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Dermatology diagnostics

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Indications:

- All cases with hair loss, scaling, crusting, papules, pustules, lichenification, or otitis should be screened for infectious organisms with skin scrapings and surface skin cytology; flea combing to screen for fleas and flea feces as well as for lice is also necessary, even in indoor pets.
- These quick and easy in-house tests will not only allow for the accurate diagnosis of the dermatitis and guide appropriate therapy, but are also revenue generators.
- Additionally, with the emerging problem of antibiotic-resistant bacterial skin infections, cytology to monitor response to antimicrobial therapy is important and can guide decisions about culture submission.
- The first important consideration is to buy a good microscope! There are numerous affordable, high quality microscopes available; I use the Swift M10 series biological lab microscope, but there are many other options.
- It is important to teach our veterinary technicians and assistants how to appropriately take care of microscopes, and there are numerous online resources including http://micro.magnet.fsu.edu/primer/anatomy/cleaning.html.

1.1 Skin scrapings (*See video on companion website*)

- Skin scrapings are used to diagnose mites such as scabies, *Demodex*, and *Cheyletiella*.
- A dulled #10 scalpel blade or medical curette/spatula and mineral oil are used to collect skin debris (Figures 1.1–1.4), which is then mixed with more mineral oil on a microscope slide and observed under 4–10×, with the condenser down for maximum contrast.
- For scabies, multiple wide superficial scrapings of crusted, papular, or alopecic lesions on elbows, pinnal margins, and ventral trunk should be obtained (Figures 1.5 and 1.6).
  - The mites live in the stratum corneum and are often few in number, and consequently false negative scrapings are common, so any animal with pruritus consistent with scabies should be trial-treated with appropriate acaricidal therapy.
- For surface living *Cheyletiella* mites, wide superficial scrapings of scaly lesions are obtained; these large surface-dwelling mites are quite visible on 4× magnification (Figure 1.7).
  - Mites can also be low in number, and as for scabies empiric acaricidal therapy is often prescribed in suspect cases.
  - In some cases, *Cheyletiella* mites can be found using multiple applications of clear acetate tape onto scaly areas; after sample collection the tape is applied to a microscope slide (no oil or stain is used) and observed under 4×.
- *Demodex* mites live in hair follicles and so require deeper skin scrapings.
  - The dulled blade is scraped briskly in one direction on the skin (be careful not to press down on the blade which could cause cutting of the skin) until capillary oozing is observed on the skin as well as on the blade (Figure 1.8). Intermittently squeezing the sampled skin area between scrapings can be helpful to express mites and increase scraping yield (Figures 1.9 and 1.10A–1.10C).
  - *Demodex* mites can also be obtained using hair plucks placed into mineral oil, then observe the roots to look for mites (especially helpful for hard to scrape areas such as paws, eyelids, and lips, or in very thickened, scarred areas of skin; Figure 1.11).

Clinical Atlas of Canine and Feline Dermatology, First Edition. Edited by Kimberly S. Coyner.

Companion website: www.wiley.com/go/coyner/dermatology

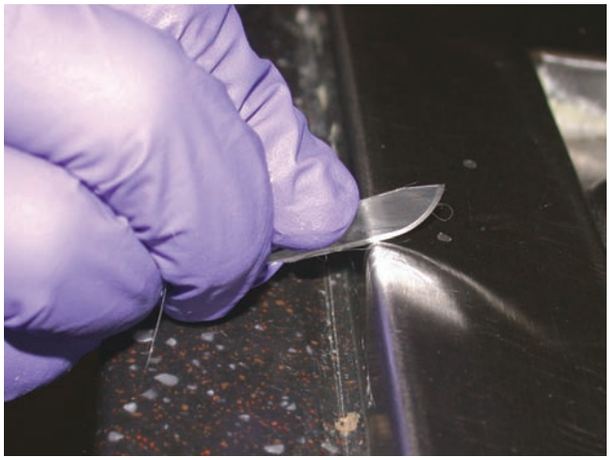


Figure 1.1 The scalpel blade is dulled by repeatedly scraping the edge on a hard surface.

