

## BSCC Code of Practice – fine needle aspiration cytology

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The British Society for Clinical Cytology Code of Practice on fine needle aspiration cytology complements that on exfoliative cytopathology, which was published in the last issue (*Cytopathology* 2009;20:211–23). Both have been prepared with wide consultation within and outside the BSCC and have been endorsed by the Royal College of Pathologists. A separate code of practice for gynaecological cytopathology is in preparation. Fine needle aspiration (FNA) cytology is an accepted first line investigation for mass lesions, which may be targeted by palpation or a variety of imaging methods. Although FNA cytology has been shown to be a cost-effective, reliable technique its accurate interpretation depends on obtaining adequately cellular samples prepared to a high standard. Its accuracy and cost-effectiveness can be seriously compromised by inadequate samples. Although cytopathologists, radiologists, nurses or clinicians may take FNAs, they must be adequately trained, experienced and subject to regular audit. The best results are obtained when a pathologist or an experienced and trained biomedical scientist (cytotechnologist) provides immediate on-site assessment of sample adequacy whether or not the FNA requires image-guidance. This COP provides evidence-based recommendations for setting up FNA services, managing the patients, taking the samples, preparing the slides, collecting material for ancillary tests, providing rapid on-site assessment, classifying the diagnosis and providing a final report. Costs, cost-effectiveness and rare complications are taken into account as well as the time and resources required for quality control, audit and correlation of cytology with histology and outcome. Laboratories are expected to have an effective quality management system conforming to the requirements of a recognised accreditation scheme such as Clinical Pathology Accreditation (UK) Ltd.

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### Introduction

This Code of Practice (COP) complements that on exfoliative cytopathology, which was published in the last issue.<sup>1</sup> The British Society for Clinical Cytology (BSCC) commissioned both these documents to be

prepared in consultation with its Council, its representative from the Institute of Biomedical Science and its wider membership through the BSCC website. The COP has been endorsed by the Royal College of Pathologists.

Fine needle aspiration (FNA) cytology is widely accepted as a first line of investigation in any patient with a mass lesion. Superficial lesions can be aspirated by palpation. Radiological imaging such as ultrasound, fluoroscopy, and computed tomography (CT) can guide fine needles to impalpable or deeply located lesions. In common with many cytopathological

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investigations, the procedure can be quick, inexpensive (apart from when certain complex imaging techniques are required) and reliable. FNA requires readily available equipment, takes very little time to perform, causes minimal trauma, and may be repeated if required. As an investigative technique it is acceptable to most patients and it rapidly provides a diagnosis on which to base further management. As Nasuti *et al.*<sup>2</sup> point out in a review of 5 688 cases, the success of FNA depends on obtaining a specimen with adequate cellularity, high-quality preparation and an experienced aspirator and cytopathologist. We address all those requirements in this COP, while recognising that some regional cancer networks in the UK currently may not have the resources to meet our recommendations.<sup>3</sup> The COP is timely because rapid access FNA cytology clinics are recommended by the National Institute for Health and Clinical Excellence *Improving Outcomes in Head and Neck Cancer*<sup>4</sup> as discussed in a recent review article in this journal.<sup>5</sup>

### Organisation of service

The provision of a comprehensive FNA service should be under the direction of a named pathologist with responsibility for overseeing all its aspects, working in conjunction with the clinicians involved in the service. The pathologist is usually the head of the cytopathology section of a cellular pathology department, which in the UK refers to combined histopathology and cytopathology departments. In large departments the responsibility may be delegated to another cytopathologist in the same department who is directly involved in FNA cytology.

### Who should take the specimen?

The responsibility for taking FNAs should lie in the hands of individuals who have a sufficient FNA workload to gain and maintain the necessary expertise and who are subject to clinical audit. In many centres cytopathologists take specimens directly from patients with palpable lesions, but in others radiologists and clinicians take most of the aspirates. There are three levels of skill required in providing a safe and reliable FNA service; taking the sample, preparing it, and interpreting the findings. The interpretation is made in the context of the clinical findings and the results of other investigations and is dependent on obtaining adequate

samples and preparing them to maximise their diagnostically relevant information. Cytopathologists should be able to acquire all these skills and are well placed to provide a high quality FNA service. Cytopathologists have been shown to perform well in terms of taking adequate FNAs<sup>6–14</sup> and it has long been known that training and experience are important.<sup>15,16</sup> It has been shown that clinicians carrying out relatively few aspirates with a high turnover of trainees may perform poorly in terms of specimen adequacy even when given prior training by cytopathologists.<sup>17</sup> Good results may be achieved in combined services where cytopathologists take FNAs from palpable lumps alongside clinicians or radiologists using US-guidance when necessary.<sup>2,9,14</sup> An important ingredient seems to be the presence of a cytopathologist to assess sample adequacy.<sup>2,14,18</sup>

Deep-seated or impalpable lesions in which ultrasound or radiological guidance is required to target the lesion are usually carried out by radiologists or, in the case of endoscopic and endobronchial US-guided FNAs, by gastroenterologists, chest physicians or specialist nurses as will be considered in a later section. Providing the aspirator is well trained, has sufficient practice to maintain his or her skills, has a close working relationship with the cellular pathology department and is subject to clinical audit, there should be no difficulty in their achieving a high quality service. FNAs should not be taken by unsupervised staff who have no training in the technique, staff who do not take such aspirates regularly, or who are unaware of the risks and complications.<sup>19</sup>

### Technical Support

Trained cytotechnologists, known as biomedical scientists (BMS) in the UK, should be available to support clinicians, radiologists or cytopathologists taking FNAs by collecting the samples in appropriate media and preparing direct smears as required. Immediate assessment of specimen adequacy may be carried out by cytopathologists or by suitably trained and experienced BMS under the guidance of cytopathologists.

