. Laboratories must have comprehensive SOPs in place and operate internal quality control procedures that audit all aspects of the process from sample collection to dispatch of the final report. It must be acknowledged that further work is required in regulating QC and QA in non-gynaecological cytology.

Quality assurance is best achieved by correlating cytology with histology results and clinical outcome, by regular audits and by inclusion of cytopathology staff at multidisciplinary meetings and in professional networks.

Quality assurance is best achieved by correlating cytology with histology results and clinical outcome, by regular audits and by inclusion of cytopathology staff at multidisciplinary meetings and in professional networks.

Naturally produced fluids

Urine

Overview. Squamous cells, urothelial cells and, less frequently, renal tubular cells, prostatic and seminal vesicle epithelial cells may exfoliate into urine. Haematuria is a common presenting symptom of urinary tract neoplasms. Urine cytology is very sensitive and specific in the detection of high-grade (*in situ* and invasive) neoplasms. The detection of low-grade urothelial neoplasms is much less sensitive. These are difficult to recognise cytologically due to the morphological similarity to normal exfoliated urothelial cells and to those seen with calculi, inflammation and instrumentation.[10]

Inflammatory and autoimmune disorders involving the kidney may produce different types of casts of renal tubules and abnormalities of red cells (dysmorphism) that are demonstrable in unfixed urine samples.[11]

Indications for cytological examination of the urine include:

1. Investigation of haematuria in suspected neoplasia of the urinary tract.
2. Follow up after treatment of *in situ* and invasive bladder neoplasia.
3. Assessment of glomerular damage in renal parenchymal disease by demonstration of urinary casts and red blood cell dysmorphism.

It is useful to audit periodically the appropriateness of clinical requests for urine cytology and adherence to agreed guidelines.[12]

Specimen procurement. Urine should be voided directly into a suitable container. If the sample is to be collected at home, containers for LBC methods with preservative fluids may be used. Providing containers with ethanol as a fixative is not recommended. The requesting clinician should explain to the

patient that the first voiding of the day is unsuitable for cytological examination due to cellular degeneration overnight in the bladder. A full voiding of urine or a sample thereof is preferable to a midstream sample. A mid-morning or random specimen is recommended for cytological testing.[10] Urine may also be collected from indwelling catheters and by washings from the bladder or upper urinary tract. These specimens are more cellular and include cohesive sheets of urothelial cells unlike naturally voided samples. Furthermore, samples from patients with indwelling catheters, with renal or bladder calculi and from ileal conduits may exfoliate urothelial cells that mimic neoplasia. It is essential that the clinician states the specimen collection method on the request form. The cytology report should reflect any difficulties in interpretation relating to the collection method of the specimen.

Specimen preparation. Cells may be harvested from urine samples by cytocentrifugation, micropore filtration or by one of the LBC methods.

Slides and stains. With LBC methods and large volume cytocentrifugation, a single wet-fixed slide is appropriate. With cytocentrifugation techniques, one wet-fixed slide is adequate[13] for diagnosis although two are prepared in many laboratories. The Papanicolaou (Pap) staining method is the most appropriate for all urinary cytology samples.

Examination of fresh urine (voided within 2 hours) for dysmorphic red blood cells using phase contrast microscopy may be performed on a wet, unstained preparation when clinically indicated.[10]

> Acceptable standard
>
> A single wet-fixed, Pap stained slide is adequate with all methods of preparation for urine cytology.

Sputum

Overview. Sputum has been defined by the RCPath as a specimen of limited or no clinical value.[14] The emphasis in respiratory cytology for cancer diagnosis has been altered by the introduction of the fibreoptic bronchoscopy.[15] The British Thoracic Society (BTS) guidelines recommend biopsy, brushings and washings at bronchoscopy.[16] However, sputum cytology has the advantage of being a non-invasive, cheap and easily repeated test.[17] It is still used by clinicians but guidelines for clinical requests should be agreed upon and periodically audited. Induced sputum cytology may be useful in patients who are unfit for bronchoscopy and has a role in immunosuppressed patients.

Specimen procurement. Patients with a productive cough should be encouraged to expectorate into a suitable collection pot. The best specimens are from the first cough after waking, collected before brushing teeth or eating breakfast. To maximise the detection of pulmonary malignancy, it is recommended that three separate sputum samples collected on different days, should be sent for cytological examination.[17]

Patients, usually immunosuppressed, or infants and the elderly with a dry cough may be induced to produce sputum by the inhalation of saline. In patients with suspected or known infections, it is essential that sputum induction is carried out in isolation and with staff taking precautions to avoid risk of infection to themselves.