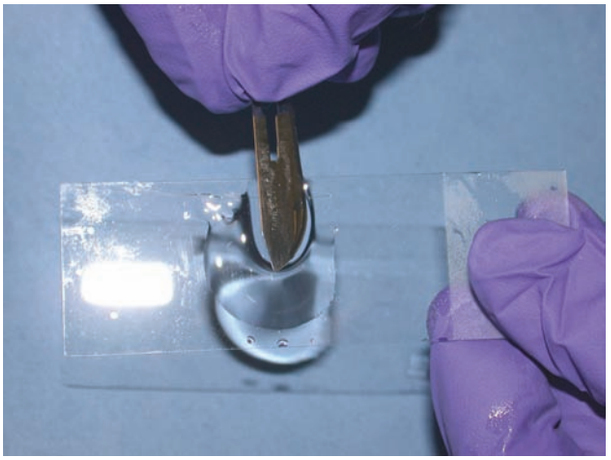


Figure 1.2 Mineral oil is applied to the dulled blade and the microscope slide.

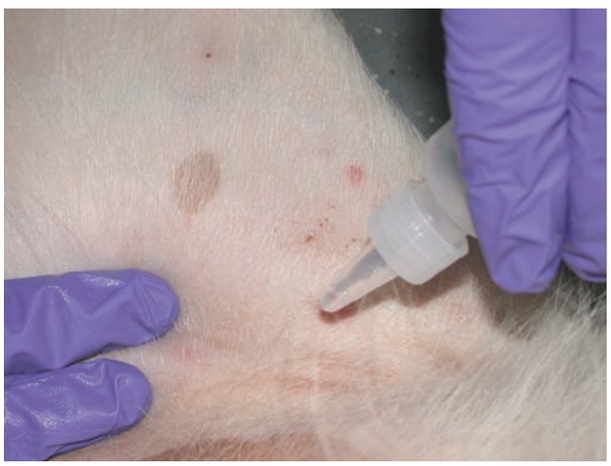


Figure 1.3 Mineral oil is applied to the lesion to be scraped.

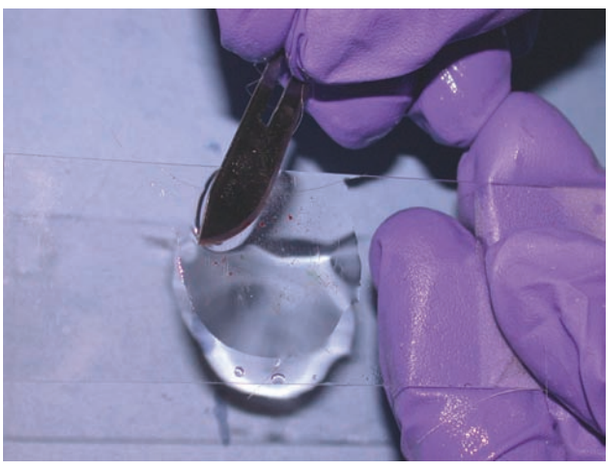


Figure 1.4 Accumulated debris on the blade is mixed into the mineral oil on the microscope slide.

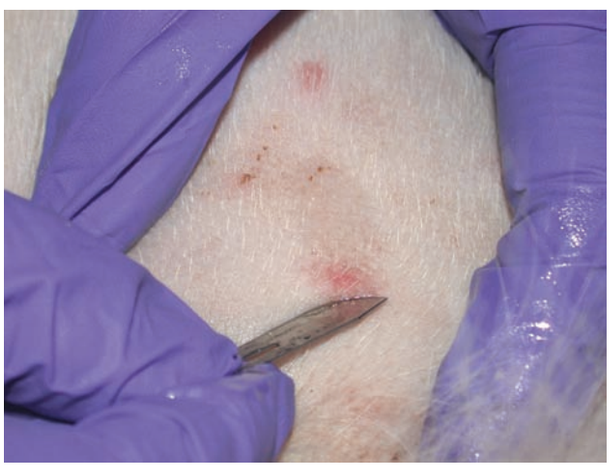


Figure 1.5 Superficial skin scraping.

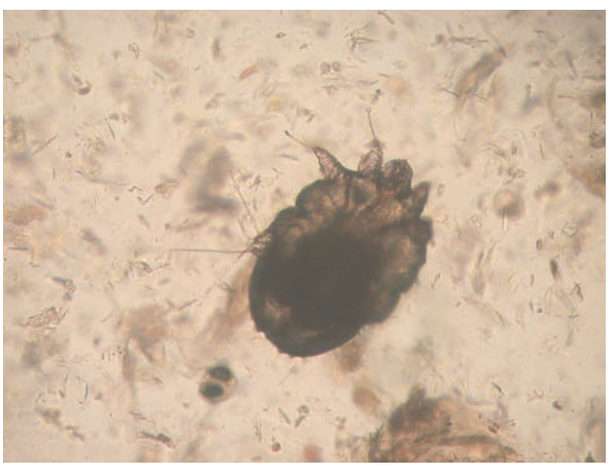


Figure 1.6 Scabies mite (10× with digital zoom).

