

DIAGNOSTIC CYTOLOGY SERVICE

What can cytology offer the practising veterinary surgeon?

Samples for cytological examination can be collected quickly, easily and inexpensively. Many can be obtained without chemical restraint. The collection techniques are less invasive than surgical biopsy and therefore give rise to fewer complications. There is no requirement for lengthy processing of the samples.

Various studies have compared the cytological with the histopathological diagnosis and/or the biological behaviour of the lesion. Accuracy varies depending on the type of lesion under investigation. Small, mobile or very firm, fibrous lesions, for example, are unlikely to yield sufficient material for cytological evaluation. In contrast, tumours of epithelial origin and discrete round cell tumours (*e.g. lymphoma and mast cell tumours*) tend to exfoliate cells in larger numbers, and the correlation between cytological and histological diagnoses may exceed 80%.

What does histology tell you that cytology cannot?

The aims of cytological evaluation are to establish an aetiological and/or morphological diagnosis and thereby obtain a more accurate prognosis. Cytology is a useful tool for differentiating inflammatory and infectious lesions from those that are neoplastic. In many cases cytology is also helpful in determining whether a tumour is malignant or benign. Cytology does however have its limitations and these should be recognised. Problems may arise when an inflammatory response results in secondary dysplastic changes which can mimic those normally associated with neoplasia (*this is particularly true of mesothelial cells in most body cavity effusions*). It is also worth noting that with poorly differentiated tumours, cytological examination may not identify the tissue of origin (*even differentiation between sarcomas and carcinomas can sometimes be extremely difficult*). Cytology, therefore, should not be regarded as a substitute for histopathological examination of biopsy specimens. Histology is more likely to provide a definitive diagnosis and, since biopsies preserve tissue architecture, grading and classification of the tumour is usually possible.

What are the indications for cytological examination?

CYTOLOGY CAN BE USED TO INVESTIGATE THE FOLLOWING:

- Cutaneous or subcutaneous masses *e.g. peripheral lymphadenopathy (fine needle aspiration or imprint cytology)*
- Intra-abdominal or intrathoracic masses (*fine needle aspiration under ultrasound guidance*)
- Haematological diseases (*bone marrow aspiration*)
- Examination of body cavity fluids (*pleural, peritoneal and pericardial effusions*)
- Examination of urine sediment (*e.g. stained preparation in addition to routine wet prep to check for neoplastic cells*)
- Joint disease (*joint tap to examine synovial fluid*)
- Neurological disease (*cerebrospinal fluid analysis*)
- Respiratory disease (*tracheal wash, bronchoalveolar lavage, and lung aspirate*)
- Prostatomegaly (*prostatic wash or fine needle aspirate of the prostate under ultrasound guidance*)
- Oestrus detection (*vaginal cytology to assess stage of the oestrus cycle*)
- Nasal disease (*nasal flushings*)
- Conjunctival and/or ocular disease (*conjunctival swab, third eyelid or corneal swab/scrape, fine needle aspirate of anterior chamber*)

COLLECTION TECHNIQUES AND SMEAR PREPARATION

The limitations of diagnostic cytology are primarily those imposed by inadequate collection techniques or staining procedures. The choice of collection technique depends on the anatomical location, the characteristics of the tissue being sampled, and also the tractibility of the patient.

Fine needle aspiration INDICATIONS: Examination of soft tissue masses (*e.g. enlarged lymph nodes, cutaneous and*

subcutaneous lesions, intrathoracic or intra-abdominal masses) and body cavity fluids. The methods used to aspirate lesions in the liver, spleen, kidneys and thoracic cavity are described in more detail in a recent In Practice article (*Collection and preparation of smears for cytological examination by Elizabeth Villiers and John Dunn, Vol 20, pp370-377*).

TECHNIQUE: Clip the area and swab with alcohol. If the sample is required for *bacteriology* (e.g. *pleural effusions or joint fluid*) surgically prepare the site. Lay out clean glass slides. Use a 10ml syringe and 1" x 21/23 g needle (larger needles are more likely to result in greater *blood contamination*). Localise and immobilise the mass with one hand. The sample may then be obtained using one of three methods.

'NEEDLE ONLY' method: This method minimises the degree of haemodilution and the cells are not damaged by suction. It is particularly useful for sampling soft tissue masses e.g. lymph nodes and masses which are highly vascular e.g. splenic tumours. A 21/23 g needle is inserted into the lesion and moved in vertical and horizontal planes, taking care not to push the needle into adjacent tissues. The sample is obtained within the hub of the needle and should be expelled gently by attaching an air-filled syringe to the needle.

