Essentials of Dermatological Diagnostic Test

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There are a limited number of tests a veterinary practitioner will be required to perform when presented with a patient with skin disease. For some of these tests, subtle and simple techniques can influence the accuracy of the results.

Cytology

Cytology is an invaluable test for a veterinarian to master. Any primary, and many secondary lesions such as papules, pustules, crust and exudate can provide helpful information. Staining with Diff-Quik® is usually adequate for most in-house cytologies. A number of methodologies are used to obtain samples, including aspiration, tape preps, swabbing, impression smears, and scraping of surface material. Some of the more important lesions to sample and what to consider include:

Pustules

Neutrophils and possibly eosinophils will be present. Are bacteria present? If so, what is the shape (rod vs. coccoid) and are they intracellular, extracellular or both? Are acantholytic cells present which would be suggestive of pemphigus foliaceus?

Nodules

Fine needle aspiration or multiple insertions of a 20-22ga needle into a nodule can yield cells and help distinguish neoplasia from inflammatory disease. If a homogenous population of cells such as lymphocytes, mast cells etc are seen then biopsies to evaluate for neoplasia are indicated. A mixed inflammatory response could direct the veterinarian to also consider infectious or sterile inflammatory conditions. Any bacterial or deep fungal infection could cause nodule formation. Sterile granuloma, sterile histiocytosis, and sterile panniculitis are examples of conditions which could cause nodule formation without microorganisms.

Exudate

Cytology of exudate can be enlightening regarding potential infectious causes. Exudate is often found in ears, interdigital areas, and the ventral neck region.

Crust

Gentle removal of the crust and sampling the material under the crust can be useful in obtaining information regarding the cause of the crust.

When microorganisms are present, it is helpful to quantify the numbers so that response to therapy can be monitored. We use a 1 through 4 plus system as defined

1+ Organisms are rare to scattered at oil power
2+ Many organisms in every field
3+ Organisms difficult to count
4+ Complete coverage of the field with organisms

Hair-pluck (trichogram)

In our practice we will pluck hairs for three reasons. Ectothrix hyphae of dermatophytes may be seen although this requires practice and should not be used alone to diagnose dermatophytosis. Plucking hair and placing in mineral oil can be useful in recovering demodex mites, especially in hard to scrape regions such as interdigital areas. Finally patients with color dilution alopecia will show melanin clumping within the hair shaft resulting in deformation of the medulla and cortex of the hair.

Skin scrapings

Skin scrapings are another commonly performed diagnostic test, and are performed to recover mites. Simple techniques to improve chances of recovery of mites start with shaving the area to be scraped. Otherwise the hair will deflect the blade off the skin, resulting in false negative scrapings. Placement of mineral oil on the skin increases the recovery of mites, otherwise the scraped material is to dry to effectively “pick up” mites. When scraping for demodex, gentle “pinching” of the skin before scraping may force mites in the follicle closer to the surface. Scrape to a depth until capillary bleeding occurs, but avoid vigorous or excessive pressure. When scraping for Sarcoptes there will often be excessive surface crust and debris present on the skin, and subsequently on the slide. Examine this debris carefully. Mites may be more motile after the slide has sat on the microscope for a minute and warmed the contents of the slide. Scraping skin with papules can also be a good area more likely to yield mites. Demodex injai inhabits sebaceous glands in canine skin, so scraping the dorsal trunk where it is most greasy will be necessary to recover this particular strain of Demodex. The numbers of recovered D. injai mites will be lower compared to D. canis populations.

Because of increasing prevalence of methicillin resistant Staphylococcus it is advisable to keep spatulas or dull scalpel blades in a cold disinfectant solution such as benzalkonium chloride with an added anti-rust ingredient

Dermatophyte culture

The most sensitive method of recovering dermatophytes in small animals is to utilize the toothbrush technique. This requires a flat DTM plate be used instead of the small jars which do not have a large enough opening to inoculate the media with a toothbrush. Any suspicious lesions should be brushed vigorously and firmly with a new toothbrush and then the ends of the bristles are gently implanted into the fungal media. In arid environments it helps to then seal the DTM in a plastic bag to reduce drying of the media. Cultures should be looked at daily. The DTM culture, with a pH indicator, will turn red early when a true dermatophyte is present. Color change after several days of fungal growth should be evaluated with caution as saprophytes will cause a color change after carbohydrates in the media are depleted and proteins then metabolized. Dermatophytes will never have dark pigments such as green, brown or black within the colony and if present it should be assumed the growing colony is a saprophyte or contaminant. Microscopic examination of the colony is easily done with clear tape and Lactol Phenol Cotton Blue stain. Plates such as Derm-Duet™ or Sab-Duet™ are available at Hardy Laboratory (www.hardydiagnostics.com)

