

Skin Cytology

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Skin lesions, from localized masses to generalized skin disease, are one of the most common complaints for which animals will be presented to a veterinary clinic, not least because they are generally highly visible to the owners! Cytological examination is a common and useful diagnostic tool in the work up of skin lesions, and the chances of achieving a meaningful result can depend to some degree on the quality of collection and handling of samples.

Obtaining a good quality, diagnostic cytology sample is something that becomes easier with trial and experience. Looking at your own samples, and feed-back from laboratory cytology reports will help you to find what works, and what doesn't! It is understandably frustrating to go to all the trouble of collecting and submitting a sample to have a "non-diagnostic" result for what may be preventable reasons.

Sampling of mass lesions:

Fine needle aspiration and non-aspiration techniques will be the most common technique used for sampling cutaneous and subcutaneous mass lesions, allowing cells to be collected from within the lesion, hopefully avoiding confounding factors such as superficial ulceration.

Equipment required includes a needle (21 to 25 gauge), a syringe (3 to 20ml) and clean glass microscope slides. As a general rule, the softer the tissue to be sampled (e.g. round cell tumours, lymph nodes), the less vacuum will be required to harvest cells, and so smaller gauge needles and smaller volume syringes can be used. Firm samples (e.g. tumours with a high fibrous content) may require a larger syringe to provide greater negative pressure. A good compromise if you're just not sure would be to use a 22 gauge needle and a 12 ml syringe.

Technique – aspiration:

The needle with syringe attached is inserted into the mass → once the needle is in place apply strong negative pressure for several seconds by withdrawing the plunger to about three quarters of the volume of the syringe → release the negative pressure on the syringe before removing the needle from the mass → remove the needle from the syringe, draw air into the syringe, replace the needle and expel any material onto a clean slide → immediately make the smear

Technique – non-aspirational:

This is similar to the aspiration technique, except that no negative pressure is applied during collection. Pre-fill a syringe with air (to allow rapid transfer of sample to slide) → the needle with syringe attached is inserted into the mass and moved back and forth rapidly in a stabbing motion → withdraw needle and express contents onto a clean slide → immediately make the smear

Trouble-shooting – potential causes for non-diagnostic sample collection:

1. Sample is not representative of the mass
 - Needle missed the lesion during collection e.g. in small lesions – e.g. the aspirate contains only underlying or surrounding fat, or superficial inflammation and infection.
 - ✓ Make and submit multiple smears
 - ✓ Ensure the needle is retained within the mass during aspiration – if the needle is removed while negative pressure is being applied, then the sample may be aspirated

- into the syringe where it is not retrievable, or cells may be harvested from tissue around the mass or from the contaminated surface.
- Needle sampled only a non-representative portion of the mass – e.g. cystic lesions, central necrosis or secondary inflammation
 - ✓ The needle can be directed in several different directions within the mass while negative pressure is maintained
 - ✓ In large masses, several different areas of the mass can be sampled from different sites with separate collection attempts
 - ✓ The centre of a mass is not necessarily the best place to sample – particularly in large or cystic masses, try to get a sample from the periphery also
- 2. Cross contamination
 - ✓ When sampling multiple masses, always use a new needle and a new syringe (and a fresh spreader slide!)
- 3. Poor cellular exfoliation
 - FACT: some lesions – e.g. those with a high fibrous content – simply do not lend themselves to effective cytological evaluation!
 - Poor cellularity may also be due to using not enough negative pressure
 - ✓ Using a larger gauge needle and/or larger syringe to provide greater negative pressure may be of benefit in firm lesions that are not exfoliating well
- 4. Blood contamination
 - Using too large a needle, or prolonged negative pressure
 - ✓ Negative pressure should not be applied for more than a few seconds in any one area
 - ✓ Release negative pressure immediately if any material becomes visible in the hub of the needle
 - Highly vascular lesions
 - ✓ Using the non-aspiration technique may be of benefit
- 5. Cellular rupture
 - Some cellular populations, e.g. lymphoid cells, are fragile and easily damaged either during aspiration or smear preparation
 - ✓ Using the non-aspiration technique may be of benefit

Preparation of smears:

The aim in making a good cytology smear is to create a thin film in which cells are well-spread in a single layer, without distorting or rupturing the cells.

