Dermatology Procedures: The What, How, When, and Why

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Introduction
The nice part about dermatology is the body organ of interest is on the surface; no need for special imaging to examine it beyond good light and good magnification. The down side, as mentioned in other talks, is many skin conditions of differing causes look alike on the outside, making special diagnostic tests necessary. This is a discussion of common dermatologic diagnostic procedures, when, why and how they are done as well as the appearance of some findings.

Skin Scrapes – Deep: Deep skin scrapes are done (usually) to look for demodex mites, the follicular mite. Demodex mites cause some variation of hair loss, red skin, infection (bumps, papules, pustules) and maybe itch. The mites, when present and causing dermatitis, are usually easy to find with properly done scrapes. We are seeing fewer cases of demodex due to the use of isoxazoline parasiticides (Nexgard, Bravecto, Simparica), which often kill demodex mites. Note: these products are not labeled for the treatment of demodicosis but we have multiple case reports of their efficacy.

Supplies needed: mineral oil, microscope slide, microscope, cooperative patient.

How: Put a healthy drop of mineral oil on a microscope slide. In area in question on the dog or cat, the skin is gently but firmly squeezed for 2-3 seconds, 1 to 2 times. Then let the skin return to its normal position. Apply a small amount of mineral oil on the squeezed area. Use a dull, clean scalpel bladed to scrape in short strokes across the surface of the skin with slight pressure until pin-point bleeding is seen. Keep the edge of the blade *perpendicular* to the skin to avoid cutting it. The material accumulating on the blade is wiped onto the awaiting microscope slide with mineral oil. Apply a coverslip. Examine the collection on 10X magnification with the condenser at its lowest position.

Skin scrapes – Superficial: Superficial scrapes are done to look for itch-causing, surface burrowing mites such as Scabies and Cheyletiella. These mites are often very hard to find; just a few mites can cause intense itch. The burrowing mites are also killed by the isoxazoline parasite control products.

Supplies needed: same as for deep scraping.

How: Where deep skin scrapes focus on a small area deeply, superficial scrapes examine a broad area of skin just on the surface. Apply a healthy amount of mineral oil to the area to be examined (usually the belly, outer ear flap / pinna, lateral elbows) and using a dulled scalpel blade helped perpendicular to the skin, collect skin cells with broad sweeping strokes. Apply the collection to a microscope slide that has a dollop of oil on it already. Examine the material at 10X magnification with the condenser at its lowest position.

Trichogram: This is microscopic evaluation of hair shafts. It is done by gently (usually with fingers) plucking hairs from area of skin in question, place them in mineral or immersion oil, cover with a coverslip on a microscope slide, and examined at 4 and 10x magnification, sometimes 40X. We may be assessing what stages of growth the hairs are in, looking for parasites (demodex around the "follicle" end), or looking for fungal spores of ringworm. This is a nice alternative to trying to scrape the face of a very wiggly puppy and a better way to find demodex in thickened skin (such as feet).

Wood's Lamp Exam: This is a type of black light emitting ultraviolet light used to look for fungal infection (dermatophytosis or ringworm). This test for fungal infection is easy but not very sensitive. Should be performed in a darkened room; move patient to a darker room if necessary. Allow the lamp to warm up for 30-60 seconds

and allow your eyes to adjust to the darkness. Shine the light over areas of skin in question and look for apple-green fluorescence of the HAIR shafts (NOT scale). When this is seen, we are virtually 100% confident this is a fungal infection; if it is negative, we have not ruled out a fungal infection.

Fungal Culture: Fungal culture is the best test for fungal infection (aka ringworm or dermatophytosis). If hairs glow on Wood's lamp exam, these are the best to submit. *Gently wipe area in question with rubbing alcohol* to remove environmental molds. Use (ideally) autoclaved hemostats and pull the hairs in question, and either place on your DTM plate or place in sterile container such as a blood tube and submit to laboratory.

If no hairs fluoresce under the lamp but the pet has suspected lesions, the best way to sample is using a new *toothbrush*. Again wipe the affected areas with alcohol. Brush the affected areas for 15 secs / several brushings. If your clinic does fungal culturing in-house, remove the hairs from the toothbrush using a sterile hemostat and place on the media and also touch the brush gently to the surface of the media. Otherwise, send the whole toothbrush to the micro lab.

If the cat (or dog) has no lesions at all, use a toothbrush and comb the whole body, especially focusing on areas where ringworm likes to be, namely the face, ears, head and feet. Process hairs as described above.

