DIY diagnostics: cytology

It is possible to take tissue and fluid samples; carry out basic preparation and staining; and evaluate the stained cells, even on a very low budget. An appreciation of the basic principles of sampling, sample preparation and cytological evaluation will assist greatly and improve the chances of gaining valuable information.

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Cytological evaluation of tissue and fluid aspirates is a relatively non-invasive and technically simple procedure, which can provide valuable diagnostic information for the clinician on a limited budget. However an appreciation of optimum sampling techniques is needed to get the most out of your samples.

Factors to consider in sampling

The clinical picture: the gross features of any lesion and presenting signs are likely to provide the clinician with a list of differentials. Consideration of your clinical suspicion should help you decide on the best approach for sampling. There are various options:

Fine needle capillary sampling (aspiration without suction)

Advised for lesions with good exfoliation, expected high fragility or hemodilution, e.g. thyroid lesions or large lymph nodes. However, for poorly exfoliative tissues, this may result in low cellular yield.

Technique

- Place needle into lesion without a syringe attached
- Redirect needle in lesion up to three times
  - Cells are displaced into the cylinder of the needle by capillary action
- Attach syringe pre-drawn with air
- Apply material onto slides after retracting the needle from the lesion
- Spread material gently to form a monolayer.

Sampling with suction

Suitable for less exfoliative lesions such as hard, subcutaneous masses, suspected fibroadnexal lesions or where there is limited aspiration on initial sampling. This procedure can produce excellent results, however it may introduce more haemodilution and may lead to cell disruption.

Technique

- Insert the needle tip into the lesion
- Retract plunger to 0.5–1 cc of vacuum
- Advance and retract needle in three directions, maintaining constant vacuum
- **Release plunger**
- Withdraw needle
- Detach syringe and pre-draw air, then reattach
- Apply specimen to slide.

Impression smears

Impression smears may be used when lesions are excised for biopsy, for rapid interpretation. They also may be used for ulcerated/surface/excoriated lesions, however samples are often not representative of the underlying pathology and may be haemodiluted if exudate/surface blood is not gently removed with a swab prior to sampling (*Figure 1*).

For surgical biopsies:

- Remember to blot excess fluid from the tissue before applying to the slide
- Avoid exposure to formalin fumes when sampling.

For flat superficial lesions:

- Remove scab
- Obtain a clean moist surface.

Staining techniques

Most clinicians will use a modified Romanowski stain such as Diff Quik, which comprises a fixative (typically methanol), primary stain (e.g. methylene blue) and eosin. Protocols are many and varied but rely on appropriate storing of samples in airtight containers to avoid excessive evaporation. Individuals should troubleshoot

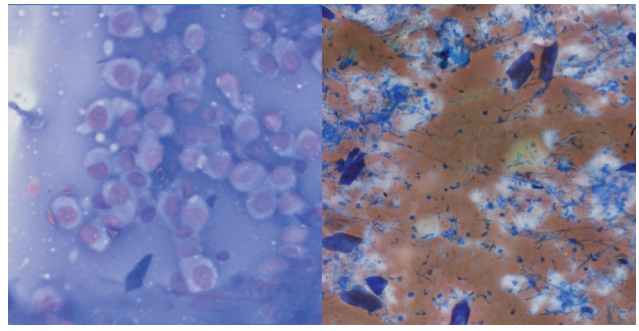

Figure 1. Cutaneous histiocytoma sampled by aspiration without suction (left). The same lesion sampled by impression smear (right).

their own protocol to establish what is working best in their lab. Critically evaluating the quality of their stain e.g. excessive blue or pink colouration is advised, and protocols can be 'tweaked' as needed. Reported protocols vary from 60–120 seconds in fixative, 30–60 seconds in solution 1 (eosin), and 5–60 seconds in solution 2 (methylene blue).

It is important to remember that cytological samples reflect just a tiny portion of the lesion (*Figure 2*). In some cases the lesion may be missed altogether, we may see only inflammation surrounding the mass or we may see one aspect only of a heterogenous population. This should be taken in to account both at the time of sampling (i.e. how many samples to take and from which areas) and when interpreting results.

Always try to produce a nice monolayer in the sample. Excessively thick smears will not stain well and cells will be insufficiently spread. So for very exfoliative lesions that are producing thick smears, try to avoid getting too much material on the slide (*Figure 3*).

Approach to cytological evaluation

A systematic approach to smear examination allows you to make the most from your cytology samples. Critically evaluating the smear as you go will help establish if this is likely to be a representative sample. Keep in mind the differentials that you have for this patient as this will often help make sense of the cytological picture.

Make a gross assessment of the smear:

- How much material?
- How well spread?
- Any issues with staining?