The availability of appropriately trained medical staff to take the aspirates, technical staff to prepare the specimens and cytopathologists to interpret the material is essential for an effective FNA service.

### Where should aspirates be taken?

When introducing comprehensive FNA services in a hospital setting previously lacking such a service, cytopathologists may often start by offering to take FNAs in a variety of out-patient clinics, wards or as a pre- or intra-operative procedure in operating theatres. As the service becomes more widely used, alternative means of service provision are often necessary.

#### *FNA in an outpatient clinic*

A common solution is to hold one or more FNA sessions in a hospital out-patient department (OPD). These may be run in association with existing clinics such as breast or head & neck clinics or run in their own right by cytopathology staff. The OPD has the considerable advantage of easy access for patients and support by dedicated nursing and reception staff. However, the clinic space will have several users and equipment must be moved before and after each session; it can also be difficult to extend the number of sessions if the service becomes overstretched due to demand.

#### *The dedicated FNA clinic*

The ideal solution for a busy FNA service is to set up a dedicated, purpose-built FNA clinic that can be operated according to a set schedule.<sup>7,8,20</sup> This must have close links with the cellular pathology department and some services have a dedicated FNA clinic located within the cytopathology laboratory. In the absence of nursing and administrative staff, the laboratory staff working in the clinic must be trained to deal appropriately with patients and should be trained in basic life support techniques. The clinic should always be attended by a medically qualified cytopathologist trained and experienced in FNA cytology. The clinic should be managed according to NHS Trust (hospital) protocols for other outpatient clinics and have waiting areas (suitable for adults and children) with toilets and fresh drinking water. Access to the FNA clinic and its facilities should be provided for disabled people.

Given the need for patient access, and the limited availability and suitability of laboratory space in many units, it may be better to site FNA clinics in an outpatient clinic. A location near or even within a radiology department is a good alternative, especially

if there is a large volume of image-guided FNAs. FNA clinics are well suited to the concept of ambulatory care in a polyclinic setting, where outpatients can be seen and investigated by as many specialists as necessary during a single visit to the hospital (providing the so called 'one-stop service').

#### *The in-patient 'bedside' FNA*

The FNA technique lends itself to near patient use in hospital in-patient wards if it is not possible for a bedridden patient to be transferred to a laboratory or OPD FNA clinic accompanied by a member of nursing staff. It is essential that a suitably trained, competent medical practitioner, be that a cytopathologist or other clinician, takes the FNA using the correct equipment. While the equipment needed to take a bedside FNA is light and easily portable, it is important to maintain health and safety precautions at all times away from the clinic. Unless an FNA trolley has a portable microscope to assess adequacy and the nature of the material on site, needle washings should be rinsed in sterile saline in case ancillary tests are needed, after preparing air-dried and alcohol-fixed slides.

### Ultrasound, other imaging suites, endoscopy and bronchoscopy suites

FNAs are increasingly taken in suites used for ultrasound, CT, stereotactic localisation, endoscopy or bronchoscopy. These procedures will be considered later but the same principles apply: radiologists, physicians and consultant nurses may be trained to take the aspirates but should work in close cooperation with cytopathologists and BMS so that facilities for making and staining the slides, preparing material for ancillary tests and assessing sample adequacy are available.

### Workload and resources

In services that lack a dedicated FNA clinic, where patients are seen by prior appointment, FNA may be limited to an *ad hoc*, on-demand service to a variety of clinics, there will be a significant additional journey time to and from the laboratory, which must be included as part of the departmental workload. With a dedicated FNA clinic the service is more time-efficient and can handle a larger volume of work. Each FNA consultation is likely to take between 10 and

20 minutes. The period of time allocated for the consultation should allow time for explaining the procedure to the patient (often with the use of specifically produced patient information in leaflet or similar form, in an appropriate language when necessary), obtaining verbal or written consent according to local guidelines, examining the patient, taking the FNA, carrying out immediate staining and microscopic assessment of sample adequacy, repeating aspirates when necessary and issuing reports in the same session ('rapid reporting') if required.

An FNA clinic session is usually followed by a formal reporting session by the consultant in the laboratory, including the review of all cases where a provisional ('rapid') result has been given. The majority of FNA clinics therefore attract a minimum of two direct programmed activities (DPA), each equivalent to 4 hours according to the 2003 NHS Consultant Contract document.<sup>21</sup> Workload should be carefully monitored to include the number of slides per case and such commitment will need to be identified within their job plan and would reduce sessional availability for 'general' reporting accordingly.<sup>22</sup> The need for additional resources such as equipment, transport or technical support should be identified before an increase in workload is undertaken as part of a specific business case. The business case should include resources for consultant and technical cover within the laboratory during the time spent outside the laboratory in the FNA clinic. This should amount to one DPA per FNA clinic. Cytopathologists must have sufficient time away from the clinic to carry out their more traditional tasks of reporting cytopathology specimens, other pathology duties (surgical histopathology and post mortems) and participating in teaching, audit, research and management; most will want to maintain an active interest in surgical pathology and autopsy services. Staffing levels should ensure that these duties, and absences during times of annual and professional leave, do not cause a hiatus in the FNA service or result in an individual pathologist performing or reporting insufficient numbers of FNAs each year to maintain their competence.