Slide over slide or “squash” preps –

This technique will be the most commonly used for fine needle preparations. Expel the material from aspiration near one end of a slide (the sample slide) → place a second glass slide (spreader slide) on top of and perpendicular to the sample slide, directly over the specimen → the specimen should spread out between the two slides with no need to apply additional pressure; very thick or “clumpy” samples that don’t immediately spread may require a light squeeze of the spreader slide onto the sample slide → lightly draw the spreader slide, without any downward pressure, across the length of the sample slide

Direct smears or the blood smear technique –

Samples with enough tissue fluid or blood can be smeared out exactly as if making a blood smear. Place a small drop of sample near one end of the sample slide → place the short end of the spreader slide onto the sample slide at a 45° angle directly in front of the sample and move it back to contact the drop → the sample should spread out along the base of the spreader slide → immediately spread the smear forward in a rapid, smooth motion

Line or “star-fish” preps –

This technique is gentle on fragile cells but can produce thick layers of cells that can't easily be evaluated. Expel the aspirate material onto the slide → drag the aspirate peripherally with the point of a needle, in a linear fashion or in several directions producing a starfish shape

Trouble-shooting – potential causes for creating non-diagnostic smears:

1. Sample dries or clots on the slide – will prevent good spreading and distort cell morphology
 - A common mistake is to spray the aspirate sample out of the needle onto the slide from too great a distance – this creates a cluster of many small dense drops that dry fast
 - ✓ Placing the drop of material gently onto the slide by holding the needle close to the slide surface is an important first step in making a good smear
 - ✓ Make smear as soon as sample is transferred to slide
2. Smear is too thick – if cells are not adequately spread they may be impossible to evaluate
 - E.g. a large or highly cellular sample, or one contaminated with excessive blood
 - ✓ Only apply a small drop of sample to the slide – e.g. as for a blood smear **if a sample extends all the way to the end of the slide it is likely to be too thick**
 - ✓ If a large sample is expelled onto the sample slide, transfer some to a second slide with the spreader
3. Cells ruptured during smear preparation –
 - Applying too much downward pressure during the “squash” prep, or even abruptly lifting the spreader slide off the sample, which can create a vacuum, may rupture cells

Techniques for evaluation of superficial skin lesions:

Impression smears can be made from ulcerative or exudative superficial skin lesions, and are most beneficial for determining if bacterial or fungal organisms are present. Making impression smears from ulcerated mass lesions most often identify only inflammatory cells – neoplastic cells will often not exfoliate with this technique. To make an impression smear, simply roll or rub a clean slide directly over the skin. Pinching or tenting the area of skin to be sampled may increase cell yield, or roughening the area gently first may get some “ooze” that will aid cells and organisms to adhere to the slide.

Scrapings can be made from mass lesions, but as with impression smears often sample only superficial contamination or inflammation, particularly if the lesion is ulcerated. Skin scrapes are most commonly used in investigation of suspected mange. Using a scalpel blade, scrape in the direction of hair growth sufficiently deep to cause some exudation of serum or blood. Material collected on the blade is transferred to a slide and spread. When scraping to look for mites, using mineral oil can be used on the scalpel blade to allow collected material to adhere to the blade and the slide.

- Where demodectic mange is suspected, organisms are often numerous but are typically located down within hair follicles – deep scrapings are therefore required, to the point of capillary bleeding, and squeezing the area of skin being sampled may help express the mite organisms closer to the surface.
- Sarcoptic mange, on the other hand, is caused by mite organisms that live in the superficial keratin of the skin surface; however severe skin disease can be caused by very few organisms. Multiple scrapings are therefore required, and are often unrewarding – a negative result does not rule out the possibility of Sarcoptic mange!

