Cytology Guide for Dermatology Cases

BY JULIA E. MILLER

Performing and interpreting cytology is fundamental in the evaluation of dermatologic cases. Without cytology, patients may be treated based on "first best guess," which may lead to misdiagnosis, and expensive ineffective treatments, and erosion of client's confidence in the veterinarian's care. The dermatologic minimum database consists of impression cytology, skin scraping, and ear cytology (if there is ear disease). Skin and ear cytology should be second nature to the practitioner and included as part of every dermatologic work up. This guide will cover the ABCs of cytology collection and interpretation for dermatology cases.

Materials needed

Diff-Quik

This standard, in-house stain is all that is required for dermatologic cases. It is important to keep the stains covered and change them regularly as debris may float in the liquid and attach to the slide creating false positive findings. Heat fixing slides with fire before staining is not required or recommended for dermatologic samples (including ear cytology) and slides are easily interpreted without this messy step.

Compound microscope

The microscope should be equipped with 5x, 10x, 40x, and 100x (oil immersion) objectives to be thorough when interpreting cytology. Be familiar with the working parts of the microscope including the iris diaphragm, as this should be closed to effectively evaluate skin scrapings. Protect the microscope by covering it with a soft fabric or pillowcase when not in use and schedule regular professional cleanings to keep the objectives in working order.

Sampling tools

Frosted glass slides offer sample security and coverslips are recommended for skin scrapings. Additional items to keep in clinic include clear acetate tape (single or double-sided), cotton-tipped applicators, toothpicks, toothbrushes, metal spatulas, scalpel blades, and mineral oil.

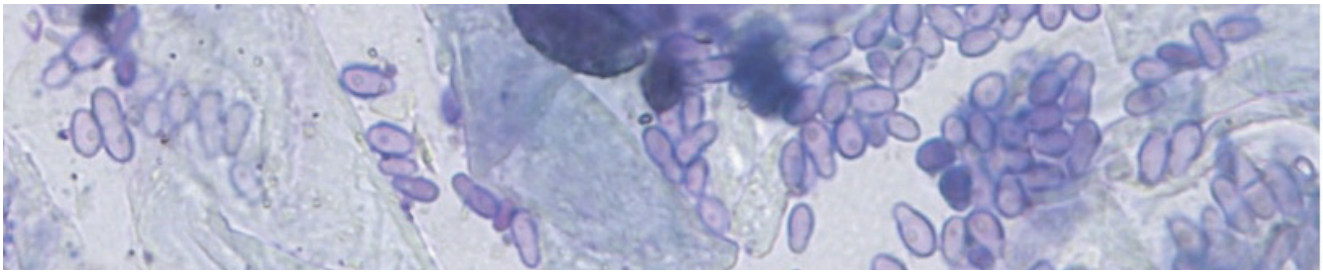
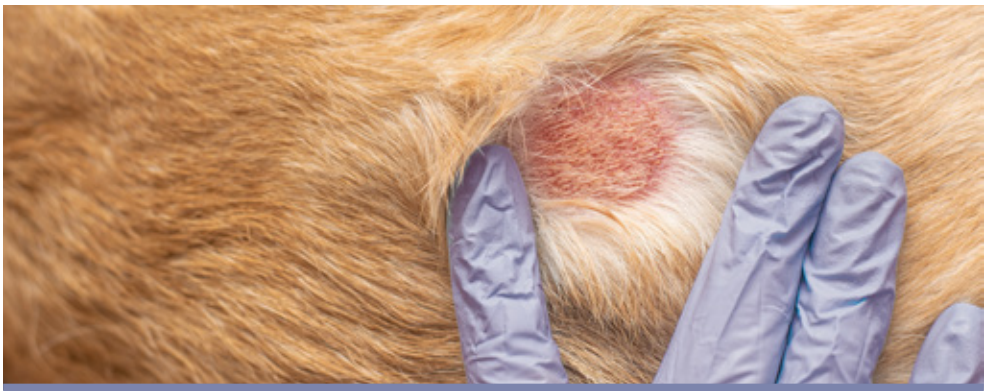


Photo courtesy of Ashley Bourgeois, DVM, DACVD

How to collect cytology samples

Techniques

There are numerous ways to collect cytology, and the method chosen should be based on the type of lesion present and the disease process suspected.