In-house fungal cultures should be evaluated daily for color change and growth; a positive results is when color change happens first or at the same time as growth of hyphae. Environmental contaminants will eventually turn change the color of the media, but not until they've been growing for several days.

Ear Cytology: This should be done on all cases of otitis to identify any parasite (Otodectes), bacterial or yeast infection so we can tailor the treatment. Before collecting ear swabs, you need to know if the doctor is looking for mites, for yeast / bacteria, or both. How the swabs are processed differs depending what you are looking for. In either case when doing swabs from canals, I prefer to use "Q-tips," not the long wooden sticks with a minimalist's amount of cotton on then end. They hurt.

For Ear Mites: Best collection done by "wetting" Q-Tip with mineral oil first before swabbing the canal for debris. Insert Q-tip into vertical ear canal, gently rub the swab on the canal walls. Roll the collected material in a healthy drop of mineral oil on a microscope slide. Examine using low magnification (4x) and condenser at lowest point – mites are easy to find; large and active.

Ear Cytology for Yeast and Bacteria: Insert Q-tip into vertical ear canal to where the canal bends, gently rub the swab on the canal walls. Gently ROLL (not smearing) the collected material on to a dry microscope slide. I use one slide and make and "R" with the swab from the right ear, "L" with the left so I only have to stain one slide.

Fixing and staining: If the swab is very waxy, it is good to GENTLY heat fix. NO ROASTING NECESSARY! Just gently heat the underside of the slide with a flame from a lighter. If the debris is purulent, do NOT heat fix. If in doubt, don't heat fix. Stain with Diff Quik as you would normally (> 30 sec in each jar). Allow to dry and examine first under low magnification to find a representative area, then progress to oil immersion to identify yeast / bacteria / inflammatory cells.

What if the doc wants to look for mites and bacteria / yeast? Easy peasy: take swab using dry Q-Tips; make your cytology slide for staining first. Then roll the swabs in a healthy amount of mineral oil on another slide.

Skin Cytology: Cytology of the skin is a non-invasive way to give clinicians useful information about their patients. We can detect various bacterial infections, yeast infection / overgrowth, and sometimes immune mediated disorders. How we do a skin cytology depends on the type skin lesions present.

Surface Skin Cytology: Tape Cytology: This is the most common skin cytology done in derm practices, usually looking for yeast or bacterial infection on the surface of red, scaly, or thickened skin. It is a great way to get interdigital cytology. Most often used on allergy patients to determine their secondary infections. Tape cytology is not good for very moist areas such as wet lip folds or oozy, ulcerated skin.

Supplies needed: roll of clear acetate tape (not invisible but clear, such as Scotch Tape), ½ to ¾" wide, microscope slide, and the second 2 stains of the Diff Quik set.

How: Tear off a piece of tape that is not longer than your microscope slide (2" is plenty). Handle the tape by the end, trying to avoid a big thumb print and human skin cells in the middle of the tape. Gently press the adhesive side of the tape to the area(s) of skin in question and remove. After I've taken my sample, I attached on end of the tape to an end of a microscope slide, just to use the slide as a way of holding the tape for transport to the lab.

Staining: There are 2 ways to do this. One is the "quick and easy but not as pretty" when you are rushed; the other "a more thorough look at the borough." Regardless of technique: Do NOT put the tape in the fixative jar of Diff Quik (the first, clear). The specimen is already "fixed" to the adhesive of the tape.

1) Quick and easy: Put 1-2 drops of the blue stain (3rd Diff Quik jar) on the microscope slide, then place the slide adhesive-side down on the slide. Gently blot with a paper towel to remove excess stain. Ready to read.
2) More thorough: You are going to dip the tape in the orange and blue Diff Quik and rinse with tap water. Again, do NOT put in the fixative. To spare you from stained fingers, use a clothespin to grasp the tape as you dip it in the individual stains. You can also use the clothespin to hold the tape on the end of the microscope slide as you stain it. After the blue stain, rinse the tape gently with tap water, then put it adhesive-side down on the slide, blot with paper towel to remove excess liquid, and it is ready to read.

Reading tape cytologies: Not easy! These are "busy" slides with many skin cells and debris from the skin.

Sometimes you find pollens and mold spores. Yeast and bacteria are best seen under oil immersion objective. It takes much practice to be comfortable with reading tape cytologies.

