Decoding Dermatology Diagnostics

Charlie Pye BSc, DVM, DVSc, DACVD
Assistant Professor dermatology, Atlantic Veterinary College

As we all know, many dermatologic diseases look identical and therefore diagnostic tests are required to enable a definitive diagnosis in many cases. Outlined below are some of the more common tests used in veterinary dermatology, technique and interpretation of results.

Cytology

Cutaneous cytology is extremely useful for identifying bacteria, yeast, inflammatory cells, neoplastic cells, and other abnormal cells such as acantholytic keratinocytes. It is also a quick, inexpensive, in-house test that can provide valuable information for treatment planning. There are many methods for performing cytology and different samples may be needed depending on the lesions you are sampling.

Cotton swab: If swabbing ears, you first take a cotton tipped applicator, insert into the ear canal until you reach the junction of the vertical and horizontal canal (there will be resistance here). Rotate the applicator a few times and remove from the ear canal. If sampling skin lesions, simply rub the cotton tipped applicator firmly onto the area of skin to be sampled. I think about it like a child colouring, keep swabbing until you get a colour change on the swab. Take the sample and then roll onto a slide. Multiple samples can be rolled onto the same slide with space between them. Don't forget to label which sample is which! Samples should then be heat fixed prior to staining. My preference is a lighter or barbeque lighter for heat fixing. A few seconds of heat fixing is more than enough until you see the ring of condensation reach the outer edge of the slide. If, after fixing, you have char on the bottom of the slide, this can be rubbed off but does indicate that the length of fixing time was too long. If epidermal collarettes are present on the skin, the applicator can be rubbed under the scaly ring surrounding the collarette for the best sample.

Tape prep: Acetate tape preparations are most useful for dry lesions or if you are looking for *Malassezia*. The tape-strip preparation has been demonstrated to be more sensitive than other cytological methods in the detection of *Malassezia* organisms.[1] Clear tape should be used so you can visualize your sample. Take a small piece of tape, with one end designated as the finger-print end. Stick the tape onto the skin to be sampled and gently remove. Stick back down a few more times to get a good sample. There are two different methods for staining tape. You can dip the tape into the pink (eosinophilic) and purple (basophilic) stain of the Diff-Quik and then rinse with tap water until the water runs clear (ie. excess satin has been removed) and put tape sticky side down onto the slide. I prefer this method as the tape is clearer to read. Another way to stain tape is to take a drop of the purple stain and place this onto the slide. The tape can then be pressed onto the slide over the stain.

Impression smear: These types of samples are great for moist or exudative lesions. Take your slide and gently press onto the skin. You can also use the corner of the slide to gently lift the corner of a crust and then do an impression smear right underneath the crust itself. Remember not to press too firmly onto the lesion or the slide can break in two. If a pustule is being sampled, the top of the pustule can be gently removed using a 25G sterile needle. An impression smear can be taken from the purulent exudate from the pustule.

Cytological samples should be stained with Diff-Quik stain, but NO fixative should be used for the tape preparation. Samples should be evaluated at lowest power first specifically searching for regions with inflammatory cells or atypical cells. Then move to oil immersion in these regions to definitively diagnose any bacterial pyoderma and

identify whether cocci or rod-shaped bacteria are present, as well as yeast. If no inflammatory cells are noted, the slide can be scanned on oil immersion.

Two newer cytological techniques appear to also be effective for the diagnosis of Malassezia and bacterial infections. Lo *et al* describe the toothpick technique for clawfolds.[2] This technique can be used for patients with paronychia where a toothpick is gently inserted into the claw fold, material is scraped from the claw, rolled onto the slide and stained accordingly. In the study they found this technique had a higher yield of *Malassezia* and cocci versus other techniques when samples were obtained from allergic and non-allergic dogs. A second study looked at a "slurry" technique where debris was collected from the skin, macerated in warm water, and then dried on the slide.[1] The slurry preparation did not differ significantly in detecting *Malassezia* but did appear to be more sensitive in detecting bacteria with a mean difference of 12.7 more bacteria per sample when compared with an impression smear.

When staining, dip into fixative (if used) and pink stain about 10 times. Then dip into the purple stain about 20 times. Yeast takes more time to "take up" the purple stain. Then rinse using tap water and a gentle stream. Rinse until the water runs clear and then dry the back using a paper towel or blot the whole slide dry using bibulous paper.