'CONTINUOUS SUCTION' method: This method is useful for aspirating firm masses which do not exfoliate cells in large numbers e.g. fibromas or fibrosarcomas. A 21/23 g needle with a 5 or 10 ml syringe attached is advanced into the lesion. Continuous suction is applied while the needle is redirected within the lesion at least three times. Suction should be released before withdrawing the needle.

'INTERMITTENT SUCTION' method: This method is appropriate for aspirating cells from small masses where it is not possible to redirect the needle without exiting the mass. A 21/23 g needle with 5-10 ml syringe attached is inserted into the mass. The plunger is withdrawn and released several times. Suction should be released before the needle is withdrawn rapidly from the lesion.

Impression smears (imprints)

Impression smears and scrapings can be made directly from ulcerated skin lesions or from the cut surface of excised tissue/biopsy specimens e.g. lymph node, spleen, liver and kidney. Use a saline-moistened swab to wipe away superficial debris; do not use alcohol or antiseptic solutions. If the surface of the lesion is covered with excessive blood or tissue fluids blot with a clean, dry swab or paper towel and then make your imprint using a clean glass slide. Do not smear the slide across the surface of the lesion. Several imprints can be made of the same slide. The disadvantages of this technique are that relatively few cells may be collected and the cells obtained from the surface of a lesion may not be representative of the whole lesion. For example, the surface of ulcerated skin tumours often becomes secondarily inflamed and infected so that the impression smear may only collect inflammatory cells rather than the underlying neoplastic cells.

Scrapings

This technique can be used for cutaneous lesions and excised tissue specimens. This is a particularly useful technique for collecting cells from masses which do not readily exfoliate e.g. mesenchymal tumours. Scraped material is smeared onto clean glass slide. The same disadvantages as impression smears apply.

Swab smears

Swab smears are useful for assessing the stage of oestrus in bitches. The swab should first be moistened with normal saline to minimise cell damage during collection. The cells are then collected from the vagina and transferred to a glass slide by gently rolling the swab along surface of slide (*do not rub*). The technique can also be used to collect cells from fistulous tracts.

Brushings

Brushings can be useful for obtaining cells from very soft friable specimens. eg splenic haemangiosarcomas. Cells are transferred from the cut surface of the excised tissue to a glass slide with a fine camel-haired paint brush.

COLLECTION AND HANDLING OF FLUID SAMPLES

The biochemical and cytological characteristics of an effusion can be used to classify the fluid and in some cases obtain a definitive diagnosis. Collect fluid samples into EDTA for total protein concentration, specific gravity, total nucleated and red cell counts, and cytology. Samples for bacteriological examination or other biochemical tests eg triglycerides and cholesterol should be collected into a plain tube. Smears should be prepared immediately after collection using a drop of thoroughly mixed fluid or the sediment from a centrifuged sample. For fluids of low cellularity e.g. true transudates, peritoneal fluid and CSF samples centrifuge at slow speed i.e. 1000-1500 RPM for 5 mins. Resuspend the sediment in a few drops of supernatant. Direct smears can be made from samples which appear visibly turbid since these will generally be hypercellular.

Thoracocentesis

The animal is placed in sternal recumbency. Cats generally require sedation. Sedation and local anaesthesia may be required in some dogs particularly if a large volume of fluid is to be drained from the chest. The site for needle insertion (*7th or 8th intercostal space in the ventral third of the thorax*) should be clipped and surgically prepared. Flexible 18-20 g plastic over-the-needle intravenous catheters are preferred since they are less traumatic once the needle has been removed and are less likely to become dislodged if the animal should move during the collection process.

The skin should be penetrated at a site 1-2 cm distal to the point of insertion through the thoracic wall. The catheter is then inserted next to cranial surface of rib to avoid the risk of lacerating blood vessels along the caudal border. The needle is removed and a three-way tap is attached to the syringe.

Abdominocentesis

Follow the same preparatory steps outlined above. With the animal standing or in lateral recumbency, insert a 1 inch x 20 g needle or a long 18-20 g plastic over-the-needle intravenous catheter through the ventral midline 1-2 cm caudal to umbilicus (*this avoids falciform fat*).