Cytology of Crusting Dermatitis
Pustules and crusts are better assessed with a more gentle direct impression rather than the "extraction" of tape cytology. Direct impression means to touch a clean microscope slide right on to the area in question. Gentle pressure is key; excessive pressure damages inflammatory cells.

For crusts: Using a hypodermic needle sideways and parallel to the skin, you can gently lift / push the crust from the skin. Gently press a slide to the exposed, now ulcerated skin. Allow material to dry and stain routinely with Diff Quik. We may be looking for bacteria (intra or extracellular), neoplastic (cancer) cells, or cells indicating an auto-immune disease (such as acanthocytes of pemphigus).

Pustule Cytology: Pustules in dogs and cats are very short-lived and it is fortunate when any are present to help with diagnostics. Using a 25g needle or smaller, very gently "lift the roof" off the pustule: Hold the needle parallel to the skin and carefully disrupt the pustule, exposing a small amount of purulent material. VERY gently touch the slide to the purulent material. (If you press firmly, inflammatory cells will rupture, altering morphology.) Sometimes the material sticks to the needle. If so, gently touch the needle to the slide. Allow to dry and stain routinely.

Skin Bacterial Culture: Multi-drug resistant staphylococcus is becoming more common; culture to guide antibiotic choice is necessary. Appropriate sampling is important to capture the suspected pathogen and avoid as many contaminants as possible. Like cytology, how a culture is taken depends on the lesion.

Culturing *from epidermal collarettes*: An epidermal collarette is the skin lesion on a dog we want to call "ringworm" but is not, it's usually staph infection. The bacteria in these lesions is found under the scale at the periphery of the lesion. The best way to culture these lesions is use a 25 or 27g needle and gently lift some scale at the edge of the lesion, then touch the culture swab to the newly exposed skin under the scale. Sample from 2-5 areas.

Culturing *from a pustule*: Like we do for cytology, gently rupture the "roof" of the pustule, then touch a culture swab to the now exposed purulent matter; can also touch the tip of the needle used to the culture swab.

Culturing *from a raised draining lesion (a furuncle):* We usually look for an area of skin not yet ruptured and draining. An area of skin that has been open to the environment for a while will have non-pathogenic, contaminating bacteria in it, and you may miss identifying the pathogenic staph.

Find a raised but intact area of skin that looks like a little abscess about to rupture; often the skin has a red to purple appearance and a soft center. Wipe the surface gently with rubbing alcohol. Carefully poke the center (where the skin wants to rupture) with a 25 or 27 g needle. If material does not ready exit the skin, apply gentle pressure on the side of the swelling. Carefully touch a culture swab to the material that comes to the surface.

Culturing *thickened skin with no exudate* or an ulcerated skin (such as lick granulomas): in this situation we often have to take a biopsy and submit the tissue for culture. Ulcerated skin will be covered with contaminants and oral bacteria.

Skin Biopsy: When the cause of a pet's dermatitis is not apparent from exam, history and other diagnostic tests, skin biopsy for histopathology is often recommended. Most skin biopsies are done with a "punch biopsy," a round, 4 to 8 mm in diameter surgical blade that takes a small core specimen. Depending on the area to be biopsied, this can be done with just local anesthesia (lidocaine or carbocaine, often diluted with equal parts of saline to decrease the sting) +/- sedation. Biopsies from tender areas such as the nose, feet, or face require heavy sedation or anesthesia.

The skin to be biopsied should have *minimal preparation*. Scrubbing the area may wash off needed information! Pathologists can get valuable information from even crusts. Best to just carefully trim hair out of the way but do not dislodge any crusts. Outline the area to be biopsied with a marker, and the local anesthetic (usually ¾ to 1 ml) is injected under this marking. If the affected area has no hair, it helps the pathologist to draw a line on the top of skin with a marker in the direction of hair growth or surrounding hairs. The small biopsy specimen should be handled gently and put into formalin promptly.

The most important part of a biopsy is picking the correct area; the second most important is giving the pathologist a history of the patient's skin problems and list of symptoms. If your doctor does not fill out the submission form, put it in front of them with a pen, or gather as much information as you can and fill out the history section. Most important are what part of body sample came from, duration of the dermatitis, current medications, response to previous meds, and degree of itch.

Intradermal (Skin) Allergy Testing: This test is done (on dogs, cats, horses) to help identify a pet's allergens (proteins that trigger their allergies) so that we may formulate allergen immunotherapy. It is uncommon for primary care practitioners to do this test and you are unlikely to be called on to do this. However it is mentioned and photos shown so you have an idea what this is when talking to clients about testing.