Bacterial Culture and susceptibility

Bacterial culture is indicated if you have a concern for bacterial resistance, the patient has previously had a resistant pyoderma, there are rod-shaped bacteria on cytology that are considered pathologic or the patient has a deep pyoderma and you are planning systemic therapy. Cytology should always be performed prior to culture to document presence of a bacterial infection. A bacterial culture taken from the skin of any dog has the potential to grow bacteria but this does not mean it is the reason for the dermatologic disease. An aerobic culture and susceptibility should be submitted for cutaneous samples. Samples should be taken from where bacteria are noted on cytology or lesions consistent with a bacterial pyoderma. If pustules are noted, the culture sample can be taken from a pustule itself (by removing the top of the pustule as described in the cytology section above). Avoid taking samples from regions where there may be contamination (if you can) such as the peri-anal region, plantar/palmar aspects of feet and around the mouth. However, sometimes cultures do need to be taken from these regions and interpreted with caution due to these contaminants. If a deep pyoderma is present and lesions include draining tracts, a culture swab should be taken from exudative tracts or, alternatively, using a punch biopsy.

Skin scrapings

Superficial skin scrapings are best obtained using a scalpel blade to retrieve skin cells and material from the top layers of the skin. Scrapings should be performed where the skin is not excoriated. Superficial scrapings can be diagnostic for certain mites (*Cheyletiella, Notoedres, Otodectes, Sarcoptes*) whereas deep scrapings are generally used to detect *Demodex* mites.[3] As *Sarcoptes* is challenging to find on even the best scrapings, if results are negative but there is a high clinical suspicion, an ectoparasiticidal trial should be instituted. For patients with demodicosis, the organisms should be readily detected when multiple sites are sampled using this technique.

To perform skin scrapings, I prefer the use of a #10 scalpel blade dipped in mineral oil and some oil placed on the spot to be sampled as well as the slide itself. Scraping should be done in the direction of hair growth whilst holding the blade at a 45-degree angle to the skin. For deep skin scrapings the skin needs to be squeezed between scrapings to try and extrude mites from the hair follicles themselves. Multiple scrapings should be performed to increase the yield. Areas with clinical signs consistent with demodicosis such as alopecia, papules and hyperpigmentation should be selected for the scrapings. For superficial scrapings the area scraped should be larger

and regions of erythema, papules, crusting, alopecia etc should be selected. Once a scraping is complete the material can be placed in the mineral oil on a slide. The slide can then be covered with a cover slip and examined on 4 or 10X power depending on the parasite you are looking for.

Another method for diagnosing demodicosis is using acetate tape. First the skin is squeezed gently, then tape is stuck onto the skin a few times, placed onto a glass slide and viewed under the microscope without staining (like a skin scraping). In one study, demodex canis was detected in 100% of acetate tape impressions and in 90% of skin scrapings. There was also a significant difference in the total number of mites detected.[4]

Wood's lamp

There are three species of dermatophytes that are the most common causes of active infection in companion animals: *Microsporum canis*, *M. gypseum*, and *Trichophyton mentagrophytes*.[5] *M.canis* is the only species that fluoresces under a wood's lamp due to its production of pteridine when metabolizing hair.[6] M.canis will fluoresce in anywhere from 30-100% cases depending on which study you read.[5] One should look for the characteristic apple-green fluoresce at the base of the hair shaft where it exits the skin. False positive fluorescence can occur with scale, certain topical agents and in other situations. A lack of fluorescence does not rule out dermatophytosis as the causative agent of the skin disease as other species will not fluoresce.

Fungal PCR

PCR detection of dermatophyte DNA can be used to aid in the diagnosis of dermatophytosis. However, one must remember that a positive PCR does not necessarily indicate active infection; dead organisms can still be detected on PCR. If clinical signs are compatible with infection and a PCR is positive, this lends more strength to the diagnosis. This PCR should not be used to monitor response to treatment due to the above concerns. A study by Jacobson *et al* looked at cats in shelters with exposure to dermatophytes or skin lesions suspicious of infection.[7] Samples were obtained for both PCR and fungal culture. The sensitivity of the PCR was 100%; correctly identifying all culture positive cats (no false negatives) and the specificity was 88.5% with 12 false positive results. They did also note that the test should not be used for identifying mycological cure as stated above.