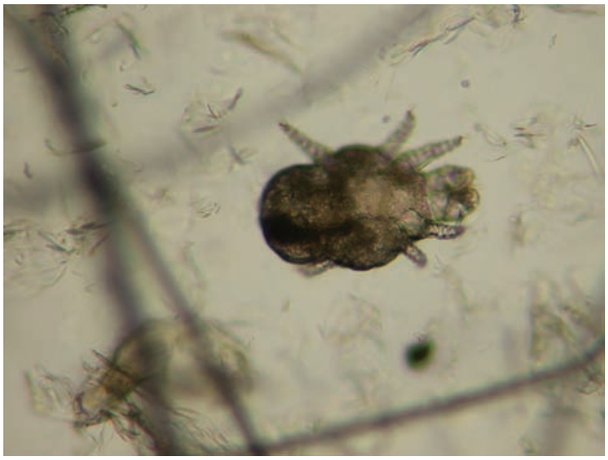


Figure 1.7 *Cheyletiella* mite (10×).

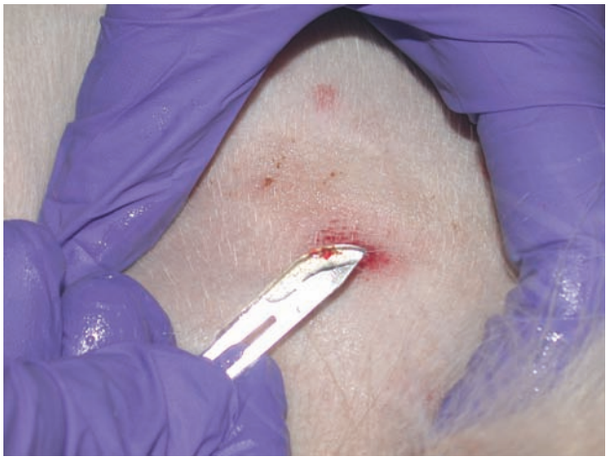


Figure 1.8 For *Demodex*, scrape until capillary oozing is observed.

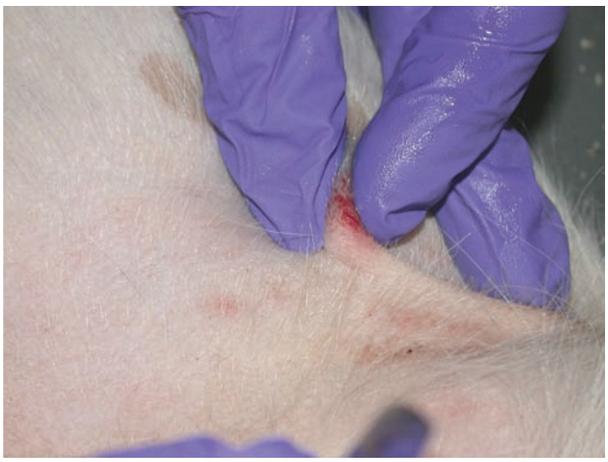


Figure 1.9 Squeeze the scraped area to increase mite yield.

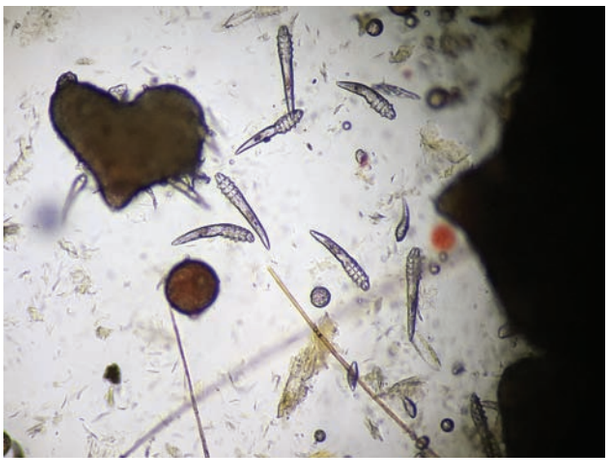


Figure 1.10A *Demodex canis* mites (4× with digital zoom).