Specimen preparation. There are several approaches to the preparation of sputum samples for cytological testing. Each has its advantages and limitations and individual laboratories must decide which method to adopt. The 'pick and smear' technique uses technical skill to identify lower respiratory tract material by gross inspection. This is selectively picked up by an orange stick (or other suitable instrument) and smeared directly onto slides, which are then wet-fixed by immersion in 95% alcohol or spray-fixed. Other methods avoid selection bias. The sample may be first mucolysed using agents such as dithiothreitol (DTT) and then homogenised (Saccomanno technique[17]). Aliquots of the homogenate are then used to make either direct smears or cytocentrifuge preparations. Alternatively, the specimen may be fixed appropriately for one of the commercially available LBC methods. These methods are particularly suited to respiratory tract specimens due to their mucoid nature that hinders rapid air-drying. Romanowsky stains are therefore not recommended.

Slides and stains. Preparation of a single wet-fixed slide is the minimum acceptable standard. Two wet-fixed slides may increase the diagnostic yield for cytospin methods. The Pap staining method is the most suitable routine stain for all respiratory tract

specimens. Additional stains such as Ziehl Neelsen (ZN) and Grocott may be necessary to establish the diagnosis particularly when infections are suspected and should be routinely prepared in the immunosuppressed.

Semen

Overview. Semen analysis involves assessment of a number of parameters, each with a wide range of normality and operator variability. Evidence of internal quality control and participation in the national Andrology EQA scheme, which is recognised by the United Kingdom National External Quality Assessment Service (UK-NEQAS), is recommended to laboratories offering this service.

Indications for semen analysis include:

1. Investigation of infertility.
2. Confirmation of absence of sperm following vasectomy.

Specimen collection and preparation. Appropriate instructions to patients are mandatory for a meaningful interpretation of the test. These include advice about a period of abstinence (2–6 days) from sexual activity, washing hands and genitalia prior to specimen collection and using a sterile, wide-neck container (available from the GP surgery or laboratory). The specimen should be collected by masturbation and not by coitus interruptus or ejaculation into a condom. Ideally, the specimen should be produced in a designated suite situated within or close to the laboratory. Where these facilities are not available, effort should be made to minimise delay in transport of the specimen to the laboratory, preferably within an hour.

Semen analysis for infertility. In the laboratory, macroscopic appearances should be recorded promptly upon receipt of the specimen. The volume, pH, viscosity and liquefaction time should also be noted. Examination of the wet preparation for sperm motility should be performed using phase contrast microscopy on a pre-warmed stage. The presence of any agglutination should be noted. The most critical parameter is the sperm count, which should be carried out using a counting chamber and attention paid to accuracy of dilutions. The reference range should be calculated within individual laboratories. In oligospermia, distinction must be made between a count just below the lower limit of the normal range and specimens in which only an occasional sperm is seen but too few for a formal count.

Sperm morphology should be assessed on a Pap stained slide. Microscopists should be aware of the full range of sperm morphology as well as the occasional appearance of sperm precursors and seminal vesicle epithelial cells.

Stringent criteria can make it difficult sometimes to identify completely normal forms as demonstrated by the wide range of variability quoted by WHO.[18] The presence of inflammatory cells should also be recorded. A new edition of the guidelines is due to be published soon.

There is variation in the quality of semen depending on general health and well-being.[19] A repeat semen analysis may be performed within 3 months of the previous if an abnormal parameter is detected.

Post-vasectomy semen analysis. In post-vasectomy samples, a distinction must be made in the report between immotile and motile sperm on the direct microscopy of the wet preparation. Examination of the centrifugate should be performed if no sperm is seen on the direct preparation. WHO guidelines should be followed for interpretation of results.

Internal quality control should be applied to each stage from receipt of specimen, preparation of dilutions for sperm count to data entry. Regular meetings with staff should be held to provide feedback on the performance of the laboratory as a whole and individually, if necessary.

> Best practice
>
> Evidence of internal quality control and participation in the national andrology EQA scheme is recommended to labs offering this service. WHO guidelines for preparation and reporting should be followed.