Clinical Differential		Collection method
Ectoparasites	*Sarcoptes scabiei*	Superficial skin scraping
	Notoedres cati	Superficial skin scraping
	Demodex canis, felis, injai	Deep skin scraping Trichogram Direct impression Acetate tape impression
	Demodex gatoi	Superficial skin scraping Acetate tape impression Fecal floatation
	Cheyletiella	Acetate tape impression Flea combing Trichogram Superficial skin scraping
Infectious – bacterial		Impression cytology Acetate tape impression Bacterial C&S
Fungal	*Malassezia*	Impression cytology Acetate tape impression
	Dermatophyte	Trichogram Dermatophyte culture +/- PCR Impression cytology Acetate tape impression
	Other	Impression cytology Fungal C&S
Autoimmune		Impression cytology
Disorders of the hair shaft		Trichogram

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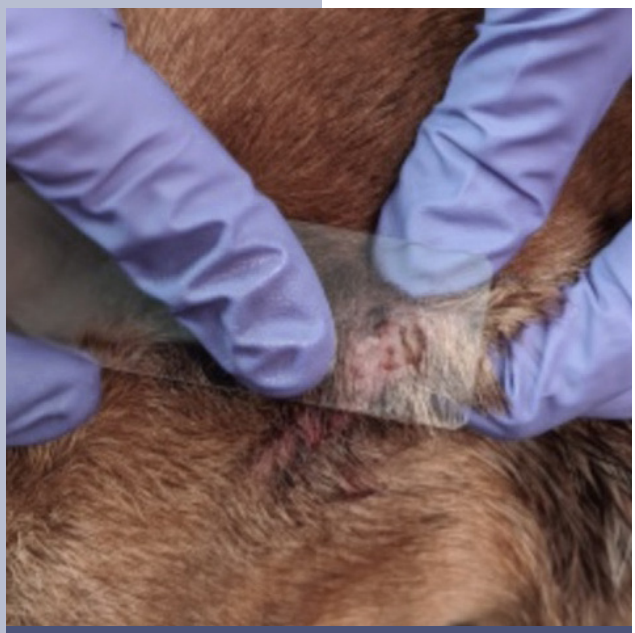

Image 1: Direct impression sampling technique.

Image 2: Toothpick impression sampling technique.

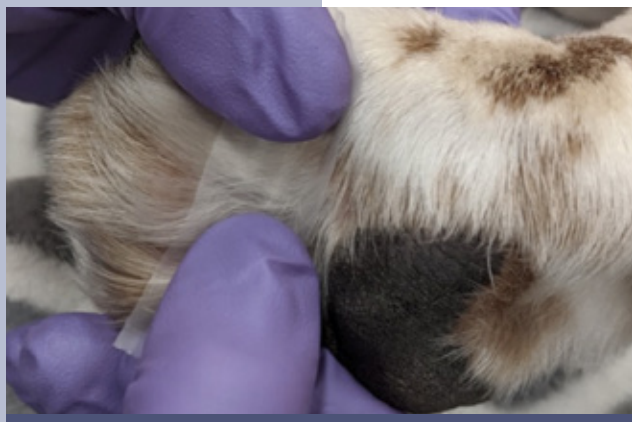

Image 3: Acetate tape sampling technique.

Direct impression smear

Often the most useful dermatologic diagnostic, this is effective for exudative lesions (pustules, exudate beneath crusts, erosions/ulcers) as well as some dry lesions. If there is a crust present, use the side of the microscope slide to remove the crust and sample the exudate beneath. Gently pinch the skin and press or smear the slide onto the skin (**Image 1**). Pressing the slide as opposed to smearing may provide better cellular morphology with less cell damage.