Many departments fail, initially at least, to appreciate the amount of work involved in establishing a high quality FNA service. FNA clinics lend themselves to direct referrals from community physicians and general dental and medical practitioners. Provided the referral pathways to GPs are clearly in place, establishing diagnoses in a primary care setting can have a positive effect on hospital referral practice, with

patients being sent to the appropriate specialists for specific investigation and treatment rather than for preliminary work-up. In the case of direct GP referrals to the FNA clinic, clear lines of responsibility for follow up and referral of patients with positive results, should be established. Although the cost of providing a satisfactory FNA service should not be underestimated, particularly when imaging techniques are used for obtaining the sample, nevertheless in terms of overall patient care it is cost-effective by reducing the number of repeat procedures and more invasive, costly and time-consuming investigations.<sup>2,7,11,15,23</sup>

- An FNA clinic introduced as an additional activity should attract additional funding.
- Adequate resources should be identified before such a service is introduced.
- Other activities such as routine exfoliative cytology reporting and other professional activities should not be compromised by the introduction of FNA services.

### Equipment

Most of the equipment needed for a high quality FNA service is inexpensive and readily available but a good quality microscope, preferably with a double-head, should be available for rapid on-site assessment.

### Needles

Needle gauge is based on external diameter. Fine needles should be 23 gauge (external diameter 0.6 mm) or less (external diameter 0.7 mm). It is important to use smaller needles for the following reasons: i) it is less painful ii) it causes less bleeding and iii) the risk, albeit rare, of tumour seeding is considerably reduced.<sup>24</sup> Thicker needles (G18, external diameter 1.2 mm, or wider) carry an ever-increasing risk of complications including significant haemorrhage.<sup>19,24</sup>

To be suitable for FNA, needles should have a long bevel giving a relatively large circumference at their cutting edge. Most disposable venepuncture and spinal needles are suitable for FNA. Longer fine needles are required for deeply sited lesions targeted by image guidance. These needles should have a stylet; several different tissues may be traversed before the needle tip reaches its target including subcutis, serosa, non-involved viscera and blood vessels. The stylet prevents

the needle sampling tissues en route and filling with blood clot or non-lesional tissue before the target is reached. Without a stylet a long needle may be uncontrollably and dangerously flexible. Variable length spinal needles (G22 or finer) are suitable but several specialist needles are also available.

For some deep site aspirates an outer co-axial needle is used to target the lesion and finer needles are inserted within its lumen to repeatedly sample the mass. In transthoracic FNAs this permits multiple passes with only a single puncture of the pleura, thus minimising the risk of significant pneumothorax. It also minimises the potential for tumour cells to seed along the needle tract.

The use of the correct needle is important and smaller diameter needles minimise patient morbidity and reduce the risk of tumour cell seeding.

#### *Syringes and syringe holders*

A syringe and syringe holder may be used for palpable lesions. However, many aspirators prefer the free-needle technique described below. If used, the syringe holder frees one hand to stretch the overlying skin and immobilise the lump and minimises the effort needed to create sufficient negative pressure. The Swedish-designed syringe holder (Cameco AB, Taby, Sweden) is suitable, although alternatives are now available. Either a 10 ml or a 20 ml sterile disposable plastic syringe can be used, depending on personal preference. BD Vacutainer<sup>R</sup> needles and collection bottles are of no value in FNA practice, as the negative pressure they produce cannot be controlled during the procedure.

#### *'Sharps' container*

A sharps container for safe disposal of needles and unused slides should be available at any site where FNAs are being carried out. In known high-risk patients, needles should not be separated from the syringe at the time of disposal into the sharps container.

#### *Slides, fixative and collection fluid*

Clean slides with frosted ends are required if direct smears are to be prepared at the time an aspirate is

taken. Direct smears can be either wet-fixed by alcohol spray or, preferably, by immersion in 95% alcohol, or rapidly air-dried. The latter, while unfixed, are potentially biohazardous and should be handled accordingly. Needle washings taken in transport media provide additional material for cytopathological study. In some centres, liquid-based methods have replaced direct smears with the entire specimen being washed into transport media. This allows aspirated material to be sent to the laboratory from clinics at sites remote from the laboratory but precludes rapid assessment of specimen adequacy and certain ancillary tests. Transport time can be significantly reduced by use of a dedicated portering service or, within a hospital, a pneumatic tube system.

There are two main types of transport media: fixation fluid that kills organisms and cells, and non-fixative/culture fluid that keeps the material viable until it can be processed. If fixed cell preparations are required then an alcohol-based fixative is satisfactory as provided with commercial LBC equipment. If only cell blocks are to be prepared then 10% buffered formalin is satisfactory. Fixation precludes the subsequent preparation of air-dried material but keeps the process technically simple. Keeping the cellular sample viable maximises the information that can be gained providing it is handled appropriately and in a timely fashion. Roswell Park Memorial Institute (RPMI) 1640 cell culture medium kept at 4 °C gives good results; this includes 10% fetal calf serum, 1% penicillin-streptomycin solution and a small amount of heparin. Cells taken into RPMI 1640 medium will survive between 12 and 72 hours at 4° C. Hank's physiological saline, which lacks antimicrobial agents, is suitable for samples requiring microbiological investigation. Sterile normal saline, which is universally available in the hospital environment, can also be used for transporting samples, providing processing is not unduly delayed.

