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## IN-HOUSE TESTING IN VETERINARY DERMATOLOGY

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A multitude of tests are used in veterinary dermatology, these include both 'in-practice' tests and those performed by a veterinary pathology laboratory. There are basic tests in which every practice interested in dermatology should become proficient and which can be performed and evaluated quickly in house, these are covered in this lecture.

### Cytology

#### A. General Comments

Evaluation of cytological samples is an inexpensive, simple and quick method to assess skin infections and otitis externa. We sample any chronic and pruritic skin condition and all cases with otitis externa (not only on the first presentation, but also on rechecks). Secondary microbial infections of the skin may be bacterial (usually *Staphylococcus* sp. in the dog and cat) or caused by yeast organisms such as *Malassezia* sp. These can be identified as cocci or yeast on cytologic preparations and appropriate therapy initiated. The importance of repeat cytology with recurrent disease is emphasized with the small group of dogs which switch from bacterial to yeast to mixed infections of both - and which may present with indistinguishable clinical signs. Cytology is also useful in monitoring the response to therapy - a decrease in the numbers of microorganisms is a positive sign of response to therapy. Particularly in cases with otitis externa we recheck every 2 weeks and routinely perform cytology to monitor the microbe population in the ear canals. Bacterial cultures should always be interpreted in the light of a cytological evaluation as overgrowth or poor sampling may distort the picture, and with multiple types of organisms growing on culture we need to know which one of them is most numerous in the lesions and thus most important to treat!

#### B. Methods

- Rubbing, or impressing a slide onto a skin surface. This obviously only works well with a moist, exudative or greasy surface.
- Rolling a cotton bud on the skin surface or inserting it in the ears (in patients with dry skin it can be moistened with saline solution)
- A direct impression technique with clear sticky tape collects the debris from the surface of the skin and is particularly useful with dry skin. It is rapid but it does take practice to establish what is 'normal'. The tape is impressed onto the skin (sticky side down) and then laid (also sticky side down) onto a drop of methylene blue or a drop of the blue stain of DiffQuick on a slide. This technique is especially useful for *Malassezia* evaluation.
- Aspiration from nodules or abscesses is done with a 22 g needle. The needle is inserted, and redirected through the same insertion point several times in different parts of the nodule. The needle is then

withdrawn and attached to a syringe with drawn-back plunger. Needle contents are blown onto a slide (hopefully). The smear is air dried.

- Skin scrapings for cytological evaluation are taken using a scalpel blade and gently scraping debris off the surface of the affected skin. This debris is then spread onto a slide in a similar fashion to buttering bread, the slide is heat-fixed or air dried and then stained.

#### *Stain*

- Modified Wright's such as DiffQuick or methylene blue can be used (much faster and easier than Gram stain) to stain the air dried slides.

### C. Evaluation

- Are the organisms yeasts (most likely *Malassezia canis*) or cocci (most likely *Staphylococcus*)? Rods in the ear are most likely *Pseudomonas* or *Proteus*.
- The number of organisms is important. Occasional cocci or yeast are not relevant. We consider one or more *Malassezia* organisms per HPF relevant, cocci should be seen in high numbers, any rods present are abnormal.
- Inflammatory cells with intracellular organisms are pathognomonic for a clinically relevant infection.

## **Skin scrapings**

### A. Superficial skin scrapings

Superficial skin scrapings are taken from large areas, usually to detect *Sarcoptes* or *Cheyletiella*. Elbows, ear margins and ventrum are commonly scraped for *Sarcoptes* mites, the back for *Cheyletiella* mites. Mineral oil or pyrethrin ear drops should be put on the scalpel blade AND the skin. Scrapings are done in the direction of hair growth. 50% of scabies cases may be negative on several scrapings. One mite or egg is diagnostic. It is important to scrape over a large area and in hairy dogs this may be easier if the hair is clipped away first. Should such clipping be necessary it is important not to remove the surface scale or crust which may be present; *Sarcoptes* mites are extremely superficially located within the epidermis and may be dislodged with such cleansing. We use scissors to remove the hair and select non-excoriated sites preferably with scale and papules as the lesions. Mineral oil is then applied to the affected skin, gently scraped off the surface, put on a slide, a cover slip is applied and the sample is evaluated microscopically.

### B. Deep skin scrapings

Deep skin scrapings are performed to detect *Demodex* mites which live in the hair follicle (often very deep). Because they are deep it is useful to squeeze the skin prior to the scraping in an attempt to push the mites out from the depths of the follicles. A survey conducted by summer dermatology students realized a 50% higher mite count when squeezed prior to scraping. A blade covered with mineral oil should be used in the direction of hair growth until capillary bleeding is observed. Feet and faces are hard to scrape, some

breeds such as the Shar Pei may be negative on scrapings and may have to be biopsied for diagnosis. More than 1 mite is diagnostic.