Impression smear

In areas where it is difficult to press a glass slide (facial folds, interdigital spaces, claw folds, ear canals) collect samples with a cotton-tipped applicator or toothpick (**Image 2**). The sample should be rolled or smeared onto the slide.

Acetate tape impression

Lesions that are dry (lichenified skin, epidermal collarettes) or in difficult locations to press a glass slide (interdigital spaces, claw folds, facial folds) may benefit from sampling with an acetate tape impression (**Image 3**). Acetate tape will provide high cellularity and may decrease false negative results, particularly when *Malassezia* dermatitis is suspected. When staining these samples skip the light blue fixative step.

Skin scrapings

Superficial

Volume is extremely important as there may be very few of the suspected ectoparasite (particularly *S. scabiei*). Using a scalpel blade (sharp or dull side) or a metal spatula, either dip the instrument in mineral oil or apply oil directly to the skin of the animal. At a 30-90-degree angle, perform a wide-sweeping, superficial scraping. No capillary bleeding is required. Collect all material by scraping the instrument on the sides of the microscope slide vigorously. Scraping multiple sites is advised and, when possible, scrape papules or light-colored crusts. Using a coverslip creates a uniform surface and ensures complete evaluation of all collected material.

Photos courtesy of Ashley Bourgeois, DVM, DACVD

Deep

To thoroughly evaluate for *Demodex canis/injai/felis* mites, it is important to squeeze the affected skin between the thumb and forefinger to extrude the mites from the hair follicle. Apply mineral oil to the skin or the scraping instrument, hold the scraping instrument at a 30-90-degree angle and scrape the skin in short, vigorous strokes until capillary bleeding is produced. Collect all material by scraping the instrument on the sides of the microscope slide vigorously. Using a coverslip creates a uniform surface and ensures complete evaluation of all collected material.

Trichography

Gentle collection of hairs is important to preserve their architecture and it is recommended to pluck hairs with fingers or rubber-covered hemostats. Mineral oil applied to the slide before collection ensures the hairs will not be lost.

How to interpret cytology results

Practice makes perfect

The key to becoming comfortable with interpreting cytology is to spend time at the microscope with every dermatology case. Pattern recognition and confidence will develop over time.

Microscope methodology

It is important to have a standardized protocol when approaching slide review. Begin with the 4x objective and perform a general scan of the entire slide. This may reveal different populations of cells and ensures nothing is missed. Evaluate all populations of cells on high power to further characterize what cells are present. Multiple high-power fields should be examined to assess general pattern.

Cell types

The most common cell types found in skin cytology include keratinocytes, neutrophils, macrophages, eosinophils, red blood cells, and infectious agents.

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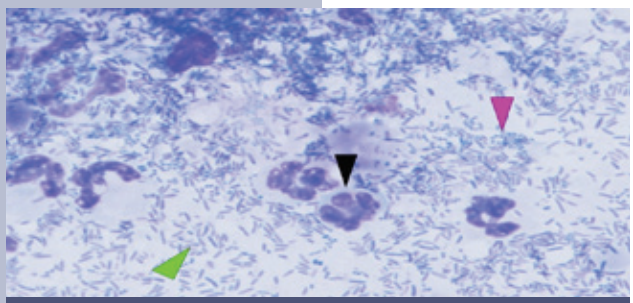

Image 4: Ear cytology with mixed infection. Black arrow: neutrophil. Pink arrow: cocci bacteria. Green arrow: rod-shaped bacteria.

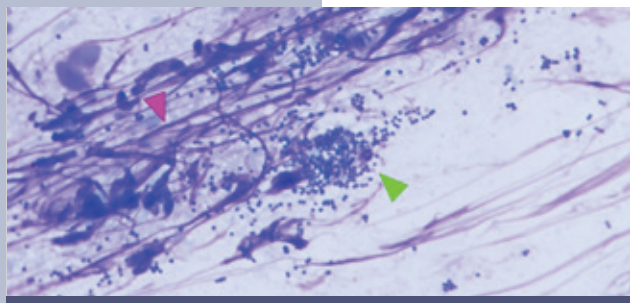

Image 5: Staphylococcal infection. Pink arrow: neutrophilic streaming. Green arrow: cocci bacteria.

