**Introduction**

The value of diagnostic procedures commonly used in veterinary dermatology is dependent upon 1) the technique, and 2) the interpretation of the results. The accuracy of a diagnostic test is only as good as the technique used to perform the test. Equally important is the interpretation of the results by the technician or clinician. As Mark Twain (American author) said, “First collect the facts, you can distort them later.”

**The Signalment**

The signalment of the patient (age, breed, sex) should be consciously noted, since certain breeds are predisposed to certain dermatologic conditions. A list of breeds and their associated dermatologic problems may be found on-line or in the textbook, Small Animal Dermatology.

**History**

The clinical history is the single most important diagnostic procedure performed in each case. The history should be obtained in a relaxed environment and should be written down. It is helpful to have a history form available for the client to fill out prior to or while they are waiting for their appointment. The form will help when records are reviewed and when the patient is seen for recheck examinations. The history includes: 1) The past medical history of the patient; 2) the environmental history, which includes a travel history of the animal, a description of the home environment, the percentage of time the patient spends indoors vs outdoors, and the presence of other pets in the household; 3) The dietary history, which should contain all information about diets the dog has received, including snacks, medications (many are flavored), and information about who actually feeds the pet; and 4) History of the present problem, which is the reason “why” the patient is presented to you. This should be taken in chronological order beginning with the owner's initial observations about the disease. It should include previous treatments (both by the owner or other veterinarians), exact dosages, length of each treatment, and the response of the animal to each treatment.

**Physical and Dermatologic Examinations**

Each patient should receive a complete physical examination. Then the skin should be systematically examined. The dermatologic examination should include examination of the entire hair coat, careful palpation of the skin, careful examination of the skin surface over several areas of the body, visual examination (otoscopic) of the external ear canals, and inspection of the foot pads. In many cases it is helpful to clip hair from the patient so that specific lesions may be seen more clearly. It is also helpful to have a form for the medical record, on which lesions may be drawn and recorded, as well as other information.
Skin Scrapings for Parasites
Skin scrapings should be performed on all animals with dermatologic disease. They are primarily used to help diagnose or rule-out the presence of ectoparasites.

Materials: Necessary supplies include a #10 scalpel blade, mineral oil, glass slides, clippers and a microscope. The scalpel blade may be cleaned with alcohol and saved for future use. (Dull scalpel blades cause less skin trauma.)

Procedures: The area to be scraped should be free of hair or clipped to remove excess hair. The skin should be squeezed gently (more of a kneading process) and released. A few drops of mineral oil are placed on a glass slide. It is helpful to scoop some of the oil onto the blade prior to scraping, as the oil helps scale and debris to adhere to the blade. The surface of the skin is then gently scraped while holding the blade perpendicular to the skin surface to avoid excessive trauma. Each scraping should cover an area of approximately 1.5 x 1.5 cm. The material collected is transferred from the blade to the slide and examined under scanning and 10x objectives.

Keys to success:
1. The area to be scraped must be clipped or free of hair. Hair blocks direct contact of the blade with the skin…and the parasites we are seeking are found primarily on the hair, burrowed in the stratum corneum, or in the follicles….not on the hair.
2. Scrapings should be made from appropriate areas such as the edge of the ear pinna, elbows, active (erythematous) areas of dermatitis, etc. A scraping made before clipping the hair may be helpful in detecting some ectoparasites, such as Cheyletiella spp. mites.
3. The skin must be gently squeezed (or rolled, kneaded) prior to scraping. This movement pushes Demodex spp. mites from hair follicles and is the KEY diagnostic step to recover follicular Demodex mites. If the skin is not squeezed, Demodex canis, injai, or cati may not be found.
4. Multiple scrapings will increase your chances of recovering mites, such as Sarcoptes spp., that may be difficult to find.

Comments: Demodex canis mites are usually easily recovered. Sarcoptes mites, D. gatoi, and D. injai may be difficult to find. Other mites are generally found easily. Most veterinary textbooks provide useful photographs and/or charts to aid in identification.

Skin scrapings are often classified by the depth of the scraping. Convention holds that a “deep” scraping (deep enough to induce so-called capillary hemorrhage) is need to recover follicular mites (e.g., D. canis). However, it is the author’s option that this is a misconception. The few studies that have looked at this process have included the squeezing process as part of the “deep” scraping…but not the “superficial” scraping. More recent data clearly show that squeezing is the key step to facilitate recovery of Demodex spp. mites.

Tape Preparations for Parasites (Demodex spp. Mites)
Recently, the use of acetate tape impressions (in conjunction with squeezing the skin) have been shown to be equal to or superior to skin scrapings for recovery of Demodex spp. mites.

Materials: CLEAR acetate tape (e.g., Scotch tape), microscope slides, mineral oil, cover slips, and microscope.