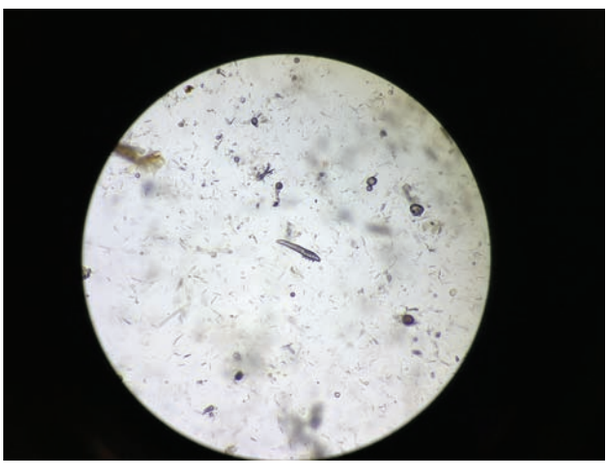


Figure 1.10B A *Demodex* mite at 4× with the microscope condenser down.

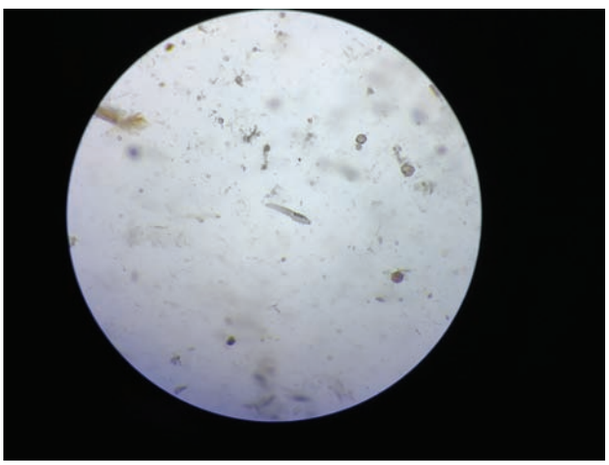


Figure 1.10C The same mite with the microscope condenser up; the mite is washed out and less visible.

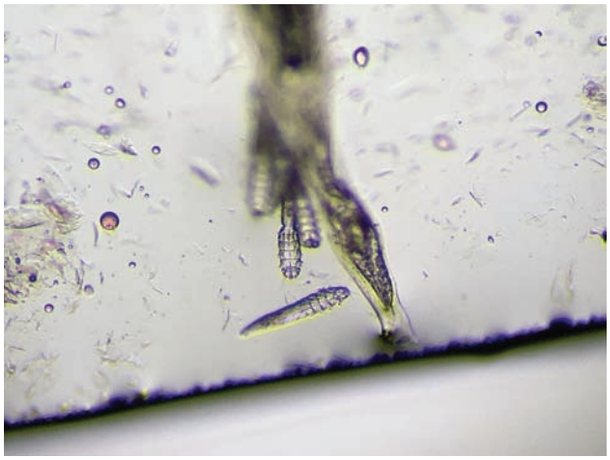


Figure 1.11 *Demodex* mites from a hair pluck, encased in keratin around a hair root (4× with digital zoom).

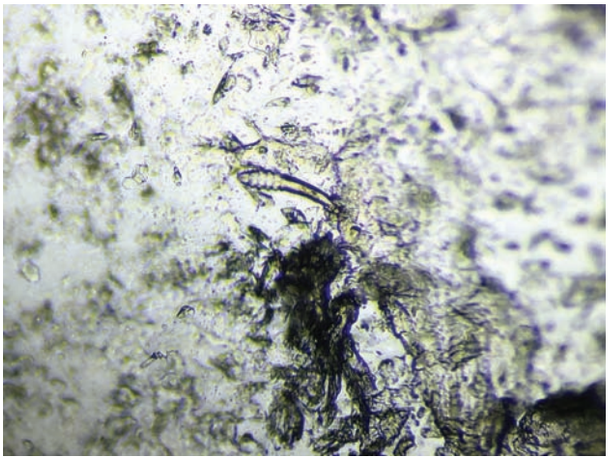


Figure 1.12 *Demodex* mite on a tape prep (4×).

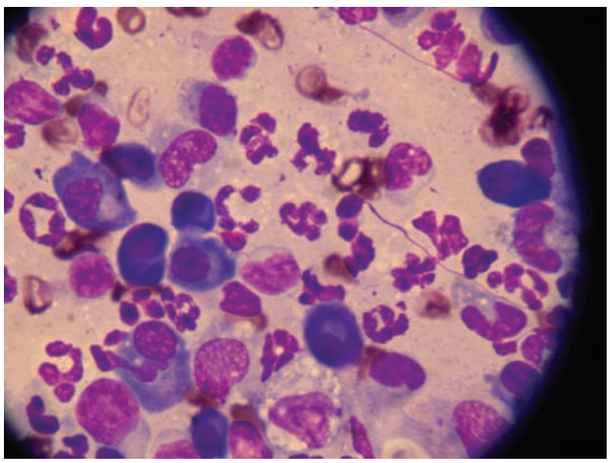


Figure 1.13 Mixed (neutrophils, macrophages, plasma cells) inflammation due to deep interdigital pyoderma (100×).