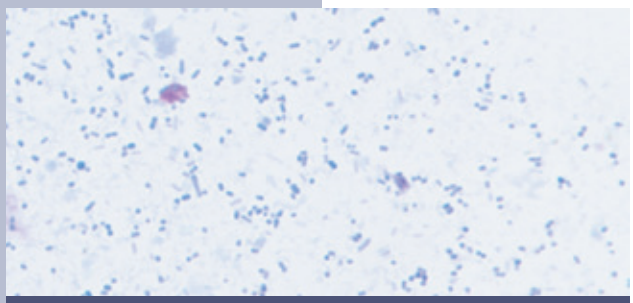

Image 6: Bacterial overgrowth.

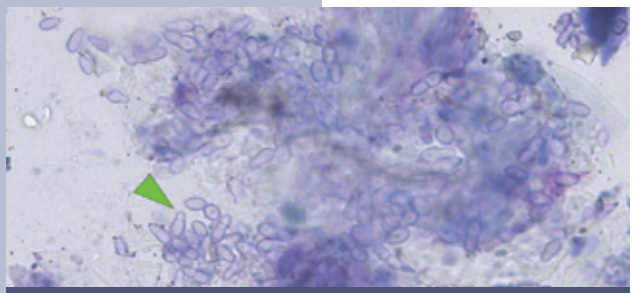

Image 7: *Malassezia spp* on acetate tape cytology. Green arrow: *Malassezia* (note light staining on tape).

Interpreting cytology is pattern recognition based on the general population of cells. A single, unknown or highly unusual cell can likely be ignored.

- Neutrophils indicate inflammation (sterile or infectious) and nuclear streaming is present when neutrophils are fragile and degenerate (or if the sample is smeared).
- Macrophages indicate chronic or deep inflammation or potentially a fungal infection. Pyogranulomatous inflammation contains neutrophils and macrophages.
- Eosinophils indicate the presence of ectoparasites, foreign bodies (including deep furunculosis), drug reaction, or allergy (cats).
- Bacteria should be identified based on morphology (rods, cocci) (**Image 4**). Pathogenic bacteria are very small and only appreciable at 40x or 100x.
- Fungi should be identified based on morphology (*Malassezia spp.*, *Blastomyces*).

Bacterial infections

To definitively diagnose a bacterial infection there must be neutrophils in the presence of bacteria, preferably intercellular bacteria (**Image 5**). Bacterial overgrowth should be diagnosed when there are many bacteria with no neutrophils present (**Image 6**).

Malassezia

Malassezia spp. typically adhere to keratinocytes and *Malassezia* dermatitis should not produce suppurative inflammation (**Image 7**). If neutrophils are noted on the cytology, thoroughly evaluate for bacteria as well.

Pyogranulomatous inflammation

Deep inflammation is characterized by neutrophils and macrophages, with variable plasma cells and eosinophils (**Image 8**). In the presence of bacteria, this is indicative of bacterial folliculitis and furunculosis. If no bacteria are noted on cytology, this type of inflammation may be due to sterile inflammation from furunculosis (ingrown hairs, demodicosis, etc.), a deep fungal infection (blastomycosis, etc.), or an autoimmune disease (sterile nodular panniculitis, etc.).

Photos courtesy of Ashley Bourgeois, DVM, DACVD

Autoimmune

Cytology findings of sterile inflammation accompanied by specific clinical findings may indicate the presence of an autoimmune disease. For example, in addition to neutrophilic inflammation, acantholytic keratinocytes (AKs) are typically present in cases of superficial pemphigus (**Image 9**). AKs are very large, round, basophilic cells. If an autoimmune disease is suspected, skin biopsies are required for a definitive diagnosis.