The FNA kit should include several 20 ml universal containers pre-filled with transport media. If Hank's or RPMI 1640 is being used it must be fresh. The pH indicator included in the fluid gives it a red colour when fresh; a change in colour to purple indicates contamination by bacteria or fungi. After drawing the transport fluid into the syringe containing the cellular sample the needle should be removed before the material is gently expressed to avoid damage to cells, which may be caused

by forceful expulsion at high pressure through a narrow gauge needle.

Cells in a balanced salt solution such as Aqsia™ (Bausch & Lomb, Kingston-Upon-Thames, UK) can be used for microbiological investigations, flow cytometry or molecular analysis. The material is preserved for up to 24 hours and may also be used later for cell blocks, cytocentrifugation or LBC methods.

The use of a balanced salt solution for needle washings allows subsequent decisions to be made in the laboratory about material to be submitted for ancillary tests.

Ideally, laboratory staff should attend FNA procedures to prepare and assess slides. When resources do not permit attendance by laboratory staff, appropriate transport medium may be used to allow preservation of material whilst it is delivered to the laboratory. The choice of transport medium should be made in consultation with the laboratory taking into account subsequent pathology investigations that may be required. In cases where an infectious cause is suspected, appropriate microbiology containers should be used having discussed the choice of these in advance with the microbiology department.

#### *Local anaesthetic*

Local anaesthesia of the overlying skin and proposed needle tract is usually only needed in especially sensitive locations such as nipple, lip or eyelid, for small children or in situations where multiple passes of the needle are planned. Thus it may be needed for FNAs of large masses, or during deep-site aspirations, but for most palpable sites it is not required. When local anaesthetic is required, a very fine needle (25G or finer) should be used to infiltrate both skin and the proposed needle track; some practitioners advocate the use of dental local anaesthetic equipment, with 30G needles and easily held metal syringes. A small volume of 2% lignocaine is generally sufficient. Application of anaesthetic cream such as Emla cream (AstraZeneca, London, UK) at the proposed puncture site according to the manufacturer's instructions before doing the FNA is helpful in children and needle-phobic patients. Ethylene spray for skin anaesthesia and needle-free commercial kits for application of local anaesthetic can also be used.

#### Best practice

- Laboratory staff should attend FNA procedures to collect material in an optimal way for subsequent processing.
- Laboratory assistance also allows assessment of adequacy of the sample and improves the chances of procuring a diagnostic specimen.

### **The FNA procedure**

A successful FNA technique is best learnt during an apprenticeship with an experienced medical practitioner. The following approach to taking FNAs from palpable lumps, which may not suit every practitioner, is intended as guidance rather than being prescriptive. Superficial palpable masses are best aspirated in a cytopathologist-led clinic in the pathology department or OPD setting.<sup>7,9,20,23</sup> The principles described below also relate to aspirates taken in other situations such as image-guided FNA sessions.

#### *Request form*

The request form serves several functions. It identifies the patient's name, address, age and gender, the person requesting the test, the type of test required. It provides clinical information about the anatomical site of the procedure and date of collection. Additional clinical information can be added if the cytopathologist has direct contact with the patient and/or clinician but this should not replace the referring clinician's responsibility in completing the request form fully and accurately.

Superficial palpable masses are best aspirated in a cytopathologist-led clinic in the pathology department or OPD setting.

#### *Talk to the patient*

The aspirator should put the patient at ease before taking a full history as necessary. The technique of FNA should be briefly explained, emphasising the purpose of the test, its limitations, its potential complications and that it may be necessary to repeat

the aspiration to obtain an adequate sample or additional material for ancillary tests. Occasional patients complain of bruising and discomfort the day after aspiration; patients should be warned about this and advised of the suitability of non-aspirin pain relief, as aspirin may increase bruising. Ask the patient if he or she has any questions or particular concerns before obtaining their consent to proceed. The patient leaflet should cover the major points outlined above, but may require modification depending on the site aspirated.

#### *Consent*

The technique of FNA has a clear purpose as well as certain limitations and complications, which should be discussed honestly and fully with the patient before he or she is asked to consent to aspiration. At least verbal consent should be sought and recorded in all cases and some NHS Trusts hospitals now require patients to sign a written consent form. Information leaflets are helpful in answering patients' questions in advance of the FNA appointment. Non-English versions of these may be required and the services of an interpreter may need to be arranged at the time of the appointment.

- Obtaining valid consent for fine needle aspiration is essential and should be recorded.
- Consent may be verbal but should be consistent with local protocols.

#### *Examine the patient*

The patient should be asked to undress as appropriate and to get on the examination couch. It is best not to try to aspirate patients sitting in an ordinary chair as they can move or faint quite unexpectedly; special chairs with head rests are suitable for some but not all situations. An assistant should be present at all times to act as chaperone for the patient, in line with local policy, and can also act as an assistant to the aspirator. This assistant should know what to expect and be trained accordingly, whether a nurse, BMS or medical practitioner.