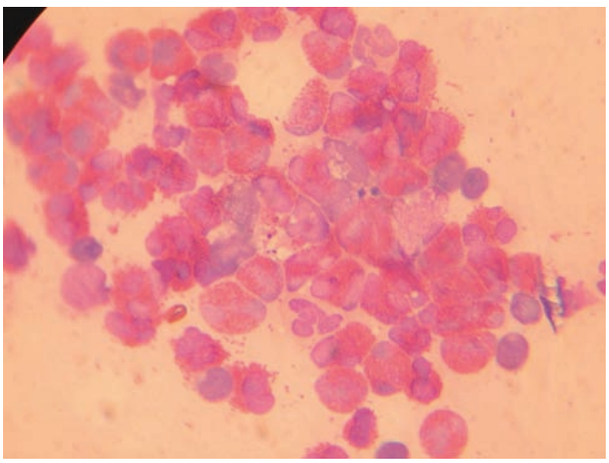


Figure 1.14 Eosinophilic inflammation found on impression smear from a feline eosinophilic plaque (100×).

  - Additionally, samples can be obtained by repeatedly squeezing the skin and then applying clear acetate tape to the squeezed area, then the tape is applied to a microscope slide (no stain) and observed under 4–10× for mites (Figure 1.12).

1.2 Cytology – Skin and ear (*See videos on companion website*)

- Skin and ear cytology can be used to obtain information on bacterial or *Malassezia* infection, as well as to characterize inflammatory infiltrate.
- Skin cytology.
  - Less than one of each type of organism (yeast, cocci, or rod) per oil-immersion field (OIF) is seen in normal skin.
  - Inflammatory cells are not found on normal skin cytology.
- Samples are applied to a microscope slide and stained with Diff-Quik or similar stain, scanned under 10× to identify an area of interest, then observed under 40–100×.
- Infectious agents which can be found on skin cytology include cocci and rod bacteria, *Malassezia* and fungal organisms, and protozoal organisms such as *Leishmania*.
- Neutrophilic or pyogranulomatous inflammation can be supportive of an infectious or inflammatory process (Figure 1.13).
- An eosinophilic infiltrate is supportive of a hypersensitivity dermatitis (Figure 1.14).
- Acantholytic cells can suggest pemphigus complex (but can also be seen with chronic bacterial or dermatophyte

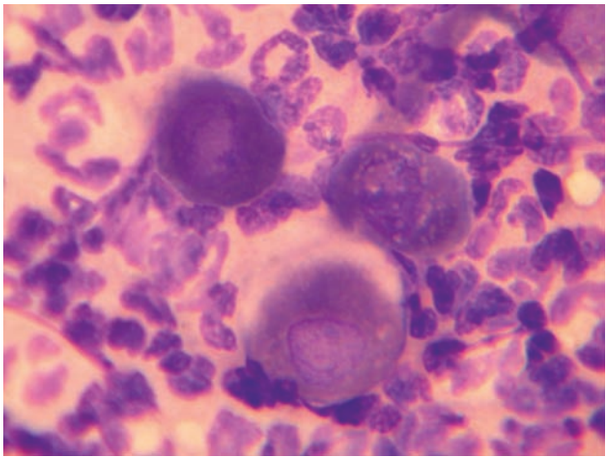


Figure 1.15 Acantholytic cells and neutrophils in a case of canine pemphigus foliaceus (100×).

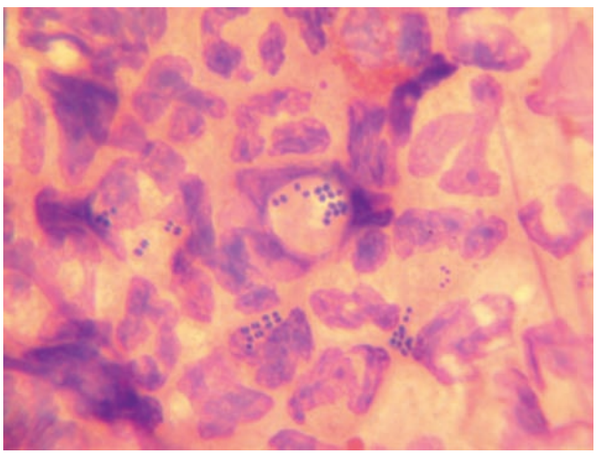


Figure 1.16B Cytologic evaluation of pustule contents reveals neutrophils and intracellular cocci (100×).