Fungal culture

Samples for a fungal culture can be either hair or skin from lesional areas of affected individuals or from unaffected suspected carriers. These samples are then inoculated onto Dermatophyte Test Medium. This media contains antibiotics to prevent bacterial growth and contamination as well as a colour indicator to allow recognition of possible dermatophytes. The colour change in the medium from yellow to red is the result of a pH change triggered by fungal growth.[5]

Sampling techniques can include plucking of hair, tape sampling or hair coat brushing using the Mackenzie toothbrush technique. The Mackenzie toothbrush technique was first described in veterinary medicine in 1965 and was previously used in humans.[8] Sterile soft bristle toothbrushes are used to brush the coat for 2-3 minutes until the bristle are full of hair. The toothbrush is then sent to the lab for inoculation onto test media. This technique is very sensitive. In one study 10.4% of cats were culture positive but only 1.67% of cats had true disease whereas the others were mechanically carrying spores on their coat but did not have active, clinical infection.[9]

Plucking suspect hairs and submitting for culture or using tape to collect hair and skin samples and sending away for culture are the two other techniques reported.

Skin biopsy and histopathology

Skin biopsy can be helpful in diagnosing many immune mediated or neoplastic conditions. They can also provide extra information if treatment is not working or a patient's health status is declining. Below are times I reach for my biopsy punch.

1. If I am suspecting neoplasia
2. If ulcers, vesicles, or aggressive and serious disease are present
3. If a patient is not responding to therapy and I need more information
4. To confirm a disease process prior to starting therapy

Ideally biopsy samples should be taken sooner rather than later. However, if your patient has a disease with a waxing and waning progression and lesions come and go; it is best to wait until lesions are present prior to biopsy. If your patient presents with concurrent skin infection, this should be treated prior to biopsy to prevent misinterpretation of the histopathology results.[10]

If an individual is on glucocorticoid therapy and a biopsy is scheduled, the medication should be discontinued for 3 weeks prior to the procedure. If discontinuing glucocorticoids is not possible then the dose should be decreased, and biopsy samples taken after a few weeks on the decreased dosage.

When selecting where to biopsy on a patient with dermatologic disease, one should aim to select primary lesions such as pustules, papules, nodules etc. If an ulcer is present, care should be taken to avoid sampling the ulcer itself and instead biopsy the edge of the ulcer. If there are multiple lesions or a large region of dermatologic disease, then ideally 3-4 samples should be taken and submitted to maximize your yield.

When selecting the biopsy punch size, if the area to be biopsied is large (e.g. alopecia on the flank of a dog), then the largest biopsy punch should be used. With most dogs this could be a 6mm or 8mm punch. This provides more tissue for assessment by the histopathologist. Samples should be centered over abnormal tissue. If a specific lesion is to be sampled, then the punch should just fit over the lesion itself with minimal space around the edges. The junction of normal and abnormal skin does not need to be biopsied. Punches are cut in half in the direction of hair growth so if normal tissue is included it is possible for the diagnostic area to be in this region and a diagnosis to not be obtained.[10] Once a lesion or region has been identified, the area does not need to be sterilely prepared. A punch biopsy can be used by pressing firmly down onto the skin and rotating the punch. Rotation should occur in one direction only. Once all three layers of the skin have been incised by the biopsy punch, the punch can then be removed, and forceps and scissors can be used to collect the sample. The site should be closed with appropriate non absorbable suture. If, during the procedure, crust overlying the skin falls off the sample, this crust should also be placed into a formalin jar to be assessed. A note should be made on the submission that the crust is separate from the skin sample.

When submitting your samples, it is recommended to provide a brief summary of the history, photos if possible and your differential diagnosis list. All of this information helps histopathologists to correctly interpret their findings and provide the most information to allow a diagnosis to be made.