The aspirator should then examine the patient as fully as necessary to determine whether FNA is appropriate and to consider where the needle should

be placed, how many aspirates are to be taken and what preparations are likely to be useful. They should use their clinical judgment to determine whether or not a referred patient has a suitable target for FNA and should not attempt to take an FNA if they consider the request to be clinically inappropriate or if the lesion is not palpable. Referral for radiological guidance may be necessary in these cases, which is facilitated by aspirates being carried out in a combined US/FNA clinic.<sup>2,9,14,18</sup>

It should be possible to determine the location, size, shape, consistency and mobility of the target by careful palpation. When the aspirator has a good idea of what is needed, slides should be laid out ready for direct smears, and containers of fixative and transport media should be made ready on a designated preparation area. Slides should be labelled prior to the procedure, including the slide used for spreading. This will minimise drying artefact and avoid specimens from different patients being mixed up.

#### *Take the aspirate*

The skin or mucosal surface over the lesion should be cleaned with an alcohol wipe. If local anaesthetic is to be used, time should be taken to allow it to achieve its full numbing effect. If required, an FNA needle, syringe and holder can be assembled during this time. When ready, one hand should be used to feel and steady the lump; the other hand is free to hold and manipulate the FNA syringe holder.

It is worth remembering that many tumours have a necrotic centre and diagnostic material may only be found at the periphery of the mass. Multiple samples may be obtained by FNA. This reduces sample error and helps to obtain sufficient material for ancillary tests, the need for which may be guided by rapidly staining and examining one of the direct smears (see below). For larger lesions it is possible to use a single anaesthetised skin site for repeated aspiration, riding the anaesthetised skin over the lump to gain several separate entry points into the mass, producing additional sampling while minimising patient discomfort.

*Using a syringe and holder.* The FNA syringe holder is held in one hand; the thumb and first two fingers of the free hand can be used to stretch the skin over the lesion and to immobilise it (acting like a bridge for the syringe as if it were snooker or pool cue). The needle should be pushed quickly through the

skin at a right angle to it and immediately into the lump. The syringe may then be drawn back using the holder to create negative pressure as required. Cells are collected by the cutting edge at the tip of the needle as it is pushed repeatedly through the lesion. A rotating or screwing action of the forearm is helpful as this helps to dislodge the cells, making them ready for aspiration through the needle. Negative pressure from the FNA syringe helps to aspirate cells into the cutting edge of the needle, and this may improve the cell yield, especially from tumours that have a prominent stromal component such as scirrhous breast carcinomas. The needle tip is moved back and forth and the needle is angled in a fan-shaped manner to sample different areas of the lesion without removing the needle from the skin.

After a few passes, or as soon as material is seen in the hub of the needle, the negative pressure is gently released and the needle, along with its sample, is withdrawn. If negative pressure is not released before the needle is withdrawn, the sample will be sucked violently from the needle along with air into the syringe; this has the effect of traumatising cells and limits the material available for preparation of direct smears. Use of an extension tube, rather than attaching the needle to a syringe directly is to be avoided. Because it often results in a prolonged procedure during which at least some undesired clotting occurs, with the result that the cells are trapped in fibrin and may be difficult to analyse.

*Using a free-needle technique.* Zajdela described the use of a fine needle without a syringe.<sup>25</sup> A modification of this technique, using an attached 2 ml syringe without a holder and without applying negative pressure, may be used to similar effect. The needle is held between forefinger and thumb and passed through the skin directly into the lesion. The needle is rotated while in the lesion to capture cells more by capillary action than by cutting action at the needle's tip. This technique is particularly helpful in aspirating vascular tissues such as thyroid or lymph nodes to limit bleeding. This technique has distinct advantages. The aspirator has more control over the needle and a better feel for the consistency of the lesion, allowing for a more vigorous manipulation with the thumb and forefinger than is possible with the syringe holder, as the latter necessitates a forearm movement. There is also less blood loss and the procedure is less intimi-

dating for the patient. As the patient is more relaxed, there is less difficulty in obtaining assent for repeated passes.

#### *Stop the bleeding*

As soon as the needle is withdrawn, the aspirator or assistant should press carefully and firmly against the aspiration site for about 1 minute to reduce bruising. Gently holding a sterile gauze or cotton wool against the skin is not enough. Failure to apply suitable pressure can lead to the formation of a sizeable haematoma. It is not good practice to expect patients to press for themselves. A plaster may be applied once haemostasis is achieved; remember to check if the patient is allergic before using an elastic-containing plaster. Patients should be warned of the possible late complications of the procedure, such as bleeding and bruising and advised to contact their local accident and emergency department in case of these complications.

#### **Preparing the specimen**

With the aspirator or assistant pressing on the aspiration site, the other is free to prepare the sample for immediate microscopy and/or transport to the laboratory. This needs to be done carefully in order to maintain safety and to make best use of the cells obtained. The type of preparation depends on the clinical situation, the information required and the resources available. If the slides are to be prepared by the assistant rather than the person performing the aspirate, the apparatus must be handed over with the needle pointing away from the assistant. Otherwise, especially if a syringe is not used, the needle should be placed in a small tray.

#### *Direct smears*

Direct smears permit rapid staining and assessment at the time an aspirate is taken and are very helpful in reducing the number of unsatisfactory FNAs (Figure 1). They are the mainstay of immediate diagnosis in the 'one-stop' FNA clinics. While direct smears may be used for some ancillary tests, their number is usually limited and a properly handled needle rinse offers a more flexible source of diagnostic material.