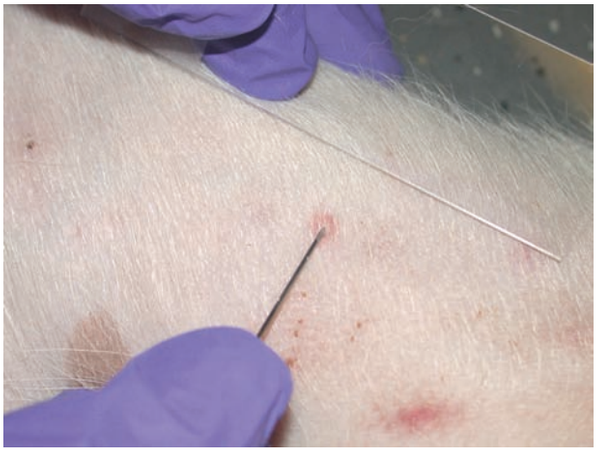


Figure 1.16A To sample a pustule, a needle is used to rupture the pustule and the contents are smeared onto a microscope slide.

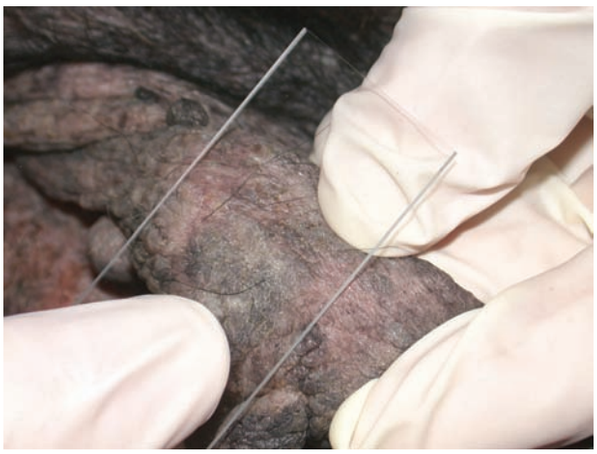
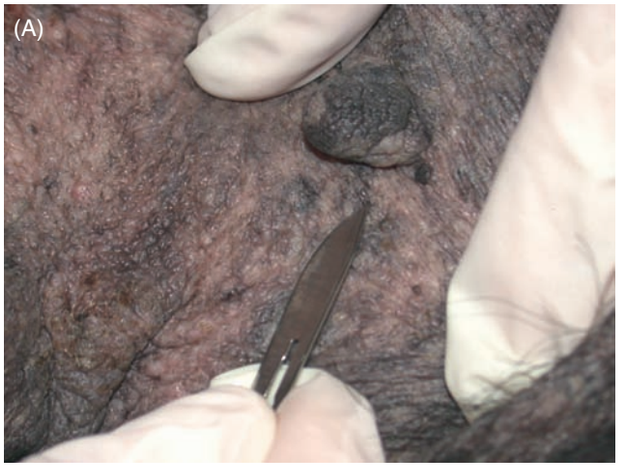


Figure 1.17 Impression smear: Lichenified skin is firmly pressed onto the microscope slide. Clinically, the lichenification and hyperpigmentation are suspicious for *Malassezia* dermatitis.

infection) and support the need for biopsy and histologic diagnosis (Figure 1.15).

- Skin cytology can be obtained in a variety of ways:
  - If a pustule is present, it can be ruptured with a needle and the contents smeared onto a slide (Figures 1.16A and 1.16B).
  - If a moist or greasy lesion is present, it can be sampled by firmly pressing a microscope slide on the surface of the lesion (Figure 1.17).
  - For dry scaling or diffuse crusting lesions, use of a dulled scalpel blade without mineral oil can be helpful to collect surface debris which is then smeared like a spatula onto the microscope slide (Figures 1.18A–1.18D). If larger crusts are present, use the blade or microscope slide edge to raise the edge of the crust and then obtain an impression smear of the exudate or debris under the crust.
  - For interdigital lesions, samples can be obtained via direct impression of the interdigital web onto a slide, cotton tipped swab of interdigital debris which is then rolled onto a slide, or by acetate tape impression (see acetate tape impressions below). In cases of paronychia, nailbed debris can be collected with a dull blade or the wooden end of a cotton swab, then smeared onto a microscope slide.
  - Acetate tape impressions can be used to sample dry, lichenified, and interdigital areas. A piece of clear (not frosted) acetate tape is firmly pressed to the lesion, then applied onto a microscope slide over a few drops of blue Diff-Quik stain (or stained using routine Diff-Quik stain omitting methanol) and observed under 40–100× (Figures 1.19A–1.19F).