Bacterial culture

Because of increasing incidence of methicillin resistant and multidrug resistant bacterial infections, it is becoming increasingly necessary to perform bacterial culture and sensitivity testing. When performing cultures of superficial pyoderma, it is sufficient to "lance" an intact pustule with a sterile 25 gauge needle and collect the pustule contents on the end of the swab. For deeper infections it may be necessary to obtain tissue for homogenization. In such cases the site is prepared with surgical scrub, and local anesthesia is used. Due to concerns that lidocaine could inhibit bacterial growth, we attempt to perform more of a ring block. A 4-6mm punch is used to obtain the tissue, usually over the site of an intact (non-draining) nodule, plaque, or changes consistent with "bulging" of the skin. The tissue is removed aseptically, and the epidermis could be trimmed if there is concern of surface bacterial contamination. The tissue is then placed in a sterile red top tube, and 1.0 cc of sterile saline is added to prevent desiccation.

If a Mycobacterial infection is suspected, we submit our samples to National Jewish Health in Denver. Many of the microbiology laboratories in the country send samples to this lab if a mycobacterium bacteria is isolated, as they excel in the identification and sensitivity testing for mycobacterium. Further information is available at www.NJlabs.org

Histopathology

The vast majority of tissue biopsies obtained can be performed with local anesthesia. Lesions to be biopsied are not prepped or scrubbed in any way except clipping of the hair. Care is taken to not disturb any part of the skin or surface lesions such as crust, pustules etc. The biopsy punch should never be less than 6mm except for biopsies of the planum, foot pad, or pinnae in which case a 4mm punch is used. The punch should only be turned one direction (eg clockwise). To remove the sample, the edge of the SQ tissue is *gently* grasped with forceps and the base of the tissue punch is clipped with curved iris scissors. Avoid crushing the tissue with the forceps which leads to artifacts. It is not necessary to place punch biopsies on "splints" as has been advocated for wedge samples. Submission to a Dermatopathologist remains one of the most important decisions of the biopsy process, but unfortunately is rarely done.

If a complete signalment, history, sites biopsied, and list of differentials is sent to the pathologist, their ability to provide more definitive answers will be greatly enhanced. Remember the clinician gets to see the entire patient and the pathologist only gets 6mm of tissue!

Food trials

Food trials remain one of the more challenging test to perform in practice, partly because of resistance on the part of both the owner and the patient when it comes to compliance. A more complete discussion is provided elsewhere, but at the core, a successful food trial will involve feeding a novel or hydrolyzed protein diet to which the patient has not been exposed to for a minimum of six weeks. Analysis of many of the over the counter foods which claim they contain only one protein and one carbohydrate has revealed contamination with beef, soy, and rice making such diets unable to definitively diagnose a food allergy. Serology and skin testing for food allergies remain inaccurate, with both false positive and false negative reactions being present.

Allergy testing

The diagnosis of atopy should be made based on history, clinical presentation and the ruling out of other hypersensitivities such as parasite and food allergy. Once a diagnosis of atopic dermatitis has been made, it may be appropriate to perform allergy testing in order to more clearly define which environmental allergens are contributing to the clinical signs. The only reason to perform serology testing, or to refer a patient for intradermal skin testing, is to follow up with allergen specific immunotherapy (ASIT). Once allergy test results are obtained, these results should always be critically analyzed to insure that the results are consistent with the patients' pruritus history. This determination must include historical information regarding seasonality. If allergy testing reveals positive reactions to only seasonal pollens in a patient which is pruritic year-round, then something is being missed! Choosing the allergens to be included in the extract is something the veterinarian should personally direct based on the specifics of each individual patient. This is where knowledge of the regional allergens is necessary. Further elaboration on this topic is available elsewhere in the proceedings.