Procedure: The hair should be clipped if possible or an area selected that has minimal hair covering. The tape is placed directly onto the skin and the skin is rolled/kneaded for several
seconds. The tape is then placed (sticky side down) onto a microscope slide on which a drop of mineral oil has been placed. The slide may then be examined as is, or a drop of immersion oil and cover slip may be placed on top prior to viewing. The slide should be viewed with the 4x and 10 objectives.

Keys to Success:
1. Clear tape must be used, not frosted tape!
2. The sticky adhesive part of the tape must be in contact with the skin (not hair) as the skin is rolled (thus expressing mites from follicles).
3. Proper rolling or kneading of the skin is important…not simply pinching the skin. Imagine that you are (which you are) trying to express the mites out of the hair follicles!

Comments: One study (Pereira AV, 2012) has shown this technique to be equal to or superior to the classical “deep” skin scraping…thus supporting the concept that the key to finding follicular mites is to push them out of the follicles.

Impressions Smears/Tape Preparations for Yeast (Malassezia spp.)
Smears for Malassezia are performed whenever yeast are suspected as a primary or secondary cause of pruritus, scaling, erythema, or seborrhea.

Materials: Glass slides, adhesive microscope slides or clear waterproof adhesive (i.e., Scotch) tape, clippers, cotton swab, a microscope slide, microscope, and appropriate stains. Adhesive microscope slides (Duro-Tak) are available from Delasco, Dermatologic Lab & Supply, 608 13th Avenue, Council Bluffs, IA 51501, 1-800-831-6273 or 1-702-323-3269 (www.delasco.com).

Procedure:
1) General suggestion: clip the hair or collect samples from hairless areas. The presence of hair prevents the slide or tape from making strong contact with the skin. This is a significant source of error!
2) The use of adhesive microscope slides is preferred by the author, since they facilitate collection of adequate cells for examination. The adhesive slide is pressed firmly onto the affected area several times in each location. Do not heat fix. Stain with Diff Quik stain, without fixing in the alcohol fixative: examine at 100 and 1000 (oil immersion) magnification.
3) Cotton-tipped applicator swabs are useful for skin fold areas. Briskly swab the skin in the affected areas. Roll (don’t rub) the sample onto a slide, pressing firmly.
4) Regular glass microscope slides may be pressed directly firmly against the skin to get impression smears of Malassezia from suspicious areas (in a manner similar to the adhesive slide collection described previously). The slide should be imprinted several times in the same area to ensure adequate recovery of epithelial cells and debris. Examination gloves should be worn to prevent your fingerprints (oils and epithelial cells) from causing confusion when you examine the slide!
4) Cells and surface debris may also be collected by pressing clear acetate (e.g., Scotch) tape against the skin forcibly. The tape is placed sticky side down on the slide (only sticking one end to the slide), then stained (like adhesive slides). The water resistant tape will adhere well to the slide, although in some cases it may be helpful to place a drop of immersion oil on top of the tape, then add a cover slip to hold the tape in place.

Keys to success:
1. If regular glass slides are used, the sample must be forcefully smeared on the slide for good adherence. The greater the amount of the sample that adheres to the slide, the better your chances are of having a significant and accurate test.
2. The cotton swab technique or use of clear acetate tape may allow better access to small
skin folds, such as in the webbing of small feet or in perivulvar and perianal areas.

3. Heat fixation is not synonymous with "cooking"! Do not overheat the slide. Heat fixing is
usually not required to make a diagnosis, but it does increase the yield on the microscope
slide. Do NOT heat fix (or alcohol fix) the adhesive microscope slides.

4. Organisms are clearer under examination using high dry (40X) objectives (400X total
magnification) when a drop of immersion oil is placed on the slide and then a cover slip
applied. The author prefers to view with the 10x objective as a scanning view, followed
by the 100x objective (1000X overall magnification) to identify Malassezia organisms.

5. A great tip to find yeast organisms is to look under low power (10x objective) for clumps
of keratinocytes that stain a rich pink-fuschia color, then examine those cells under oil-
immersion. For some reason, we often find large numbers of yeast adhering to those
cells, even when there are few yeast elsewhere on the slide!

Comments:
1. *Malassezia pachydermatis* is part of the normal flora of canine skin and ears but can overgrow
when exposed to excess moisture, wax, and inflammation. The presence of any yeast in
conjunction with clinical signs of Malassezia dermatitis (or otitis) is significant.

2. The adhesive microscope slides are far superior to a tape method for recovering yeast from the
skin. The tape preparations tend to have distortion when viewed through the microscope. The
adhesive slide preparations are clear. Tape may be superior when sampling the dorsal interdigital
spaces on toy-breed dogs, where it is tough to get the slide into that space to achieve firm
pressure on the skin.

**Trichograms (hair "plucks")**
The trichogram is useful to evaluate the integrity of hair shafts and as a test for demodicosis and
dermatophytosis.