Pericardiocentesis

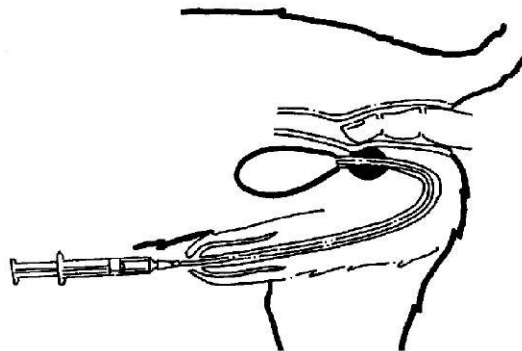
Pericardiocentesis may be therapeutic as well as diagnostic. Light sedation is generally required to drain the pericardial sac. The animal is placed in lateral recumbency and the area over the 3rd-7th intercostal spaces is clipped from the sternum to costochondral junctions. Inject local anaesthetic over site before making a small stab incision in skin. Use a 16 g x 15-20 cm long over the needle IV plastic catheter. The point of entry should be determined from the dorsoventral radiographs or the point of maximum intensity of cardiac impulse on palpation. This is usually between the 4th and 6th ribs. When the catheter has been placed into the pericardial sac the needle should be withdrawn before attaching a three-way tap and 50 ml syringe. The majority of pericardial effusions are port-wine coloured and should not clot (*the PCV should be different from that of blood*). Cytology rarely differentiates neoplastic from benign effusions, and typically there is wide variation in the protein content, red cell and nucleated cell counts.

Prostatic washes

Cytological examination of a prostatic wash is a useful technique for investigating prostatomegaly. For example, it can be used to differentiate benign prostatic hyperplasia from bacterial prostatitis, but is a less reliable method for confirming prostatic neoplasia since not all prostatic tumours necessarily exfoliate cells into the prostatic urethra (*fine needle aspiration of the prostate under ultrasound guidance is a more reliable diagnostic tool in this respect*).

TECHNIQUE

- * The dog is positioned in lateral recumbency and the bladder is catheterised and emptied.
- * Ideally this catheter should be withdrawn and a fresh catheter inserted to the level of the prostatic urethrae; the position of the catheter can be gauged per rectum.
- * The prostate is then massaged per rectum for approximately 30 seconds (*an assistant will be required to do this*).
- * 5-10 mls of normal saline is flushed into the urethra with a 10 ml syringe and aspirated back immediately. Suction should be removed while the catheter is withdrawn.
- * The contents of the catheter and syringe are collected into an EDTA tube for cytology and a plain, sterile container for bacteriology if infection is suspected.



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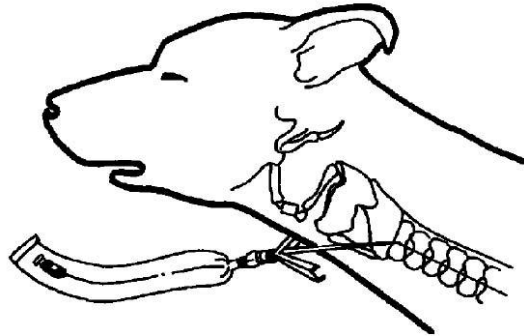
PROSTATIC MASSAGE

A modification of the prostatic wash technique is to collect cells simply by massaging the prostate. The tip of the urinary catheter is placed within the prostatic urethra as described above. Constant suction is applied with a 10 ml syringe by an assistant while the prostate gland is massaged per rectum. A small amount of material will be retrieved in the tip of the catheter and this should be transferred to clean glass slides. If necessary the material should be expelled by flushing air or saline through the catheter.

Smears are prepared using the squash preparation technique described on pages 9-10.

Transtracheal and bronchoalveolar washes

Both these techniques can be used to collect material from the tracheobronchial tree for cytology and bacteriology.

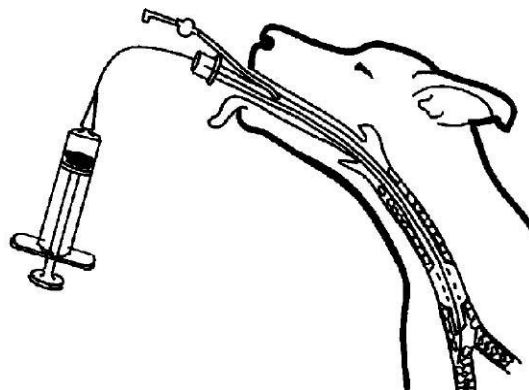


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TRANSTRACHEAL WASH

This is a percutaneous technique which can be performed without general anaesthesia. Sedation may be necessary in some animals. The degree of oropharyngeal contamination is minimised using this technique. Possible complications include haemorrhage, subcutaneous emphysema and pneumomediastinum.