Trichogram

A trichogram is performed by plucking hairs from an alopecic or hypotrichotic lesion. Numerous hairs should be plucked and then placed in a drop of mineral oil on a glass slide. A cover slip can then be added prior to microscope evaluation. This diagnostic technique is minimally invasive and can be used to identify certain parasites such as

demodex. However, lack of visualization does not rule out these organisms as the causative agent of disease.[11] Skin scrapings are still considered the gold standard for the diagnosis of demodicosis but trichograms can be useful in regions of the body where scraping is difficult. Trichograms are also used to identify broken hair shafts in self-induced alopecia or melanin clumping in follicular dysplasia.[3]

Diet trials

Multiple tests exist to "diagnose" cutaneous adverse food reactions in companion animals but unfortunately these tests are not accurate and do not correlate with clinical findings.[12,13] A novel protein or hydrolyzed restricted diet trial is still the preferred method for diagnosing a cutaneous adverse food reaction in dogs and cats. These trials should be performed for 8 weeks using a veterinary brand diet. One study showed that over the counter diets can be contaminated with protein sources not listed on the ingredients due to cross contamination from other diets during manufacturing.[14] Client compliance during diet trials can be challenging so clients should be advised that this diet trial is a form of diagnostic test.

Intradermal Allergy Testing and Serologic Allergy Testing

Intradermal allergy testing is a specialised procedure available through a board-certified veterinary dermatologist and is still regarded by most as the gold standard for allergy testing. Small volumes of allergens are injected into the skin to document positive wheal and flare reactions to these allergens. Results of this testing can be altered by certain medications and this test does require sedation. Serum allergy testing is a laboratory test where blood is sent away and IgE levels for allergens in the blood are measured. Most of these tests are based on ELISA testing. There are a few different laboratories that offer this testing and there is often inter-laboratory disagreement with positive results.[15]

Results of both tests should always be interpreted considering a patient's clinical signs, history and allergen exposure. Allergy tests should not be used to diagnose environmental allergies but to select allergens for inclusion into immunotherapy.

References

1. Rich N, Brune J, Duclos D. A novel cytological technique for bacterial detection on canine skin. Vet Dermatol. 2022;33(2):108-e30.
2. Lo KL, Rosenkrantz WS. Evaluation of cytology collection techniques and prevalence of Malassezia yeast and bacteria in claw folds of normal and allergic dogs. Vet Dermatol. 2016;27(4):279-e67.
3. Scott DW, Muller WH, Griffin CE. Small animal dermatology. 6th edn. WB Saunders, Philadelphia, 2001;423–516.
4. Pereira AV, Pereira SA, Gremião IDF, *et al*. Comparison of acetate tape impression with squeezing versus skin scraping for the diagnosis of canine demodicosis. Aust Vet J. 2012;90(11):448-450.
5. Moriello KA, Cyner K, Paterson S, *et al*. Diagnosis and treatment of dermatophytosis in dogs and cats. Vet Dermatol 2017;28(3):266-e68.
6. Foresman A, Blank G. The location of the fluorescent matter in Microsporum infected hair. Mycopathol Mycol Appl 1967;31:314-318.
7. Jacobson LS, McIntyre L, Mykusz J. Comparison of real-time PCR with fungal culture for the diagnosis of Microsporum canis dermatophytosis in shelter cats: a field study. J Feline Med Surg. 2018 Feb;20(2):103-107.
8. Mackenzie D. Hairbrush diagnosis in detection and eradication of non-fluorescent scalp ringworm. Br Med J. 1963;2:363-365.

9. Moriello K. Feline dermatophytosis aspects pertinent to disease management in single and multiple cat situation. J Feline Med Surg. 2014;16:419-431.
10. CAVD bulletin, Erin Locke, Optimizing Skin Biopsy Submissions, 2018. Volume 29 Number 2. Page 2-7.
11. Mueller RS, Rosenkrantz W, Bensignor E, *et al*. Diagnosis and treatment of demodicosis in dogs and cats: Clinical consensus guidelines of the World Association for Veterinary Dermatology. Vet Dermatol. 2020;31(1):5-27.
12. Udraite Vovk L, Watson A, Dodds WJ, *et al*. Testing for food-specific antibodies in saliva and blood of food allergic and healthy dogs. Vet J. 2019;245:1-6.
13. Coyner K, Schick A. Hair and Saliva test fails to identify allergies in dogs. J Sm Anim Prac. 201;60(2):121-125.
14. Raditic DM, Remillard RL, Tater KC. ELISA testing for common food antigens in four dry dog foods used in dietary elimination trials. Journal of Animal Physiology and Animal Nutrition. 2011;95:90–97.
15. Hensel P, Santoro D, Favrot C. *et al*. Canine atopic dermatitis: detailed guidelines for diagnosis and allergen identification. BMC Vet Res. 2015;11:196.