After aspiration the needle is removed from the syringe, air is drawn into the latter and the needle is



**Figure 1.** Air-dried EBUS-FNA direct smear stained with Hemacolor® (Merck Chemicals Ltd, Nottingham, UK) for rapid on-site assessment.

re-attached. Taking great care to minimise spray, a small amount of material is delivered onto a glass slide a third of the way from its frosted end. A single slide may be used to pick up material and used to transfer this in small aliquots onto several slides for spreading. The spreading slide is placed on the specimen slide with one edge resting well below the material to act as a pivot; its free edge is brought gently down to just touch the wet material and at the same time this spreading slide is moved quickly back to spread the material by capillary (not dragging or crushing) action. In this way the cellular content of the material is subjected to minimal trauma. With practice a single aspirate can yield sufficient material to produce many direct smears. However, if needle rinses are used it is recommended that most of the sample is committed to fluid, limiting the direct smears to one alcohol-fixed and one air-dried.

In very bloody aspirates a spreading slide can be used to separate fluid blood from more solid fragments of tissue; holding the slide at a slight angle allows blood to drain away from the cellular material, which can then be picked up by a second spreading slide and directly smeared onto additional slides. Heavily bloodstained aspirates are best committed to the needle rinse fluid for processing in the laboratory to separate blood from diagnostically useful cells.

The patient's identity should be written in pencil on the frosted end of all prepared slides. This should be done prior to the procedure. Ideally, both Papanicolaou and Romanowsky stained slides should be available for microscopy. Provided there is sufficient material, at least one slide should be wet-fixed by spraying or immersion in 95% ethanol or its equivalent; the rest should be spread rather more thinly to facilitate rapid air-drying. Holding a slide still with its back against the warmth of a (gloved) hand, near a light bulb or on a small warming plate facilitates

rapid (and safe) air-drying. All unused slides prepared for the procedure, including the spreader if this is not needed, should be discarded in a 'sharps' container.

#### *Needle rinses*

Needle rinses are especially useful for ancillary tests, such as special stains, immunostaining, microbiological investigations, flow cytometry or molecular testing. Needle rinses may be heavily bloodstained and include necrotic debris as well as viable cells of diagnostic interest. Needle rinses taken into normal saline or other transport media can be manipulated in the laboratory to produce slides free of blood and debris using centrifugation with gradient gels. Alternatively cell blocks may be prepared<sup>19,26</sup> or the sample may be split for several uses.

#### **Rapid on-site assessment of specimens**

One or more air-dried direct smears may be post-fixed by immersion in 100% methanol and rapidly stained using a water-based Romanowsky method such as Diff-Quik or Hemacolor® (Merck Chemicals Ltd, Nottingham, UK) (or toluidine blue on alcohol-fixed smears) for immediate microscopic examination, having removed the gloves before touching the microscope. This enables immediate recognition of inadequate aspirates so that the procedure can be repeated if necessary. This is the critical procedure for reducing inadequate cytology rates<sup>2,8,10,14,18</sup> and is equally necessary for FNA of palpable lumps and for those taken with image guidance. Immediate assessment may be carried out by BMS with experience and training in non-gynaecological cytomorphology. It is possible to use liquid-based cytopspin preparations for immediate assessment.<sup>27</sup>

If a diagnosis is possible on the rapidly stained smears then no further material is taken. A further aspirate may be taken if ancillary testing is considered necessary, with subsequent material being washed directly into fixative or transport medium as required. A small direct smear should be made from each additional aspirate to decide adequacy of the sample.

#### **Ancillary tests**

Immunocytochemistry is increasingly used in FNA cytology to confirm the difference between broad groups of tumours such as carcinoma, lymphoma and

melanoma and to indicate the site of origin of metastases. While immunocytochemistry may be carried out on direct smears and liquid-based cytology slides, there are advantages to making cell blocks from needle washings and processing them as for histological sections.<sup>19,26</sup> Needle washings may be made after direct smears have been prepared but additional material should be aspirated in any clinical situation where immunocytochemistry is likely to help. Although primary diagnosis of lymphoma usually requires a formal histological biopsy, recurrences and deep-seated lesions may be diagnosed by cytology especially if material for flow cytometry is collected at the time of aspiration.<sup>2,10,28</sup> In many clinical situations, particularly lymphadenopathy in the head & neck and mediastinum, culture and sensitivity is essential to the diagnosis of mycobacterial and other granulomatous infections. In these situations needle washings should be collected into sterile saline at the time of aspiration and submitted immediately for microbiological analysis. Immediate assessment of direct smears allows cytopathologists to make decisions about optimal selection of material for ancillary.

#### **FNA performed using imaging guidance**

Radiologists will carry out most image-guided FNAs themselves, using their own guidelines. The following guidelines are designed to inform pathologists and BMS attending the sessions where aspirates are taken, but should be made available to radiologists and discussed with them, especially when combined radiology/pathology FNA sessions are set up. In some instances, cytopathologists or physicians may be trained to take FNAs with ultrasound guidance under the direction of a radiologist.