- The dog is allowed to sit or is placed in sternal recumbency with the neck extended.
- The skin over the larynx is clipped and surgically prepared. A small volume of local anaesthetic is infiltrated into the skin and subcutaneous tissue over the cricothyroid ligament which is located just cranial to the cricoid cartilage.
- A long 18 g jugular catheter is inserted through the cricothyroid ligament. Alternately a 3.5 French urinary catheter can be passed through a 14 g needle.
- Flush 1-2 mls of normal saline/5 kg BW and aspirate immediately (*note that this should induce a cough reflex*). Transfer into EDTA and plain tubes for cytology and bacteriology respectively. Apply a gauze wrap over puncture site for 12-24 hours. To avoid some of the problems associated with ageing of these samples during transit to the Lab, it is always advisable to make fresh air-dried smears of any material which can be extracted from the wash. If you have a centrifuge it is also helpful to make some concentrated preparations of a sample of sediment from the wash which are air-dried on slides and sent in with the wash samples.



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BRONCHOALVEOLAR LAVAGE

This is the preferred technique for small dogs and cats. General anaesthesia is required. It provides a more reliable method of collecting cells from the lower airway. The obvious advantage of this technique is that the operator can visualise the airways so that the catheter can be directed to collect cells from specific areas of interest. If necessary, biopsy material can also be obtained. The major disadvantage is that the tip of the *catheter (and hence the sample obtained)* is more likely to be contaminated by oropharyngeal material as it is passed through the endotracheal tube or bronchoscope.

A long 14 g urinary catheter is passed either via a cuffed endotracheal tube, or rigid or fiberoptic bronchoscope to the level of the carina and into a mainstem bronchus. Flush 1-2 mls of normal saline and aspirate immediately. The yield of cells may be enhanced by gently redirecting the catheter within the airway lumen as the fluid is aspirated.

HORSE

Samples for tracheal wash (*TW*) and bronchoalveolar lavage (*BAL*) cytology can be obtained via the transtracheal route or fiberoptic endoscope. The techniques for these procedures will not be described here.

Aspiration of synovial fluid

Cytological examination is helpful in the differentiation of inflammatory (*e.g. immune-mediated*) arthropathies from degenerative joint disease. Adequate restraint and sedation are essential in order to minimise the amount of blood contamination. This will depend on the joint(s) under investigation and the tractability of the patient. Most joints can be tapped with the animal in lateral recumbancy and the affected limb uppermost. Routine aseptic technique is advised.

A one inch 20-22 g needle with 2 ml syringe attached is gently advanced into the joint space, gentle suction should be applied until synovial fluid appears in the hub of the syringe. It is often helpful to have an assistant manually flex and extend the joint, and also to help immobilise the joint as the needle is inserted. Synovial fluid is usually easily aspirated from joints which are visibly distended (*joints which are difficult to aspirate often yield synovial fluid which is normal!*). Suction should be released before withdrawing the needle in order to minimise the amount of blood contamination. Normal synovial fluid should not clot. It is more likely to do so if the protein content is increased due to inflammation or if it is significantly contaminated with blood. The aspirate should be placed into an EDTA tube for cytological examination. Joint fluid which appears visibly turbid and/or which is of decreased viscosity is abnormal. In addition to cytology you may wish to submit such a sample in a plain tube for bacteriological examination.

Cerebrospinal fluid

Cerebrospinal fluid analysis is an important part of the investigation of most central nervous system diseases. The collection of CSF is contraindicated in conditions where increased intracranial pressure is suspected (*e.g. cerebral oedema or intracranial haemorrhage due to trauma, or hydrocephalus*), since the sudden release of CSF pressure may result in herniation of the brain stem through the foramen magnum.

CSF is normally collected from the foramen magnum at the atlanto-axial articulation. Subarachnoid puncture at the lumbar cisterna may be more useful diagnostically if a single thoracolumbar spinal cord lesion is suspected. The techniques for collecting CSF will not be described here.