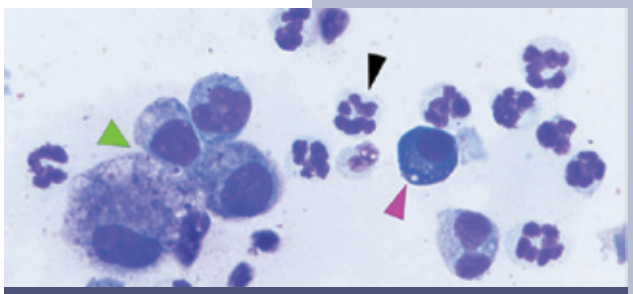

Image 8: Sterile pyogranulomatous inflammation. Black arrow: nondegenerate neutrophil. Green arrow: macrophages. Pink arrow: plasma cell.

Ectoparasites

When assessing a skin scraping, or trichogram, close the iris diaphragm on the microscope to allow for better visualization of the mites (**Image 10**). Examine the entire slide at 4x as there may be very few ectoparasites, eggs, or feces. Negative skin scrapings never completely rule out ectoparasites and if clinical suspicion is high but the skin scraping is negative, a treatment trial is warranted.

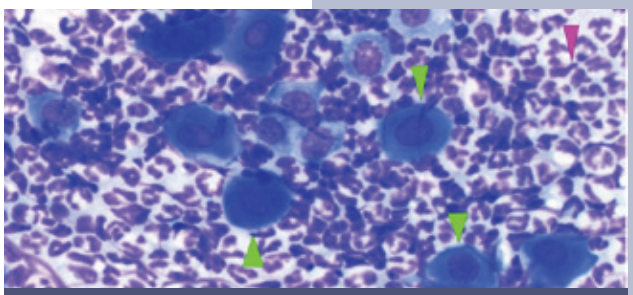

Image 9: Sterile neutrophilic inflammation with AKs. Pink arrow: nondegenerate neutrophils. Green arrows: AKs (note very large size).

Trichography

To interpret a trichogram it is important to have a foundational knowledge of the normal hair structure. Normal hair shafts are uniform in diameter and taper at the tip. A trichogram may reveal abnormalities in the pigmentation of the hair (color dilute alopecia), abnormalities in the structure of the hair (dermatophytosis, **Image 11**), broken hairs (feline traumatic hypotrichosis), and/or ectoparasites (*Demodex canis*, **Image 10**).

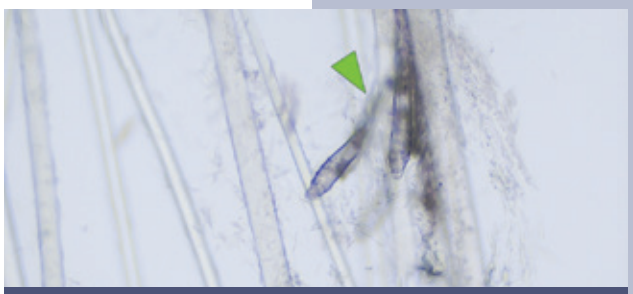

Image 10: Demodex mites from a trichogram (green arrow).

Use your resources

Cytology and dermatology textbooks are very helpful and it is recommended to keep a user-friendly reference within easy reach. Additionally, there are automated resources being developed that may provide a simpler approach to interpreting cytology.

As with all things in veterinary medicine, a definitive diagnosis requires diagnostics, and dermatology cases are no different. The more cytology is performed, the more comfort and expertise a practitioner will gain. Cytology everything.

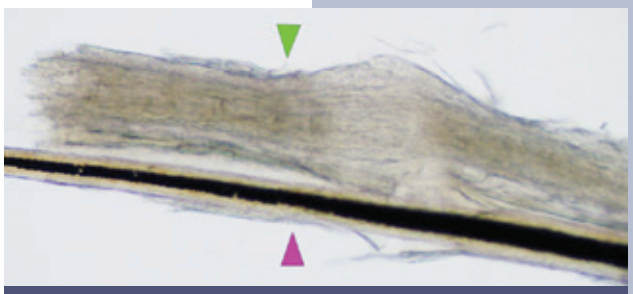

Image 11: Dermatophytic hair (green arrow), normal hair (pink arrow)