Drained fluids

Serous (pleural, peritoneal and pericardial) fluids

Overview. Cytological examination of serous fluids is most frequently performed to detect malignant cells and where possible, confirm or suggest possible primary sites of malignancy. Some inflammatory effusions (tuberculosis) and those associated with

systemic autoimmune disorders (rheumatoid disease and systemic lupus erythematosis (SLE) may produce specific appearances in pleural fluid samples. A commonly encountered problem in malignant effusions is the distinction between adenocarcinoma and mesothelial cells (reactive and neoplastic). Good cytological preparations and the availability of reliable ancillary methods (including appropriate immunocytochemical panels) are prerequisites for accurate diagnosis as it is increasingly necessary for clinical management to identify the type and primary site of malignancy in serous fluids.

Indications: Pleural and pericardial effusions are aspirated for clinical treatment and are used specifically for the diagnosis of

1. Malignancy.
2. Infections, especially tuberculosis.

Peritoneal (ascitic) fluid is aspirated for clinical relief of distension and for diagnosis of malignancy, and is used for staging in certain gynaecological malignancies. Peritoneal dialysis fluid may be sent for comment on eosinophil content, which may be estimated on a Romanowsky stained slide. However, in general cell counts are best carried out in the haematology department.

Specimen procurement. Fluid is collected, sometimes under image guidance, into sterile containers. It is advisable to send 20 ml of fresh sample to the cytology laboratory, regardless of how much fluid was aspirated although smaller or larger volumes may be sent. Only a single sample should be assessed when draining effusions related to cardiac failure or chronic liver disease.[14]

Specimen preparation. Direct smear, cytocentrifugation and LBC methods are all suitable preparatory techniques. The method of choice will depend on the amount and nature of the deposit after centrifugation. Any clot in the fluid should be prepared histologically as a cell-block, which should be reported at the same time as the cytological preparations. If ancillary testing is likely to be required, an aliquot should be retained for preparation of a cell-block or for additional slides following cytocentrifugation.

Slides and stains. It is recommended that where possible both Pap and Romanowsky stained slides should be prepared; with direct smears and cytocentrifugation preparations, one slide of each is recommended as the minimum. Romanowsky stains are preferable for diagnosis of lymphoma/leukaemia, their distinction from reactive lymphocytic effusions, and for the recognition of eosinophils. LBC methods are limited to stains requiring alcohol fixation such as Pap stain. One slide is sufficient as routine. Sections from cell-blocks and clots should be stained with haematoxylin and eosin (H&E).

Special stains for microorganisms may be helpful in relevant cases. Cytocentrifuge preparations, LBC slides and cell-block sections are all suitable for special and immunochemical stains, and preparation method is largely a matter of local choice. It may depend on the panel of antibodies required. In many instances, a large panel of antibodies may be required and a cell-block may provide more material than cytocentrifugation or LBC preparations. Separate aliquots must be retained and diluted if LBC is to be used for immunochemistry.

Cell-blocks may be made from natural fibrin clots, bacterial agar and clots induced with thrombin or commercial kits.[20] Cell-blocks made using commercial kits may be compromised if phosphate buffered saline is used in processing. Cytocentrifuged material should be prepared on coated slides using a carefully controlled dilution and choosing post-fixation techniques to optimise antigen retrieval for immunochemistry.

> Best practice
>
> For serous effusions, both Pap and Romanowsky stained slides should be prepared. One slide of each is recommended.

Cerebrospinal fluid (CSF)

Overview. Many diseases affect the CSF compartment of the central nervous system; these range from inflammatory conditions to metastatic tumours. Their nature can be suggested by the cytological examination of CSF. Aspiration of CSF is not without risk, especially in patients with raised intracranial pressure, and repeat sampling may be difficult. CSF samples are therefore, precious and should be handled with extra care. Due to the scanty nature of the cellular material, carryover can be a particular problem and meticulous steps must be in place to avoid this.

Indications of CSF examination include:

1. Diagnosis of certain infections, particularly in the immunosuppressed.

2. Distinction between reactive lymphocytosis and lymphoma.
3. Diagnosis of meningeal involvement by metastatic carcinoma.
4. Staging of certain central nervous system tumours.

Specimen procurement. CSF is usually obtained by lumbar puncture under aseptic precautions. Correct choice of special investigations is paramount and aliquots of samples should routinely be sent to Clinical Chemistry, Microbiology and Cytology. If pyogenic meningitis is suspected clinically, preference must be given to Microbiology especially if the volume of the sample is minimal.