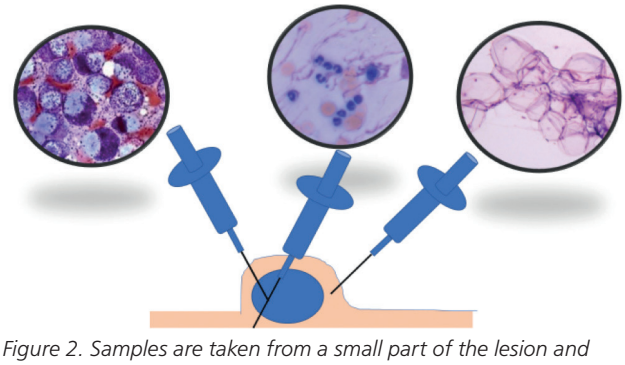

Figure 2. Samples are taken from a small part of the lesion and may be unrepresentative.

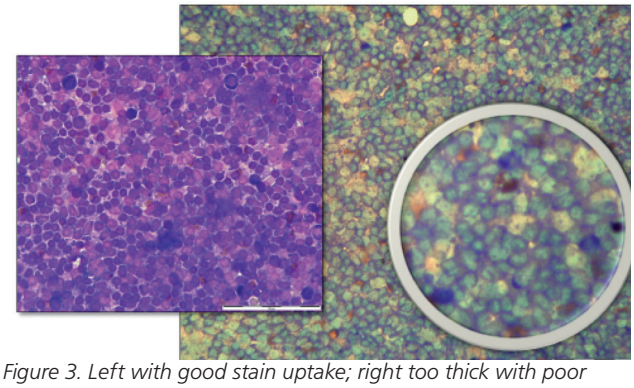

Figure 3. Left with good stain uptake; right too thick with poor stain uptake.

Examine at low power to find the best areas.

- Is the sample homogenous?
- Is the preservation uniform or better in some areas?
- Are the cells well spread?

Skim across the whole smear at low power then go back to the best areas.

Assessment at low power

How are the cells arranged?

- **Discrete:** typically round cell populations e.g. lymphocytes/plasma cells/histiocytes/mast cells
- **Clusters:** epithelial populations, forming cell:cell junctions
- **Aggregates:** typically mesenchymal (connective) tissues; may or may not be associated with matrix.

Assessment at high power

- Is there an inflammatory population?
  - Nature of the inflammation?
  - Infectious agents?
- Do you suspect neoplasia?
  - Is the lineage identifiable?
  - Are there features of malignancy?
- How do the findings correspond with the clinical picture?

Cytological features of malignancy

The following features may be noted in smears from malignant lesions:

- Anisocytosis
- Anisokaryosis
- Coarse chromatin
- Prominent nucleoli
- Atypical nucleoli
- Multinucleation
- Nuclear moulding
- Intracellular anisokaryosis
- Satellite nuclei.

Assessing cytological features of malignancy can be very helpful in establishing a diagnosis of neoplasia and in some cases predicting if a tumour may be more clinically aggressive. However there are numerous examples of tumours that will display overt pleomorphism without necessarily indicating malignant

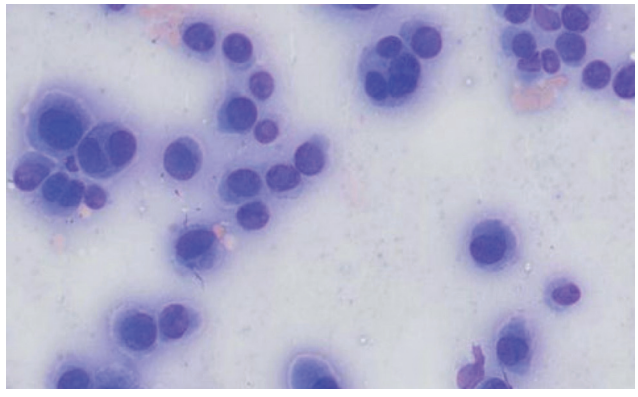

Figure 4. Cutaneous plasmacytoma showing anisocytosis, anisokaryosis and multinucleation.

behaviour, e.g. cutaneous plasmacytoma (*Figure 4*). Keeping in mind the gross features of the mass and your list of differentials can be helpful in such cases.

Inflammation and neoplasia

Cytological interpretation can be challenging when there is significant inflammation associated with a tumour. Differentiating whether cells are hyperplastic and/or dysplastic due to chronic inflammation may not be possible cytologically. Well differentiated squamous cell carcinoma can be one such example (*Figure 5*). Gross features of the lesion, anatomic location etc. may be helpful however in some cases histopathological evaluation of tissue architecture is needed to be definitive.

