On a cellular level

By Arefeh Ravanbakhsh

Tips and tricks to improve the diagnostic quality of cytology samples.

Diagnostic laboratories are in a unique position to be able to collate and document large numbers of pathological specimens.

Obtaining a good-quality cytology sample is a crucial first step to maximising the chances of a correct cytological diagnosis. This article provides some suggestions to help obtain better quality cytology samples, largely focusing on fine-needle biopsy (aspiration and non-aspiration techniques).

Labelling slides

As with any procedure, having a systematic approach to collecting cytology samples will help reduce the chances of forgetting a step. Before collecting a cytology sample, it is a good idea to have the slides ready and labelled. Wiping them with a tissue prior to transferring the material onto the slides will help remove debris and microscopic glass particles that can interfere with the spreading procedure.

Labelling the smears is very important to avoid any confusion about the site sampled. At a minimum the slide should include the site sampled (for example, the left popliteal lymph node, right submandibular lymph node, left hock joint, right flank mass) and ideally the name/ID of the patient. Use pencil or solvent-resistant slide-specific markers to avoid the labels rubbing off during staining.

If you are sending the smears to a referral laboratory for examination, avoid labelling only the slide holders. Labelling the smears directly will reduce the chances of a mix-up when they are removed from the holders.

Collecting specimens

Two common approaches to collecting cytological specimens for cutaneous and subcutaneous masses are fine-needle aspiration and fine-needle capillary sampling (aka the non-aspiration technique).

As a brief overview, with fine-needle aspiration the tip of the needle is inserted into the tissue of interest and the plunger of the syringe is retracted slightly to allow for negative pressure. While still submerged in the mass, the needle is advanced and redirected several times. Only gentle, constant suction of the syringe is needed (Raskin and Meyer, 2016); vigorously pumping the plunger multiple times should be avoided, as this will result in ruptured cells and excessive blood contamination.

After redirecting the needle within the mass, the negative pressure is released, then the needle is withdrawn from the mass. To transfer the material to the slide, the needle is removed from the syringe and air is drawn into the syringe. The needle is subsequently replaced into the syringe and the cellular material is propelled onto the slide through the depression of the plunger. If fluid is retracted, consider putting some of the sample on a slide for a fresh smear and placing the rest in an EDTA tube. A butterfly needle attached to a syringe can also be used to enhance operator flexibility.

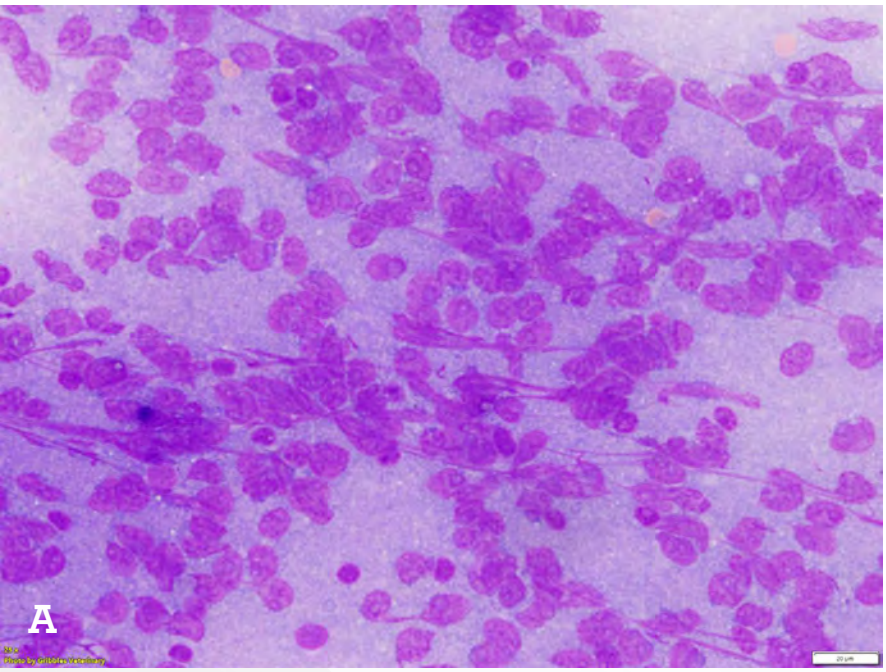
Fine-needle capillary sampling involves using just the capillary action of the needle, without the use of negative pressure from a syringe. The process for transferring the material onto the slide is similar to that explained above. The main advantage of this technique is that it allows for less blood contamination, and it may also aid in reducing the likelihood of ruptured cells. Using the needle alone can allow for better control of its placement and movement, and is particularly useful when sampling lymph nodes in suspected lymphoma patients, as immature lymphocytes can easily rupture (figure 1).