In some centres, the entire sample is sent to central pathology reception for division within the laboratory. Where this is the normal practice, the cytology laboratory should be contacted promptly so that processing of the specimen is not delayed. In other centres, the sample is divided at source. If delay is anticipated, (for example, samples collected at weekends), storing the sample at 4 °C may help preserve cells for up to 24 hours. Laboratories may choose to have the sample packed on ice if delay during transport is expected.

Specimen preparation. A 2 ml sample is usually sufficient for cytological examination. However, smaller volumes should be accepted for processing. Specimens should be prepared and examined as quickly as possible. Macroscopic appearances should be recorded. Care must be taken to optimise the centrifugation time and speed for CSF samples. The addition of albumin helps protect cells as they are spun on to their target slides, however, albumin leaves a background and other treated slides may be used.

Slides and stains. At least one air-dried slide is recommended as Romanowsky stains are preferable for examining lymphoid cells.[21] In selected cases where there is sufficient material, additional slides may be helpful. If additional stains are likely to be needed, spare slides should be prepared at the outset. Useful special stains include ZN for acid-fast bacilli (AFB), and diastase periodic acid Schiff (DPAS), Grocott and Mucicarmine for fungi, for example, Cryptococcus. Immunochemistry may be helpful in selected cases. Flow cytometry may be performed if non-Hodgkin lymphoma is suspected[21] even on small volumes of sample as long as these are sufficiently cellular. Fetal calf serum should be added for cellular preservation.

Synovial fluid

Overview. Synovial fluid supports and lubricates the articular cartilage in joints. Diseases that affect joints may cause effusions into the joint space. Some of these are associated with deposition of crystals in the joint. These crystals can be characterised by examining a wet preparation using polarised microscopy.

The main indication for examination is to distinguish inflammatory (septic, gout and rheumatoid) from degenerative (osteoarthritis) arthropathies.[22]

Specimen procurement. Fluid is aspirated and may be sent fresh or with an added anticoagulant such as lithium heparin.[22] Aspirated material may also be sent for microbiological investigation if clinically indicated before adding an anticoagulant.

Specimen preparation. A wet preparation should be examined to determine the presence and type of any crystals. Birefringerent crystals may be seen in both gout (urates) and pseudogout (pyrophosphates) and should be distinguished by their shape and colour under polarised light using appropriate filters. The wet preparation also allows identification of cartilage cells in osteoarthritis and ragocytes in rheumatoid arthritis.[22] The remaining sample may be prepared to a known dilution and stained with a supravital stain such as methyl violet for a nucleated cell count in a haemocytometer chamber. Either direct smears or cytocentrifuge preparations should be made for assessing the cell content further and to exclude the occasional occurrence of malignant cells.

Slides and stains. One unstained wet preparation and one Romanowsky stained slide are recommended. The latter should be examined to assess the degree and type of inflammatory infiltrate. A supravital stained preparation may be prepared if nucleated cell count is indicated. Specific features such as iron-laden macrophages seen after haemorrhage may be identified and confirmed by Perl's stain if necessary.

Best practice

The minimum recommendation is one unstained wet preparation and one Romanowsky stained slide.

Cyst aspirates

Overview. Cysts from a variety of anatomical sites may be aspirated for symptomatic relief and for diagnosis. Many clinically benign cysts may be aspirated to dryness and require no further intervention. Any residual lump after drainage of a cyst should be re-aspirated as it may represent the solid component of the same lesion.

The aspirator may discard breast cyst fluid[23] if it is not bloodstained but should be examined if received in the laboratory. Thyroid cyst fluid should be examined cytologically as papillary carcinoma always needs to be excluded. Ovarian cysts may be neoplastic or functional. Where there is a high suspicion of malignancy aspiration of the cyst is contraindicated because of the risk of rupture and tumour implantation in the peritoneum. Patients being treated for subfertility are prone to develop exaggerated functional cysts and cytological examination of these is of limited value.[14] Necrotic tumours may present as cystic masses in the neck, chest or abdomen. Benign cysts in an intra-abdominal location include pancreatic pseudocysts and mesothelial cysts. Parasitic (e.g. hydatid) cysts may be encountered in the liver and spleen. These should be aspirated with great caution as spillage of contents within the abdomen may provoke a severe allergic reaction.