CSF should be collected into EDTA for cytological examination and a plain tube for bacteriology. The sample should be examined/prepared as soon as possible after collection because cells in CSF may degenerate rapidly. If you are sending CSF in for analysis at AXIOM it is advisable to make some sedimented or gently centrifuged air-dried smears of the CSF to send with the fluid sample. Normal CSF is water clear, colourless and should not clot. Blood tinged taps in most cases are due to faulty technique resulting in blood contamination. CSF becomes visibly turbid when the nucleated cell count exceeds $0.3-0.5 \times 10^9 / l$.

Bone marrow aspiration/biopsy

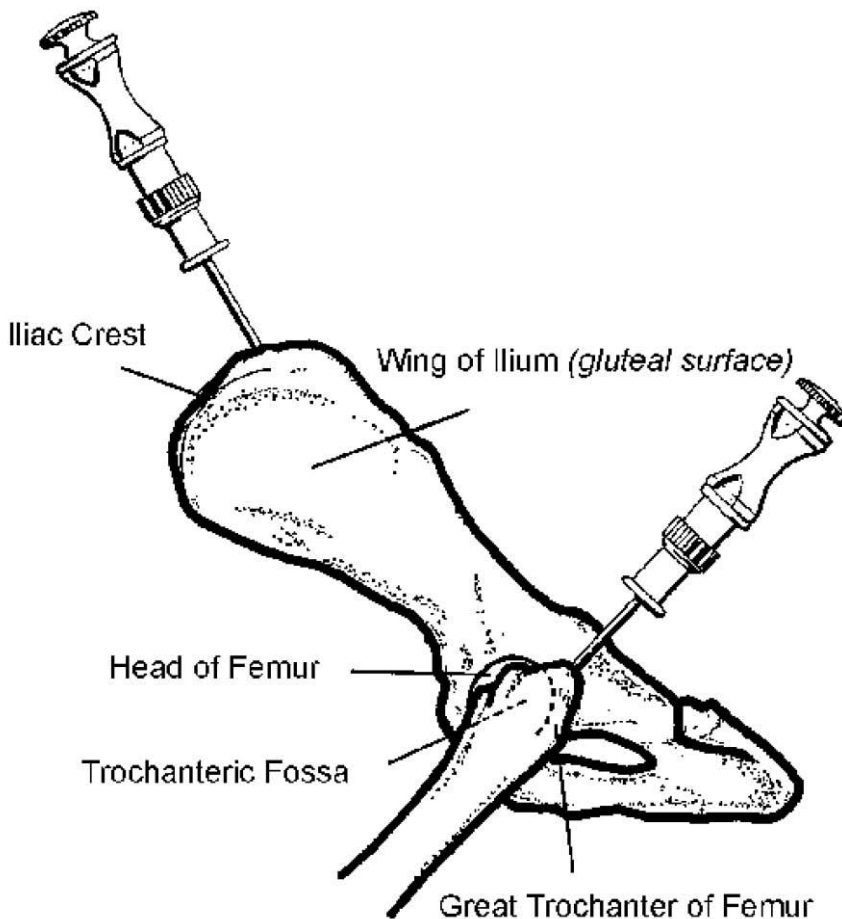
Bone marrow evaluation is an important part of the investigative approach to many haematological disorders. Bone marrow may be evaluated by two methods:

- I. cytological examination of an aspirated sample and
- II. histological examination of a core biopsy. Both these procedures can be performed under sedation and local anaesthesia.

ASPIRATION

Suitable aspirates for cytological examination may be obtained from the iliac crest (*medium and large dogs*) or from the trochanteric fossa (*small dogs and cats*) using a Klima or Rosenthal biopsy needle with interlocking stylet (*see diagram*). The marrow smears can either be prepared immediately without anticoagulant or 1 ml of 3% EDTA solution may be used as an anticoagulant in the barrel of a 10 or 20 ml syringe. Appropriate needles can be ordered on request from Axiom Laboratories.

- To obtain a sample from the iliac crest the animal is placed in sternal recumbancy. The site is clipped and the skin, subcutis, and periosteum are infiltrated with local anaesthetic.
- After surgical preparation and draping, a small stab incision is made in the skin through which the needle is advanced into the cortical bone using alternating clockwise-counterclockwise rotations. The needle is advanced perpendicular to the long-axis of the wing of the ilium. Care should be taken to ensure the stylet remains in situ otherwise the lumen of the needle may become plugged with cortical bone.



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Left lateral aspect of the os coxae and proximal femur showing points of entry for bone marrow aspiration.