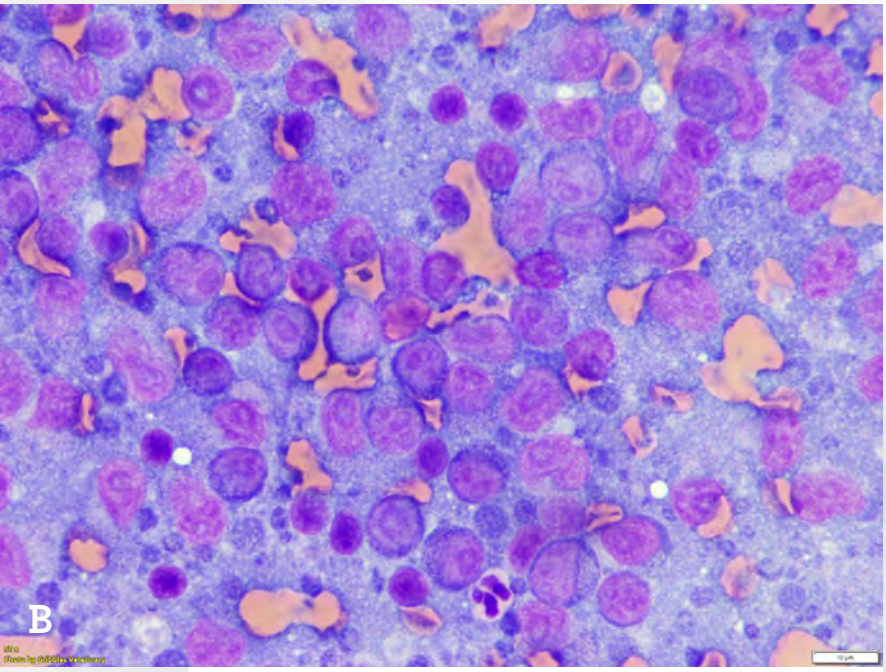
User preference can dictate which technique is more frequently used. If the patient is cooperative, both techniques can be used to increase the chances of getting a diagnostic sample.

The collection of cytology specimens from small animals typically requires a 22–25 gauge needle with a 3–20ml syringe (Valenciano and Cowell, 2019). When using the aspiration technique, a 3ml syringe is generally adequate for softer masses, while for firmer lesions a larger syringe should be used. If the texture of the lesions is unclear, a 12ml syringe would be a good choice (Valenciano and Cowell, 2019).

In a 2019 study comparing the quality of cytological samples obtained via 22- and 25-gauge needles in dogs and cats, it was found that the samples

IMAGERY: SUPPLIED





FIGURES 1A AND B: Figure 1A, image of fine-needle aspirate from an enlarged lymph node. Although the cellular yield is adequate, almost all of the exfoliated cells have ruptured, rendering the sample non-diagnostic. Figure 1B also shows fine-needle aspirate on an enlarged lymph node. Although occasional cells are ruptured, most are intact.

Obtaining a good-quality cytology sample is a crucial first step **to maximising the chances of a correct cytological diagnosis.**

collected via 25-gauge needles resulted in less blood contamination but increased cellular trauma compared to samples collected with 22-gauge needles (Shiori et al., 2019). The gauge of needle used did not make a significant difference to the overall ability to attain a diagnosis in this study (Shiori et al., 2019). The result of the study did suggest that using a high-gauge needle in highly vascularised lesions, and a lower-gauge needle when sampling lesions with fragile cell populations (for example, lymph nodes in suspected lymphoma patients) may be beneficial in attaining good-quality samples.

Once the sample has been transferred to the glass slide, the last step is to distribute the material evenly on the smear. There are a few techniques for smearing cytology samples; refer to the numerous cytology textbooks for more information.

The squash technique is often favoured for spreading fine-needle biopsy cytology samples evenly. One way to perform the squash technique is to keep the slide on a flat surface. After the material is expelled onto the slide, a second glass slide (spreader slide) is placed gently on top of and perpendicular to the slide containing the sample. The thumb and pointer finger are placed on either side of the spreader slide and used to push the spreader slide gently across the length of the bottom slide. Just the weight of the spreader slide alone should be enough to provide an even smear in most cases. Very light pressure can be applied to the spreader slide in cases where samples are very thick.