Examination of cyst fluid is indicated:

1. To exclude or confirm the presence of malignant cells in the cyst fluid.
2. To perform microbiological and cytological examination if infection is suspected.

Specimen procurement. Palpable cysts (breast and thyroid) may be aspirated freehand using a needle and syringe attached to a holder. Deep-seated and visceral cysts are aspirated under ultrasound or CT guidance. Ovarian cyst fluid is usually aspirated under transvaginal ultrasound guidance. Sometimes fluid is aspirated under direct vision during laparoscopy.

Specimen preparation. Suitable methods include centrifugation with direct smearing of the cell button, cytocentrifugation and LBC methods. Gross appearances should be recorded and may be of diagnostic importance (dermoid and endometriotic ovarian cysts).

Slides and stains. One wet-fixed slide is appropriate for LBC methods. Cytocentrifugation methods may be preferable in that they can produce both wet-fixed and air-dried slides; one of each is recommended as a minimum for Pap and Romanowsky staining.

> One wet-fixed and one air-dried slides is recommended as a minimum for Pap and Romanowsky stains.

Endoscopic specimens

Endoscopic brushings

Overview. Lesions that visibly involve the bronchial, gastrointestinal and urinary outflow tracts may be brushed under direct vision during endoscopy. The cells obtained by brushings are often better preserved compared to cells that have exfoliated.

Indications:

1. Endoscopic brushings are used in the investigation of symptoms or radiological suspicion of *in situ* and invasive malignancy involving a mucosal surface.
2. They may be used to diagnose specific infections at certain sites (e.g. Candida in the oesophagus and other opportunistic infections in the respiratory tract).

Specimen procurement. The brush should be firmly rolled along the mucosal surface whilst ensuring good contact with the bristles.[15] This may produce some bleeding that can limit further endoscopy and sampling, but gentle strokes of the brush are unlikely to yield diagnostic material. Biopsy should be performed after washings and brushings have been collected.

Specimen preparation. If direct smears are required, they must be prepared at the time of endoscopy. At least two wet-fixed slides should be prepared. In some units, a BMS attends the procedure to prepare, rapidly stain and microscopically assess smears for adequacy at the time of endoscopy.[15] This can benefit the patient and the clinical team by maximising the cell yield from diagnostic samples and minimising inadequate samples by prompting repeat brushing when necessary at the time of the procedure. Immediate staining of alcohol-fixed slides can be carried out with a modified Pap stain or toluidine blue.

An alternative approach is to agitate the brush in a transport medium or, preferably, to remove the

unsheathed brush and place it in a transport medium for dispatch to the laboratory. Cells are then disaggregated from the brush (ultrasonic vibration may be helpful) and harvested from the fluid. Cytocentrifugation or an LBC method may be used to produce suitable slides.

Slides and stains. With LBC and large volume cytocentrifugation methods, a single wet-fixed slide is usually adequate; with direct smears from cell deposits and conventional cytocentrifugation techniques, a minimum of two wet-fixed slides is recommended for examination using Pap staining. Immunocytochemistry may be carried out on LBC slides, cytospin preparations or sections of cell-blocks prepared from centrifuged deposits.[20] This is increasingly used in lung cancer to help the distinction between small cell and non-small cell carcinoma as well as between adenocarcinoma and squamous cell carcinoma. Preparation methods for immunocytochemistry will also be considered in the forthcoming FNA COP.

Endoscopic washings

Overview. Mucosal surfaces may be washed to collect exfoliated cells. These are likely to be less well preserved than the cells abraded by a brush but are less prone to sampling bias by the endoscopist. Washings have the additional benefit of sampling cells beyond the direct vision of the endoscope.[15]
Indications:

1. Endoscopic washings are used in the detection of malignant cells exfoliating into the lumen of a hollow viscus.
2. They may also be used in the investigation of infections by direct demonstration of organisms on special stains or by microbiological culture.

Specimen procurement. Saline is washed over the mucosal surface and then collected through the endoscope. The washings should be sent directly to the laboratory. If there is any delay, they may be stored at 4 °C for up to 24 hours to preserve the cells. Gastric and biliary tree washings should be taken to the laboratory for immediate processing to prevent the rapid degradation of cell content that invariably ensues from their storage in a medium contaminated by enzymatically active gastric or pancreatic juices. If any delay in transport is expected, a fixative fluid should be used.