- With the needle in the marrow cavity, the stylet is withdrawn and a 10ml syringe is attached to the needle hub. The marrow is then aspirated by several, quite forceful, withdrawals of the plunger; if this fails to produce marrow the needle should be withdrawn slightly before reapplying suction. Depending on the level of sedation, and assuming that the needle is correctly placed, an animal will show a transient pain response as the marrow is aspirated. If repeated attempts to obtain marrow from the iliac crest fail, the needle should be withdrawn, and the stylet replaced before redirecting the needle into a different anatomical site e.g. the tibial crest or proximal humerus.

When the femur is used the same preparatory procedures should be carried out. With the animal placed in lateral recumbency care should be taken to ensure that the local anaesthetic infiltrates the deeper subcutaneous tissues and periosteum. The greater trochanter is palpated and the needle is directed medial to this into the trochanteric fossa. Once in the trochanteric fossa, the needle is advanced, parallel to the long axis of the femur, into the medullary cavity.

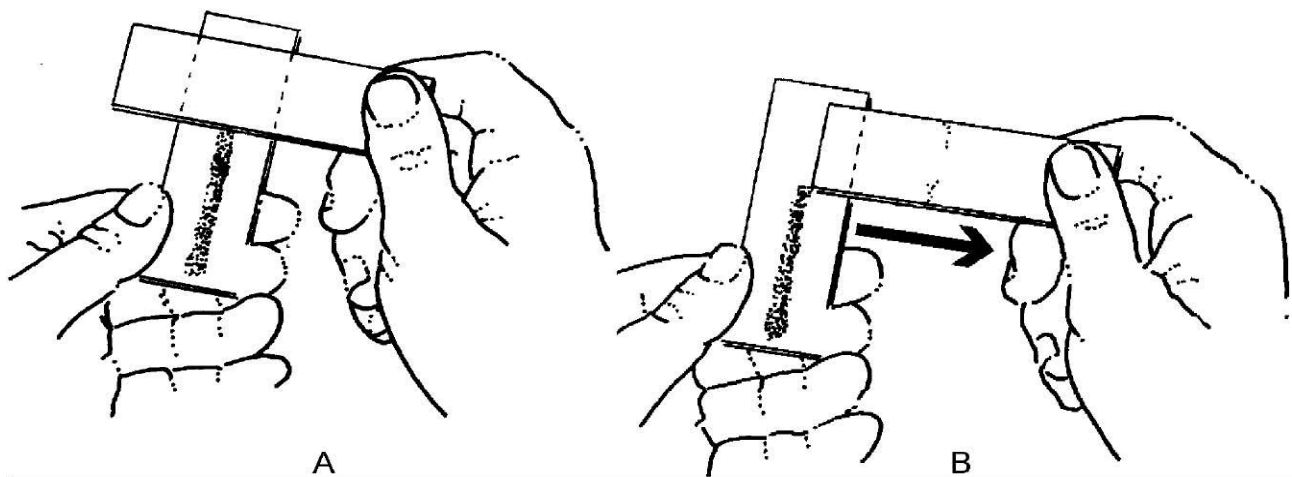
- When marrow appears in the syringe the negative pressure should be released immediately since continued and vigorous suction only results in haemodilution of the specimen. In smaller animals the volume of marrow obtained may be less than 0.5 ml.
- The needle should be withdrawn with the syringe attached and a drop of marrow is expelled onto a series of clean glass slides which are tilted at an angle. This allows blood to gravitate downwards whilst marrow spicules remain at the top of the slide.
- A suitable smear may be obtained by gently crushing the spicules with another glass slide which is then pulled across the bottom slide in a horizontal plane (*see diagram of squash preparation*).
- Note that if EDTA is not used as an anticoagulant smears of the fluid marrow should be prepared before the sample clots (*usually less than 30 seconds*). The smears are then air-dried. Thick smears may need to be fixed by immersing in methyl alcohol for three minutes.

CORE BIOPSY

Following repeated 'dry' taps or when a hypoplastic or aplastic marrow is suspected, a bone marrow core biopsy should be taken for histopathological examination. The main advantage of a core biopsy is that it preserves the normal architecture of the marrow cavity and provides a more representative picture of the distribution of haematopoietic cells in relation to the non-haematopoietic elements of the marrow stroma. A major disadvantage is the inevitable delay associated with decalcification of the specimen and preparation of the sections.

Cores of marrow suitable for histopathological examination are best obtained from the iliac crest of larger dogs using a Jamshidi needle (*appropriate needles can be provided on request from Axiom Laboratories*).