Close cooperation between radiological and cytopathological teams is essential for a high quality image-guided FNA service. It is very helpful to have an experienced BMS and cytopathologist on-site to prepare and rapidly examine aspirates. A mobile minilab, complete with slides, stains and other consumables, together with a suitable microscope, is easy to maintain in the radiology department and provides an excellent forum for teaching as well as reporting. Health and safety precautions should be observed at all times, and are particularly important in thoracic aspirates where there should be a high index of suspicion for mycobacterial infection in mass lesions.

#### **Ultrasound-guided FNA**

Although most superficial palpable masses can be aspirated without radiological guidance, ultrasound may be useful even when abnormalities are palpable. It can be used to ensure that the target has been appropriately sampled and that vital structures such as the carotid artery are avoided. US is readily available and provides a rapid, safe and inexpensive means of guiding FNAs and is increasingly used for breast, thyroid and head & neck aspirates.<sup>2,9,14,18,29,30</sup>

It is important to remind radiologists to wipe off the ultrasound gel from the puncture site prior to the FNA as it can produce fixation artefacts in the cell preparations. The gel can be wiped off carefully with a swab, but a no-touch technique with both hands behind the needle is essential to avoid the risk of needle-stick injury. During aspiration, sonographic contact can also be provided by sterile water or skin disinfectant without the need for gel. At certain anatomical sites, especially the head and neck, it may be technically difficult to have an ultrasound transducer on the skin and to needle the lesion at the same time. Even then, ultrasound confirmation of the lesion, and particularly measuring its depth from the skin surface, can be very helpful.

For superficial targets, a 3 cm long 23G venepuncture needle is usually suitable. For deeper targets, it is best to use a 22G spinal needle complete with stylet. As soon as the needle is in the target its stylet is removed, a syringe is attached and negative pressure applied as the needle is moved rapidly back and forth within the mass. Negative pressure is not always required and is best avoided in vascular lesions, such as in the thyroid gland, where capillary pressure alone is sufficient. If a cytopathologist or cytotechnologist is present, the needle should be passed to them (in a tray) to prepare the specimen. Needles washed into fixative should not be used to repeat the aspiration; a new needle should always be used.

#### **Computed tomography (CT) guided FNA**

Lesions less than 1 cm in diameter located deeply within the body can be reached with precision using this technique. The other advantage over US guidance is for deeper lesions, particularly those obscured on US by intervening gas (for example, the lung or abdomen). This is a more time consuming and expensive

modality for guidance. The other disadvantages are the temporal delay between needle positioning and imaging and also the limitation to the axial plane for approach.

### Magnetic resonance image (MRI) guided FNA

MRI is rarely used to guide sampling because patient access is limited by the scanner; special coils giving reasonable operator access, may be used to guide breast aspirates, if the abnormality is only visible using this modality, using non-magnetic needles.

### Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) and endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA)

EUS-FNA (transoesophageal, transgastric or transduodenal) is used to evaluate upper abdominal masses including lymph nodes, pancreas and the adrenal gland. EBUS-TBNA is used to evaluate mediastinal and other masses in the staging of lung cancer. Lesions of less than 1 cm diameter may be sampled in 'real-time'<sup>31</sup> and the techniques may be used for other clinical indications such as the diagnosis of granulomatous inflammation.<sup>32</sup> The procedures are performed by gastrointestinal and chest physicians trained in these specialist techniques. Assistance from the cytology laboratory is central to these expensive investigative procedures, the success of which is determined by the optimal collection and appropriate preparation of cytological diagnostic material. There is already strong support for these investigations when performed with rapid on-site evaluation (ROSE) of aspirated material<sup>33</sup> and with a cost saving from reduced numbers of staging procedures such as mediastinoscopy.<sup>34</sup>

The role of the BMS and cytopathologist attending these procedures is no different to that in other clinical areas although the specimens may be regarded as precious in view of the relatively invasive nature of the procedure and the time and expense spent in obtaining and preparing them. It is vital that for best practice to flourish, close cooperation should exist between the physicians who obtain the sample and the cytologists who prepare them. This starts with the inclusion of the cytology department in discussions at the time of planning such a service and continues with the monitoring of its effectiveness.

#### Best practice in EUS and EBUS FNA

- Close co-operation should exist between the physicians who obtain the sample and the cytopathologists who interpret them.
- The cytology department should be included at the time of planning the launch of such a service and should be involved in continually monitoring its quality.

### Reporting

The FNA cytology report is a consultant's opinion based on the cytological appearances and assessment of the clinical information provided. The final cytological opinion should aim to be as clear as possible. At times, it may not be definitive and, for example may raise a suspicion of malignancy or other pathological process rather than yield a firm diagnosis. To this end, it may be useful to use established schemes such as the C1–C5 numerical reporting scheme used for breast and thyroid needle aspirates.<sup>14,35,36</sup> It is important that the referring clinician understands the pathologist's diagnostic confidence or degree of suspicion in order to decide what the next step in the diagnostic pathway should be. Even when C1-C5 codes are used pathologists should always make clear their degree of certainty or otherwise, using a final (bottom line) assessment of 'malignant cells present', 'suspicious of malignancy', 'equivocal', 'probably benign', 'benign' or 'inadequate' in addition to a free text report. SNOMED coding greatly aids correlation with histology and outcome, clinical audit and comprehensive quality management of the FNA service. The coding should include a procedure code for FNA (P1144 or similar) for ease of data retrieval.