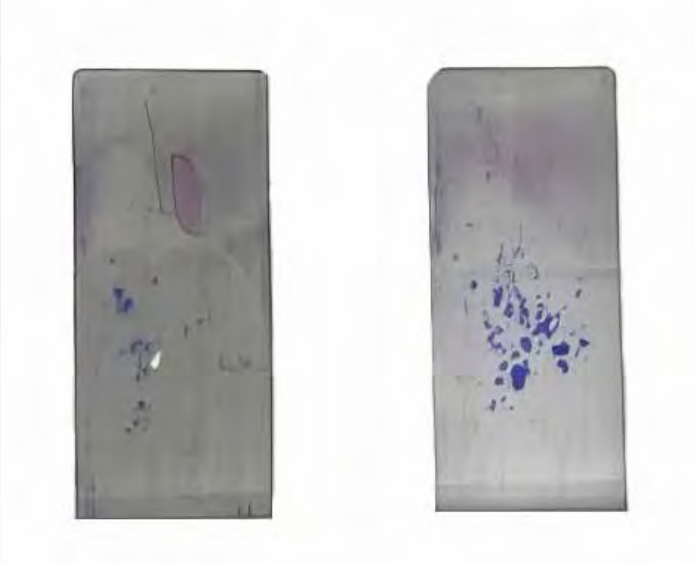


FIGURE 2: The 'spray-and-pray technique'; an example of a poorly smeared sample. The sample was sprayed on the slide and no further smearing was performed. On cytological examination the cells were present in dense clumps, obscuring cytological detail.

It is very important to smear cytology samples evenly. Just spraying a specimen on a slide without spreading it evenly, aka 'the spray-and-pray technique', is strongly discouraged, as the cells are often present in dense clumps that obscure cytological detail (figure 2).

Whenever possible, sampling multiple areas of a mass is recommended. The greater the area of a mass sampled, the higher the chance that a representative cell population will exfoliate. For example, some tumours will be surrounded by fat. If only one fine-needle aspirate is collected, only the surrounding fat may be sampled, without exfoliation of the true neoplastic population, resulting in a misleading cytology sample. Another example would be a tumour with areas of necrosis. If only the necrotic portion is sampled, the cytology smear would reveal mostly amorphous debris and degenerate cells, which would preclude further cytological characterisation of the mass.

When sending smears to a referral laboratory for examination, providing a concise history (including a description of the lesion, relevant clinical examination findings and the signalment of the patient) is highly recommended, as it will provide crucial ancillary information to help with a correct cytologic interpretation. Helpful information to provide in the history includes a description of the lesion (size, location, cutaneous versus subcutaneous, well demarcated versus infiltrative, firm or soft, presence of ulceration or inflammation, etc), the duration of the lesion, change in size (slow growing versus rapid enlargement) and response to previous therapy, if applicable.

Cytology samples sent with formalin-fixed tissue should be kept in separate bags, as formalin fumes can cause rapid cell fixation resulting in reduced staining quality.

As with any diagnostic procedure, the more often it is attempted the easier it will become. It is important to keep in mind that some lesions are inherently poorly exfoliative or otherwise poor candidates for cytological assessment (for example, highly necrotic tumours). So even if the sampling technique is perfect, it may still result in limited cytology samples. This should not discourage the use of cytology as a diagnostic tool, as in many cases cytology can be rapid, safe and relatively inexpensive and provide valuable information to aid in case management and decisions on the next steps. VS

Arefeh Ravanbakhsh is a clinical pathologist at Gribbles Veterinary.

FURTHER READING:

Raskin RE, Meyer DJ. *Canine and Feline Cytology: A Color Atlas and Interpretation Guide*. 3rd Edtn. Elsevier St. Louis Missouri, USA, 2016

Shiori A, Rist P, Clancey N, Gilroy C, Stryhn H, Amsellem P. Fine-needle aspiration of cutaneous, subcutaneous, and intracavitary masses in dogs and cats using 22- vs 25-gauge needles. *Veterinary Clinical Pathology* 48, 287–2, 2019

Valenciano AC, Cowell RL. *Cowell and Tyler's Diagnostic Cytology and Hematology of the Dog and Cat*. 5th Edtn. Elsevier St. Louis Missouri, USA, 2019