Specimen preparation. Washings of mucosal sites may be treated in one of the following ways, largely depending on the viscosity of the specimen. Unfixed specimens may be prepared by centrifugation and direct smears made from cell deposits. Alternatively, cytocentrifuge preparations from unfixed fluids or spun deposits may be re-suspended in transport medium such as RPMI or a balanced electrolyte solution.[20]

Specimens may be fixed as soon as they are collected or received in the laboratory and slides prepared by one of the LBC techniques, which tend to require specific fixative/preservative solutions. These must be stained by Pap stain as Romanowsky stains cannot be performed on these preparations. These techniques are modified and adapted as required for different specimen types.

Slides and stains. One wet-fixed slide is appropriate for LBC methods or large volume centrifugation preparations. Two wet-fixed slides are generally prepared as direct smears or by cytocentrifugation methods. As with brushings, material may be saved for immunocytochemistry if required.

Bronchiolo-alveolar lavage (BAL)

Overview. Bronchiolo-alveolar lavage is a specialist technique of sampling the distal airways of the lung for obtaining cytological material for diagnosis and occasionally for therapeutic reasons in conditions such as alveolar proteinosis.[17]
Cytological examination of BAL is indicated:

1. For diagnosis of diffuse malignancy such as bronchiolo-alveolar cell carcinoma, lymphoma/leukaemia, opportunistic infection or aspiration pneumonia involving the lung parenchyma.
2. For monitoring the activity of interstitial lung disease, though not for its initial diagnosis.

Specimen procurement. The endoscope is impacted as far into the respiratory tract as possible and an aliquot of saline is introduced into the most distal airways and then re-aspirated. In this way, a fluid sample of inflammatory cells from the alveolar spaces and pulmonary interstitium is obtained.

Specimen preparation. Cytocentrifugation and LBC are the most appropriate methods of preparation of BAL

samples. The specimens may be treated with mucolytic agents if necessary.

Slides and stains. A minimum of one wet-fixed, Pap stained slide (LBC or direct smear from cell deposit) is recommended. In interstitial lung disease, an estimate of the cell content may be helpful. It should be borne in mind that samples prepared by LBC methods are not suitable for performing cell counts as they substantially exclude inflammatory cells.

Special stains for microorganisms may be helpful. Immunostaining may also be helpful in cases of malignancy. Flow cytometry may be used in cases when differential counting of CD4 and CD8 positive T cells is required, or when lymphoma is suspected. Oil Red-O staining may be used to identify lipid-laden macrophages in aspiration pneumocytosis.

Best practice

The minimum is a single wet-fixed slide for Pap staining. With cytocentrifuge techniques, an additional slide may improve the diagnostic yield.

Skin and mucosal scrapes

Overview. Scrapes of skin lesions, including those involving the nipple, may be helpful in identifying basal cell carcinoma,[22] adenocarcinoma and some parasitic infestations (e.g. Leishmaniasis). A similar scraping technique may be used for intra-oral mucosal lesions. Scraping of melanocytic lesions is not recommended.

Specimen procurement. The site to be scraped is wiped clean. There is no value in examining superficial squamous cells or an inflammatory exudate and a robust approach to cleaning is essential. A sterile scalpel blade is held perpendicular to the surface and dragged firmly across the lesion, gathering cells against the blade. A good sampling technique will usually cause some bleeding. In some centres, cytopathologists perform skin/mucosal scrapings alongside the FNA service.

Specimen preparation. The cells should be either spread directly on to a slide and wet-fixed or air-dried. Where this is performed by the cytopathologist for on-site assessment, this may be offered using air-dried or wet-fixed slides using rapid staining methods.

Slides and stains. A wet-fixed and one air-dried slide are generally adequate for Pap and Romanowsky stains respectively.

A single slide stained with either Pap or Romanowsky stain according to local preference is the minimum required.

Summary

Exfoliative cytology of all types requires similar skills in slide preparation and microscopy involving close cooperation between BMS, laboratory assistants and cytopathologists. Although ancillary tests are increasingly used, accurate diagnosis is underpinned by cytomorphology, which itself depends on high-quality preparation and staining of cells.