#### Rapid reporting of FNA specimens

There is an increasing demand for instant diagnoses, and it is possible to offer these by using quick staining with Romanowsky stains (on air-dried smears) or toluidine blue (on alcohol-fixed smears). Papanicolaou stains may also be used in a rapid setting but take a little longer to prepare. Whilst advantageous for the patient and the referring clinician, there are potential pitfalls in offering such a service. All members of the team, including the cytopathologist and the requesting clinician, must know the limitations of rapid stains and

be prepared to await routinely stained preparations in difficult cases. While a positive diagnosis of malignancy may be secured on a single slide, the reverse is not true; a negative report can only be issued after all the material has been examined. It is important that standards are not compromised in order to achieve rapid reporting. It should always be possible to defer the final report in difficult cases. If it is laboratory practice to prepare both Papanicolaou and Romanowsky stained slides for routine reporting then these should both be made available for rapid reporting. Clinical demands for rapid reporting must not be detrimental to the quality and reliability of the service.

#### *Preliminary reports provided on-site*

An immediate preliminary assessment given to clinicians or radiologists, made on rapidly processed slides from an FNA, should be recorded in a hand-written format and should not only be verbal. The preliminary report should be included in the final cytology report, as is done for frozen sections in surgical pathology practice. Any discrepancies between the immediate and final reports should be highlighted and explained (if possible) in the final report. If this change of report may affect clinical management then the relevant clinician must be contacted as soon as any such discrepancy is identified. The provision of a rapid, 'one-stop' FNA service has obvious workload implications, as mentioned above in the 'Workload' section.

### **Health and safety**

Because all fresh body fluids may harbour unexpected biohazards, such as mycobacteria or hepatitis B, C or human immunodeficiency virus (HIV), it is essential that appropriate precautions are taken to prevent infection of staff and patients both during aspiration and slide preparation. If surgical gloves are used, latex-free gloves should be available for sensitised staff or patients. In high-risk cases, masks, eye protection and an apron or gown should be used. Patients with active infections should themselves wear masks. There are no absolute contraindications to making direct smears, which are essential for rapid assessment, but appropriate care must be taken to minimise risk. FNA procedure does not need to be carried out in premises specially designed for handling hazardous materials. It can be carried out in any standard consulting room. However, the use of 'special procedures' room is preferable since this provides better conditions for

health and safety, such as space to access the patient and the equipment easily.

Careful needle disposal, avoiding re-sheathing, should be carried out and staff should be aware of local arrangements for needle stick injuries. Written protocols should be available as part of the laboratory special operational procedures (SOP) manual for dealing with needle stick injuries at all sites where FNAs are carried out. Medical and non-medical staff should receive instructions for management of such injuries, including guidelines for requesting blood tests from patients under some circumstances, as part of their training and induction for working in FNA clinics. Advice from the local infection control team may be of use to ensure these aspects are suitably addressed.

Health and safety risks inherent in handling fresh body fluids and chemicals used in rapid processing must be recognised and appropriate procedures must be in place to minimise these risks.

### **Contraindications and risk management**

Other than a patient refusing informed consent, there are no absolute contraindications to FNA of superficial masses. There are specific contraindications and potential risks to deep site aspirates and these depend to some extent on site.

#### *Uncorrected coagulopathies*

Anticoagulant therapy and intrinsic bleeding problems increase the risk of bruising and haemorrhage – this information must be sought prior to the FNA. While local bleeding can be controlled by applying pressure to superficial targets, deep site aspirates should only be performed after the bleeding abnormality is corrected in consultation with the relevant clinical team.

#### *Cough and other respiratory problems*

Intractable cough and poor respiratory function are absolute contraindications to transthoracic FNA. Some degree of pneumothorax is almost inevitable when even the finest of needles breach the pleura; while this only occasionally requires treatment even a minor pneumothorax in a respiratory cripple carries a significant risk of mortality.

### Parasitic cysts

US-guided FNAs of suspected and unsuspected hydatid cysts have been reported without serious consequences<sup>37</sup> but the potential risk of anaphylactic shock should be recognised in view of a reported case of this occurrence, which was not fatal when managed appropriately.<sup>38</sup>

### Carotid body tumour

FNA of carotid body tumours may cause catecholamine release with the potential risk of hypertensive crisis; for this reason, suspected carotid body tumours are usually not aspirated. However, it is impossible to practice FNA cytology in a head & neck clinic and not aspirate an unsuspected carotid body tumour occasionally. There are no reports of adverse reactions to FNA in the cytology literature.<sup>39</sup>

### Audit and external quality assurance (EQA)

A comprehensive FNA service requires sufficient time for audit and quality assurance. Correlation of cytology with subsequent histology and clinical outcome should be carried out on an on-going basis or by regular directed audits. The NHS Breast Screening Programme<sup>35</sup> requires detailed monitoring of breast FNA reporting patterns, positive predictive values and other quality parameters that can be used to monitor non-screening cases and also as a model for auditing FNAs from other organ sites. At present there is no national EQA programme specific for FNA cytopathology. Specialist cytopathologists should have prior training in all types of diagnostic cytology. Pathologists should participate in and contribute to EQA activities relevant to their specialist areas.

#### Audit of FNA service

- Correlation of cytology with histology and clinical outcome and regular audits are essential.
- Time for audit must be appropriately resourced.
- Numbers and results of FNAs carried out and reported by individual consultants should be audited.

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