Swabs for cytology are used most commonly for sampling the external ear canal, vagina, or exudative (e.g. fistulous) inflammatory lesions. Again, these are most useful for identifying the presence of infectious organisms. A sterile cotton swab is used. If the area appears dry the swab can be moistened with sterile saline. Once the sample is collected, the swab is gently rolled across the surface of a slide. **smearing the swab may disrupt any cells collected**

Sellotape preparations may be useful for examination in clinic for superficial “free roaming” parasites such as Cheyletiella. We seldom find these preparations of diagnostic value when submitted through the lab. A piece of Sellotape is pressed onto the surface of the skin, and then placed on a slide.

Staining:

Samples may be stained in clinic for in house examination, or, as cells may not be grossly visible during sample collection and slide preparation, may be performed in clinic to ensure there is something there, before going to the expense of submission to a lab.

Whatever stain that is used in clinic (usually Diff-Quick or some variation) will have instructions according to the manufacturer, which users should become familiar with. However, it is important to remember that these are not absolute: thicker or highly cellular smears may require more time in the stains to achieve adequate contrast.

Trouble-shooting – potential reasons staining techniques may prevent a sample from being diagnostic:

1. Inadequate drying – may distort cell morphology or produce distracting artefacts
 - ✓ Leaving a smear to air-dry also aids adherence to the slide
2. Under-stained smears – this is the most common problem we see in pre-stained smears that are submitted to the lab
 - Thick or highly cellular smears may require longer periods in the stain solutions
 - Stains that are not changed regularly may become diluted over time, meaning that using the same number of dips results in increasingly inadequate staining
 - ✓ If you look at a smear and it seems excessively “pink”, or nuclear detail is not evident although cells can be identified, take it back to the blue stain for a few more dips **try to assess this before you have put immersion oil on the slide as the oil will interfere with re-staining!**
3. Bacterial contamination –
 - Organisms may shed off a slide, e.g. from “chunky” samples, and can contaminate or in some cases even colonize stains and then may appear on subsequent smears confounding results
 - ✓ Regularly change stains – regardless of how often they are used
4. Formalin exposure –
 - Exposing a cytological smear (or a blood smear) to formalin or even formalin fumes interferes with staining, resulting in a smudgy, characteristic blue-green appearance to the smear and disrupting cell morphology to the point that it is often non-diagnostic
 - ✓ Do not send smears in the same package as formalin samples

Submission of smears to the laboratory:

If smears are submitted to a lab without being examined in clinic, it is preferable not to stain them first. If slides are going to be examined in clinic with the plan to also submit to the laboratory, making multiple smears and staining only one or two, so that some unstained can also be submitted is appreciated. This allows the pathologist to stain smears with the type of stain they are used to viewing.

1. Submit air-dried smears in rigid plastic slide mailers to prevent damage in transit
2. Use slides with a frosted end so that they can be easily labelled

- Labelling the slide containers is not always effective, as on a busy day in the lab the information may not be noticed and transferred to the slides
 - ✓ Label each smear with patient name, +/- site of aspiration (particularly if more than one site has been sampled) in pencil *pen ink, including "permanent" marker, will dissolve in the fixative*
- 3. Accompanying history is very important for cytology samples!
 - Because cytology allows excellent examination of cell morphology but not of tissue architecture, where there are abnormal cells present it is not always possible for the pathologist to tell through examination if a sample has come from a skin mass, a lymph node, or an internal organ
 - ✓ Site of sampling should be the bare minimum of information provided
 - ✓ Ideally, as full a description of the lesion as possible can be very helpful to interpretation – e.g. location in skin (dermal versus subcutaneous), size, description (polypoid versus plaque-like), duration, rate of growth, presence of absence of ulceration, response to treatment, even colour...

Always remember you can call the lab to discuss what may or may not be a useful or suitable sample, the best way to handle or send it, and also to discuss the results afterwards!

References (and useful resources):

Meinkoth and Cowell. Sample collection and preparation in cytology: increasing diagnostic yield. *Veterinary Clinics of North America: Small Animal Practice* 32 (2002), 1187-1207

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