- The biopsy needle is advanced with the stylet in situ through the cortical bone and into the medullary cavity using alternating clockwise-counterclockwise rotations.
- The stylet is then removed and the bevelled cutting point of the needle is advanced a further 1-2 centimetres into the marrow cavity. The needle is then rotated vigorously in one direction about its long axis before removing it from the bone to ensure that the core is sectioned at its base.
- The core is expelled by inserting the long blunt-ended probe through the distal cutting end of the needle; since the Jamshidi needle tapers towards its cutting point pushing from the proximal end of the needle may compress and damage the specimen. Before fixing in neutral buffered formalin, impression smears may be made by gently rolling the core on a clean glass slide.



MAKING SMEARS FOR CYTOLOGY

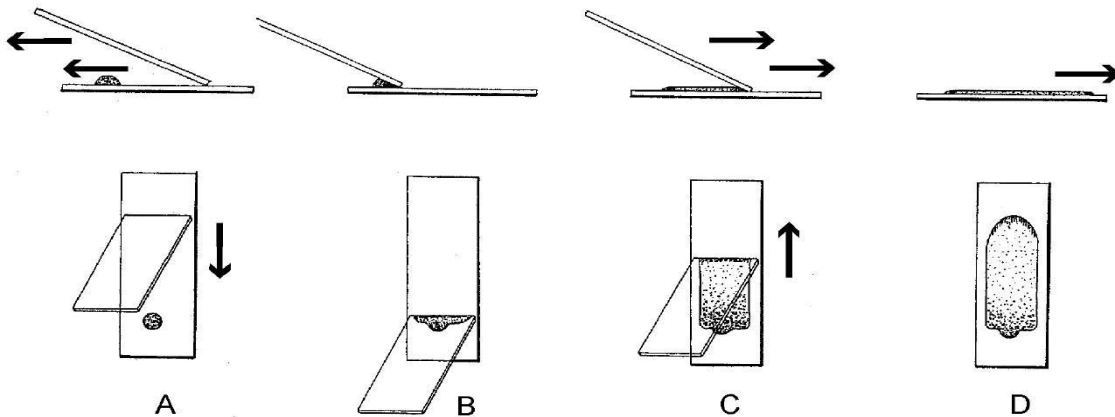
A poor quality smear makes interpretation difficult. Potential causes include dirty slides, water artefact, a smear that is too thick or heavily contaminated with blood, and poor smear preparation resulting in large numbers of smudged cells, bare nuclei or strands of nuclear protein.

Having obtained your aspirate remove the needle and draw air into syringe. Replace the needle and expel the aspirate onto clean glass slides. Make as many smears as possible using one of the methods described below. The cellularity of fluid samples can be gauged by assessing the degree of turbidity. Samples which are clear generally contain very few cells compared to those which appear turbid in which case the nucleated cell count usually exceeds $0.3 \times 10^9/l$.

SQUASH PREPARATION TECHNIQUE

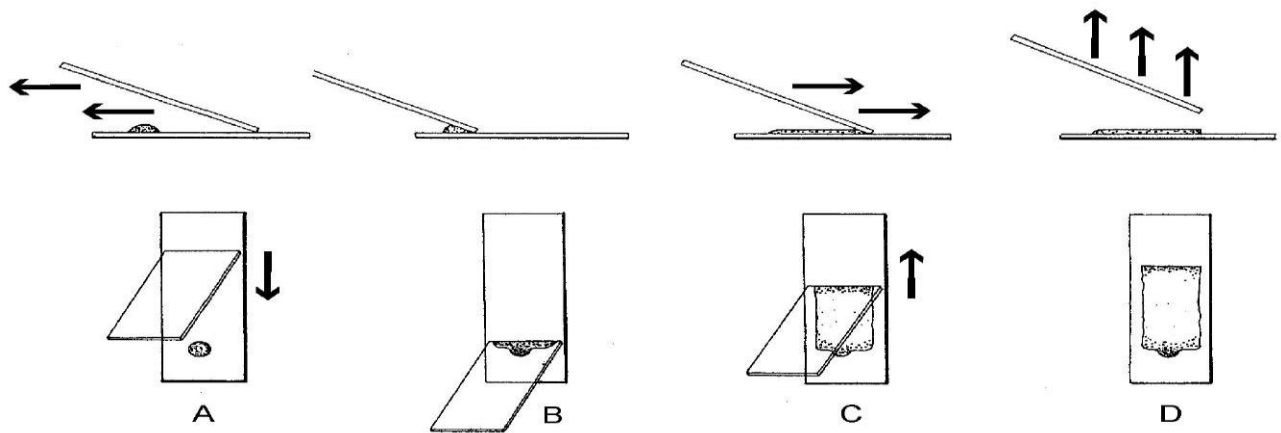
If right handed, hold the slide with the aspirated material in your left hand. A second slide, held in the right hand, is placed flat at right angles to the first slide. This spreads out the aspirate. The top slide is drawn smoothly across the bottom one to produce the smear on the lower surface of the top slide (*see diagram*). Excessive downward pressure should not be applied during the smearing process since this will result in cell rupture and the artefacts described above.