When evaluating Demodex scrapings it is important to assess and to note the site of scraping, the relative numbers of adults, larvae / nymphs and eggs per field. In subsequent visits assessment of response to therapy relies on the comparison of such numbers, we routinely repeat scrape the same sites monthly when monitoring our demodicosis cases.

### **Wood's light examination**

In 50% of all infections with the dermatophyte *Microsporum canis*, greenish fluorescence of tryptophan metabolites is seen. This fluorescence runs along the hair shafts (rather than fluorescing individual occasional scales that will be seen in normal animals and humans as well). Drugs, soaps and bacteria such as *Pseudomonas* may fluoresce as well but are usually not associated with the hair shafts. It may be helpful to warm up the lamp for 5 minutes before use.

### **Fungal culture**

- Hairs and scale from the edge of a lesion (preferably the ones fluorescing under Wood's light) should be taken. If lesions are not well circumscribed or asymptomatic carriers are suspected, the McKenzie tooth brush method is recommended. In this technique the hair is brushed with a mycologically sterile toothbrush (and any tooth brush bought in the supermarket is mycologically sterile) and the tooth brush, scale and loose hairs gently imprinted onto the agar.
- Sabouraud agar is the most common agar for fungal cultures, however, in practice dermatophyte test medium is commonly used. This is a Sabouraud agar with added ingredients to inhibit overgrowth with saprophytes and bacteria as well as a color indicator. These are sold in small jars (agar 'slopes') or Petri dishes which are stored in the refrigerator and have a reasonably long shelf life (months). Once inoculated the culture agar should be incubated at 20 - 25°C, or in a warm, dark corner with the lids not screwed down tight. PH change (and subsequently color change) which occurs as the colony is growing is indicative of dermatophytes. It is imperative that the color change be observed coincidentally with the development of the colony as color changes will also occur in association with mature (large) saprophyte colonies. It is impossible to distinguish on gross appearance whether a mature colony with significant red pigmentation to the underlying and surrounding agar is a pathogenic or saprophytic fungus.
- Always check the colony under the microscope. Clear sticky tape is impressed gently onto the culture (sticky side down) and then laid onto a drop of methylene blue (also sticky side down) on a microscope slide and evaluated under the microscope. The surface of the sticky tape

acts as its own cover slip and if required, microscope oil can be placed directly onto the surface of the tape.

- *Microsporum canis* has a white, woolly colony with a yellowish reverse pigment (which may be difficult to assess if grown on dermatophyte test media). Abundant spindle-shaped macroconidia with knobs at the ends and typically greater than six internal compartments are seen. *Microsporum gypseum* has granular, beige cultures with yellowish reverse pigment and thin-walled macroconidia with less than six internal compartments. *Trichophyton mentagrophytes* grows in variable colonies which characteristically have very few, cigar-shaped macroconidia and small, round microconidia.

## Trichogram

- A forceps is used to forcefully pluck hairs in a partially or completely alopecic area. The hairs are then placed onto a slide and evaluated under low power. I usually use mineral oil and a cover slip to prevent hair blowing all over the table rather than remaining under the microscope.  
A trichogram is taken to evaluate:
- if a cat or dog creates hair loss by licking or rubbing or if the hair falls out due to other reasons. If the animal is pruritic and licks the hair off (or if the hair shafts are damaged due to dermatophytes), the tips of the hairs are broken off. If the hair falls out for other reasons, the tips are tapered.
- if demodicosis is present (then you will find *Demodex* mites hanging on the hairs and you do not need to perform a skin scraping). This is particularly useful when sites close to the eyes are affected or the lesions are very painful. However, only a positive result is diagnostic.
- if dermatophytosis is present, you may see fungal spores efface the clear shape of the hair shaft (as if somebody has smeared caviar onto a chopstick). However, only a positive result is diagnostic, a negative result necessitates fungal culture.

In summary, evaluating hair tips, this is an easy and fast test. It's especially useful to determine if bald cats which present with a history of non-pruritic alopecia are so-called 'closet lickers' or 'hidden groomers' in which case the hair shaft ends will be fractured, or if the hair falls out without any licking, in which case the hair shafts will be tapered. The only exceptions to that rule are cats with dermatophytes or anagen defluxion, which have broken tips as well. The latter should be easily revealed on history, the former is identified by fungal spores surrounding the hair shaft or much more reliably by fungal culture.

## References

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