Figures 1.18A and B The lichenified skin is scraped with a dry, dull scalpel blade.

Figure 1.18C The accumulated debris is smeared onto the microscope slide.

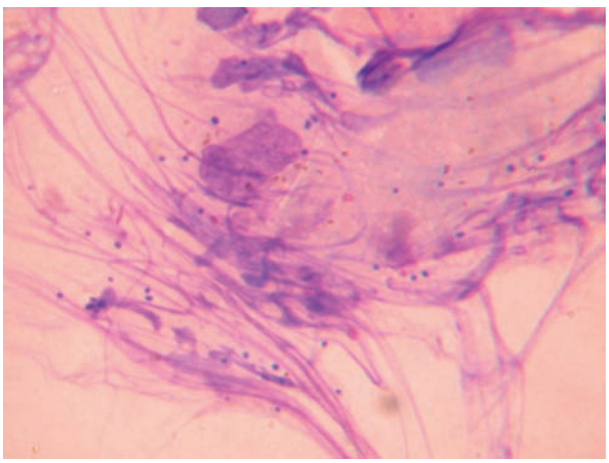
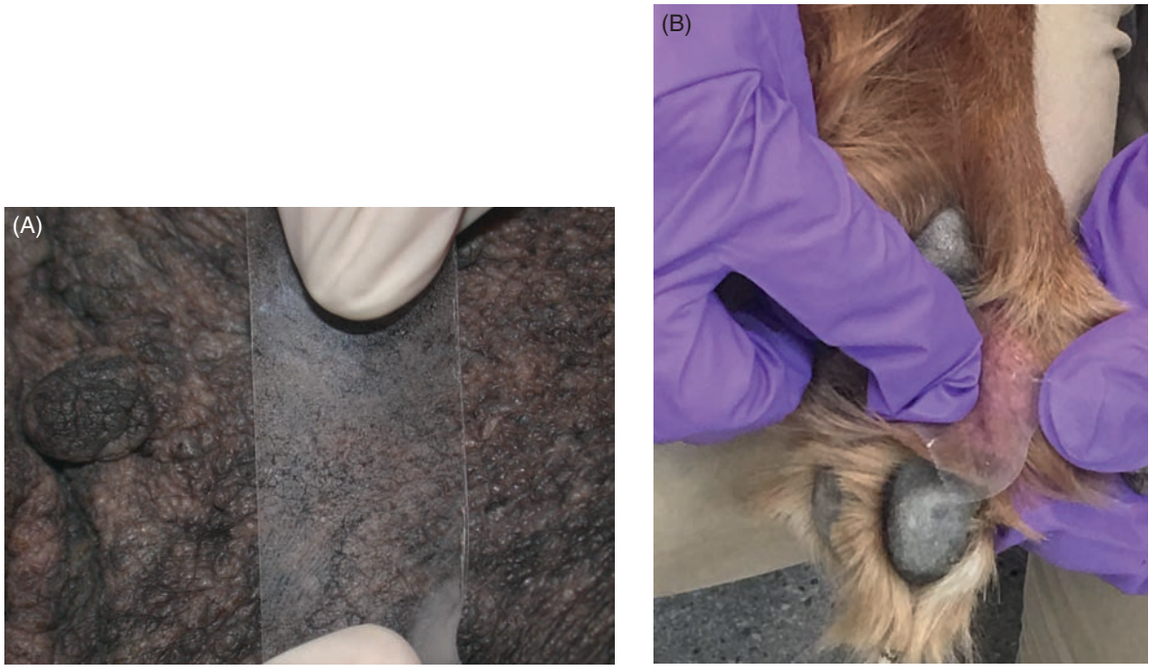


Figure 1.18D Cytology reveals streaming neutrophilic debris and bacterial cocci, but no *Malassezia* are found (100×).