This is the preferred technique for aspirates which are semi-fluid in consistency e.g. lymph node and bone marrow aspirates.



BLOOD FILM TECHNIQUE

This technique is used primarily to prepare films from fluid samples but can also be used for lymph node aspirates. A drop of fluid is placed at one end of the slide. A spreader slide is placed at a 30 to 40 degree angle in front of the sample and drawn backwards until it makes contact with the drop of fluid. The fluid will then spread along the sample slide. The spreader slide is then advanced forwards to make a smear with a feathered edge (*see diagram*). The blood film technique is less suitable for samples of low cellularity. Note: A spreader slide can be made by breaking off the corner of a glass slide, having first scored it with a diamond writer or glass cutter.

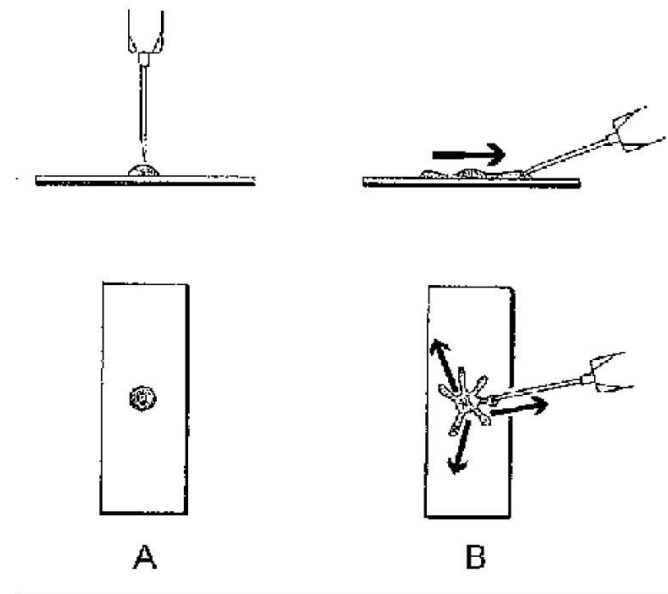


LINE CONCENTRATION TECHNIQUE

This method is more suitable for fluids of low cellularity but may not sufficiently spread cells from hypercellular samples. The technique is similar to the blood film technique described above except that the spreader slide is raised abruptly upwards once it has been advanced approximately three quarters of the distance along the bottom slide (see *diagram*). Cells are concentrated in a line along the end of the smear.

STARFISH PREPARATION

This technique minimises the amount of trauma to fragile cells and is useful if only a small volume of material is aspirated. The aspirate is gently 'blown' onto the slide and the material is dragged peripherally in several directions with the tip of a needle to produce a 'starfish' appearance. This method tends to spread out the cells less adequately than the other methods described above and may result in thick areas where morphological detail is obscured by tissue fluid.



Are my smears suitable for more detailed cytological interpretation?

Having prepared your smears you may wish to examine these under the microscope to ensure the quality of the specimen is sufficient to permit meaningful cytological interpretation. Diff Quik-type stains are perfectly appropriate for routine use in a practice laboratory. First scan the slide under low magnification ($\times 10$) to identify areas of increased cellularity or areas with different staining characteristics. Crystals, foreign bodies and parasites are usually visible at low magnification. Change to $\times 20$ objective and assess the cellularity and cellular composition i.e. assess the relative numbers of inflammatory cells, epithelial cells etc. Finally change to $\times 40$ and then $\times 100$ oil to evaluate cell morphology in greater detail.

This method tends to spread out the cells less adequately than the other methods described above and may result in thick areas where morphological detail is obscured by tissue fluid.