References

1. CPA (UK) Ltd. *An Approach to Medical Audit in the Laboratory*. Version 1, 2000: CPA (UK) Ltd; www.cpa-uk.co.uk.
2. Health and Safety Executive. Control of Substances Hazardous to Health. The Control of Substances Hazardous to Health Regulations 2005. Approved Code of Practice and Guidance L5 (Fifth edition). Norwich: HSE Books; 2005.
3. CPA (UK) Ltd. *Standards for the Medical Laboratory*. Version 2, 2004: CPA (UK) Ltd; www.cpa-uk.co.uk.
4. Johnson J, Patrick J. *Achievable Standards, Benchmarks for Reporting and Criteria for Evaluating Cervical Cytopathology*, 2nd edn. NHSCSP Publication no.1. Sheffield: NHS Cancer Screening Programmes; 2000.
5. The Royal College of Pathologists. *Guidelines on Staffing and Workload for Histopathology and Cytopathology Departments*, 2nd edn. London: The Royal College of Pathologists; 2005.
6. The Institute of Biomedical Science. *Patient Sample and Request form Identification Criteria*. London: The Institute of Biomedical Science; 2003.
7. The Institute of Biomedical Science. *Error Logging in Clinical Laboratories*. London: The Institute of Biomedical Science; 2003.
8. *Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer Screening*. NHSBSP Publication no. 50. 2001.

9. SNOMED CT. Copenhagen: International Health Terminology Standards Develpment Organisation, 2007. http://www.ihtsdo.org.
10. McKee G. Urinary tract cytology. In: *Diagnostic Cytopathology*. Gray W, McKee G (eds). Edinburgh: Churchill Livingstone; 2003: pp. 471–97.
11. Koss LG. Tumours of the urinary tract in urine and brushings. In: *Koss*' Diagnostic Cytology and its Histopathologic Basis. Koss LG, Melamed MR (eds). Philadelphia: JB Lippincott Co; 2006: pp. 777–846.
12. Allen DJ, Challacombe B, Clovis JS *et al.* Urine cytology: appropriate usage maximises sensitivity and reduces cost. *Cytopathology* 2005;**16**:139–42.
13. Burton JL, Goepel JR, Lee JA. Demand management in urine cytology: a single cytospin slide is sufficient. *J Clin Pathol* 2000;**53**:718–9.
14. The Royal College of Pathologists. *Histopathology and Cytopathology of Limited or No Clinical Value*. London: The Royal College of Pathologists; 2005.
15. Sterrett GF, Frost FA, Whitaker D. Tumours of lung and mediastinum. In: *Diagnostic Cytopathology*. Gray W, McKee G (eds). Edinburgh: Churchill Livingstone; 2003: pp. 71–131.
16. British Thoracic Society bronchoscopy guidelines committee. *British Thoracic Society Guidelines for Diagnostic Flexible Bronchoscopy*. *Thorax* 2001, **56** i1–21.
17. Gray W. Normal respiratory tract and inflammatory conditions. In: *Diagnostic Cytopathology*. Gray W, McKee G (eds). Edinburgh: Churchill Livingstone; 2003: pp. 17–69.
18. World Health Organisation. *Laboratory Manual of the WHO for the Examination of Human Semen and Sperm-cervical Mucus Interaction*, 4th edn. Cambridge, UK: World Health Organisation; 1999.
19. Tomlinson M, Sakkas D, Barratt CLR. Laboratory semen analysis and sperm function testing. In: *Diagnostic Cytopathology*. Gray W, McKee G (eds). Edinburgh: Churchill Livingstone; 2003: pp. 641–8.
20. Bales CE. Laboratory techniques. In: *Koss*' Diagnostic Cytology and its Histopathologic Basis. Koss LG, Melamed MR (eds). Philadelphia: JB Lippincott Co; 2006: pp. 1569–621.
21. Koss LG. Cerebrospinal and miscellaneous fluids. In: *Koss*' Diagnostic Cytology and its Histopathologic Basis. Koss LG, Melamed MR (eds). Philadelphia: JB Lippincott Co; 2006: pp. 1023–53.
22. Freemont AJ, Denton J. The cytology of synovial fluid. In: *Diagnostic Cytopathology*. Gray W, McKee G (eds). Edinburgh: Churchill Livingstone; 2003: pp. 929–39.
23. Lindholm K. Breast. In: *Fine Needle Aspiration Cytology*, 4th edn. Orell SR, Sterett GF, Whitaker D (eds). Philadelphia: Elsevier; 2005: pp. 165–225.