**Materials:** Forceps, mineral oil, glass slides, cover slips, and a microscope.

**Procedure:** Hair samples are collected from patients by using mosquito forceps or other forces
to firmly grasp several hairs and firmly pull them (i.e., "pluck") from the follicle. The hair
samples should be placed in a drop of mineral oil on a microscope slide and then covered with a
cover slip. The slide is examined immediately. Hairs are examined for 1) type: primary vs
secondary, 2) structural damage to hairs supporting the presence of pruritus (e.g., fractured hairs
from chewing) or other hair shaft abnormalities (various diseases), 3) evidence of
dermatophytosis (e.g., hyphae or spores), 4) ectoparasites, primarily *Demodex* species (also
*Cheyletiella* spp. eggs, nits, or fur mites), 5) the stage of hair growth (anagen vs telogen), and 6)
pigmentary abnormalities (e.g., clumping of melanin associated with some follicular disorders
and color dilution alopecia).

**Keys:**
1. It is probably best to collect samples from more than one body area.
2. A firm grasp of the hairs to plucking will ensure removal of all hairs in the sample area,
not just the hairs in telogen (that are easily removed, even with fingers).
3. All hairs should be orientated the same direction (faster, easier exam) on the slide.

**Comments:** Abnormalities may often be subtle and a trichogram may vary from animal to
animal and at different seasons. However, gross abnormalities or the presence of parasites or
infectious agents may provide significant information to the general practitioner. It is an
excellent method to find *Demodex spp.* mites, especially in cats.
**Fungal Culture**

Fungal cultures should be considered for all dermatology cases; especially if there are circular, crusty or scaly lesions, or if broken hairs are present. There are crucial in geographic areas where dermatophytosis is common.

**Materials:** Forceps, culture media, glass slides, stains, cover slips and a microscope.

**Procedure:** Hair samples, crusts from lesions taken by skin scraping or nails should be collected and gently applied to the surface of the selected culture medium. Broken hairs are preferred for culture, if present. Generally, 8-20 hairs are cultured, spread out over the medium. The medium and samples should then be incubated at 30°C and 30% humidity. Samples should be observed daily for growth for at least 10 days and then periodically for a total of four weeks. Samples may be incubated at room temperature if the humidity is controlled.

After the colony grows on the medium, hyphae should be teased apart and placed on a glass slide with a stain such as lactophenol cotton blue, and a cover slip added. The sample should be examined microscopically and the fungus identified. Alternatively, a piece of clear acetate tape can be touched to the surface of the Sabouraud's Dextrose Agar colony and placed on the slide as a coverslip over the drop of stain. This can then be examined under 10X and 40X.

**Keys:**
1. It is essential to examine cultures daily if Dermatophyte Test Medium (DTM) is used. This medium contains phenol red, a color indicator that turns red because alkaline metabolites are produced by pathogenic fungi. Saprophytic fungi use the carbohydrate in the medium, producing acid metabolites and no color change. However, after the carbohydrate is exhausted, saprophytes will utilize protein and a red color change will be seen. Also, some saprophytes do cause a red color change during normal growth. Therefore, all fungi should be identified by microscopic examination!
2. Plates grown in your hospital should be kept in an incubator or in a closed drawer with some additional source of humidity added (e.g., sponge wetted daily) to prevent drying of the agar.

**Comments:**
1. It may not be in the best interest of most veterinary clinics to perform these tests in-house. The zoonotic nature of this disease probably warrants use of a professional laboratory whenever possible.
2. Many different culture media are available. Sabouraud's dextrose agar is the standard in mycology. Other media containing bacterial and fungal inhibitors and/or pH indicators are available. Dermatophyte Test Medium and Mycosel agar are two such examples. Potato dextrose agar promotes rapid sporulation making identification possible at an earlier time than when using standard culture media.
3. An excellent source of culture media is Hardy Diagnostics (800-266-2222). Derm-Duet™ II (DTM and a rapid sporulating medium) is an excellent product.
4. It is helpful to have an identification manual for fungi handy when examining the culture.

**Other Diagnostic Tests**

There are, of course, many other diagnostic tests than can help with dermatology cases. Cytology of pustules, papules, and nodules is a KEY diagnostic tool in dermatology. I refer the reader to other notes/reading materials and continuing education presentations for more details and training.
Bloodwork (CBC / biochemistry profile) and a urinalysis are indicated when the patient is elderly or when skin conditions are accompanied by non-specific signs of general illness, such as depression, lethargy, anorexia, or whenever system-specific signs are present. These tests are recommended prior to initiation of immunosuppressive therapy. Fecal flotation can also be a valuable test in dermatology, since many parasites (including Demodex and Sarcoptes mites) are often ingested as a consequence of licking or chewing. These mites will often transverse the gastrointestinal tract and will float using standard fecal techniques. The sensitivity is low, but the specificity is high!

Summary
The key, the real key, to dermatology cases is to be organized and to stay with a routine that prevents missing simple problems. Dermatology cases are complex and they are dynamic: they WILL change from presentation to presentation. The dermatology data base is a collection of tests that are necessary in the workup of patients with skin disease. These tests should be done on every case...at the first visit and at every recheck...because skin diseases are dynamic and frequently change.

Selected readings and references.