Mesenchymal proliferation

Cytological specimens from dogs with soft tissue sarcoma are often presented for evaluation (e.g. *Figrure 6*). In some instances, there may be enough to reach a presumptive diagnosis. However, the potential for mesenchymal tissue to be markedly proliferative and to demonstrate overt cytological atypia in response to chronic inflammation/trauma or foreign body reactions etc. must always be borne in mind (*Figure 7*). Essentially, evaluation of tissue architecture is needed to be definitive and histopathological grading is critical in establishing a prognosis, as the clinical behaviour of soft tissue sarcomas can vary widely dependant on grade. Always exhibit caution when there is inflammation accompanying mesenchymal proliferation, even when significant cytological atypia is apparent. CA

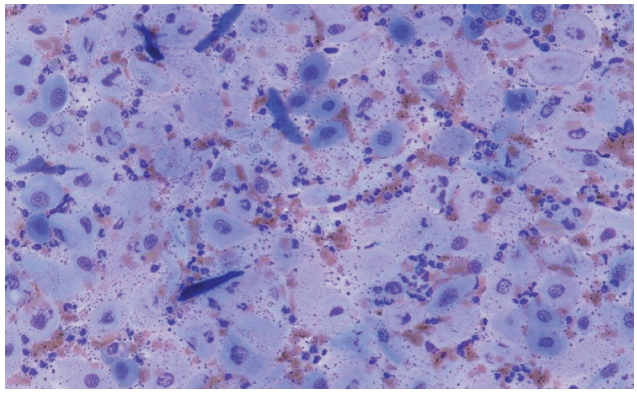

Figure 5. Squamous cell carcinoma with suppurative inflammation.

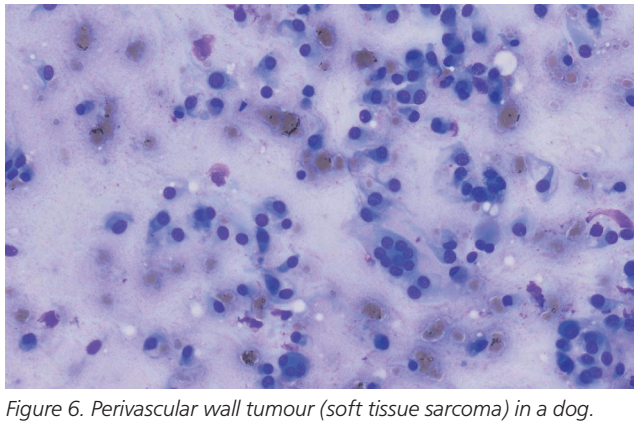

Figure 6. Perivascular wall tumour (soft tissue sarcoma) in a dog.

TAKE-HOME MESSAGES

- Manage your (and your client's) expectations.
- It is common for clinicians to become frustrated when samples are inconclusive or non-diagnostic, but don't dismay! Cytology is a very valuable part of your diagnostic toolkit; don't always expect a definitive diagnosis but hope that careful analysis of a good quality sample will help you make an informed decision for your patient.
- Even a poorly-cellular sample is probably telling you something about that lesion.
- Always relate your cytological findings back to the clinical picture and your initial differentials. Two very different lesions can look cytologically similar, so the interpretation must take into account gross features of the lesion and clinical history, particularly anatomic location, association with surrounding anatomy and results of imaging, where relevant (*Figure 8*).

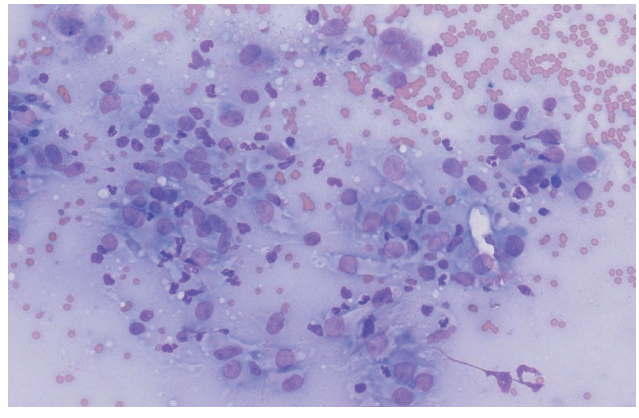

Figure 7. Pleomorphic stromal cells in an inflamed digital swelling.

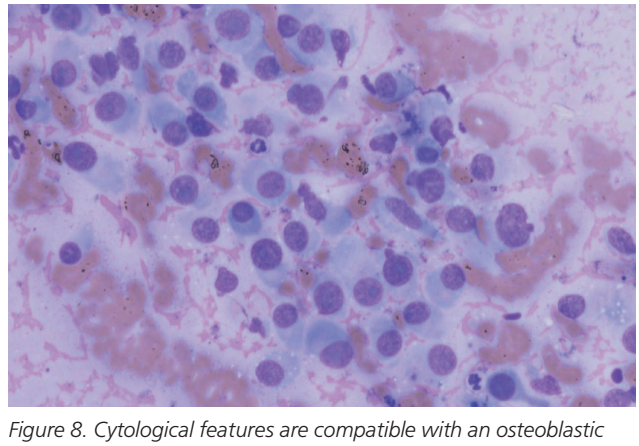

Figure 8. Cytological features are compatible with an osteoblastic osteosarcoma; a thorough gross and radiographic description adds significant weight to this interpretation.

The Art of Streetvet-ing 2019

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