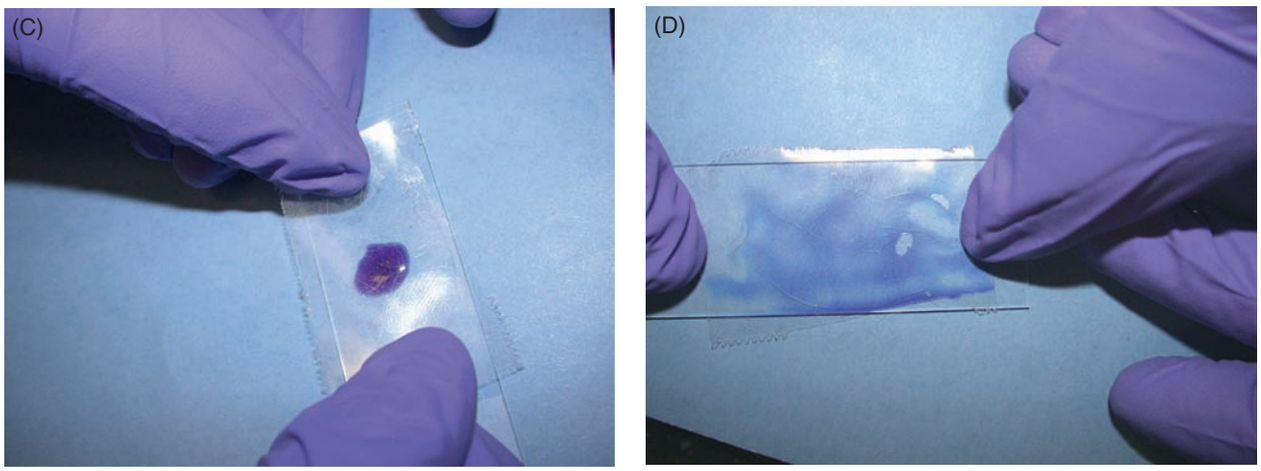
- For otic cytology, gently obtain a swab of external ear debris (insert the swab into the canal no further than the proximal vertical canal, Figure 1.20), then roll the swab onto a microscope slide; both ear samples can be placed onto one slide with each side labeled (Figure 1.21A and 1.21B).
  - Otic cytology:
    a) >3 yeast/OIF in dogs and >1 yeast organism/OIF in cats may be considered abnormal (Figure 1.22).
    b) >5 cocci/OIF and >1 rod/OIF is considered abnormal (Figures 1.23 and 1.24).
    c) Presence of inflammatory cells is also abnormal.
- Red herrings: Non-significant cytology findings which can mimic infectious organisms include:
  - Melanin granules (Figures 1.25A and 1.25B).
  - Keratohyalin granules (Figure 1.26).
  - *Simonsiella* oral bacteria, huge rod bacteria, often found on the lips and paws (Figure 1.27).
  - Pollen (Figures 1.28A–1.28C).
  - Saprophytic fungal spores (Figure 1.29).
  - Stain precipitate (Figure 1.30; to avoid this, stain solutions should ideally be changed weekly).

1.3 Cytology – Mass aspirates (*See videos on companion website*)

- Ideally, every new mass should be evaluated. Dermal or subcutaneous masses can be aspirated for in-house cytology in order to determine:
  - If the mass is inflammatory vs. cyst vs. potential neoplasia.
  - Potential need for biopsy or tissue cultures (if neoplastic cells or inflammatory cells are seen).
  - If sample submission to a reference laboratory for pathology evaluation will be needed or diagnostic (if suspected neoplastic cells are found).



Figures 1.19A and B For a tape prep, the clear acetate tape is repeatedly impressed onto the lichenified skin; to sample interdigital areas, the web is pressed up onto the tape.



Figures 1.19C and D The tape is then applied onto the microscope slide on top of a drop of blue Diff-Quik stain.

- Procedure:
  a) Use a 25–22 g needle and a 3–6 cc syringe.
  b) Insert needle into lesion, aspirate, redirect, and aspirate again (Figure 1.31A; if lesion is vascular, then only insert needle in once then withdraw to avoid blood dilution).
  c) Take needle off syringe, draw air into syringe, replace needle and expel needle contents onto slide (Figure 1.31B).
  d) Use a second slide for gentle squash prep to form monolayer of cells (Figure 1.31C).
  e) Stain with Diff-Quick, observe under 10× to find a diagnostic area, then observe under 40–100×.
- Appropriate action based on findings:
  a) Potential "wait and see" nodules: Follicular cysts (Figure 1.32), lipoma (Figure 1.33), histiocytoma (Figure 1.34).

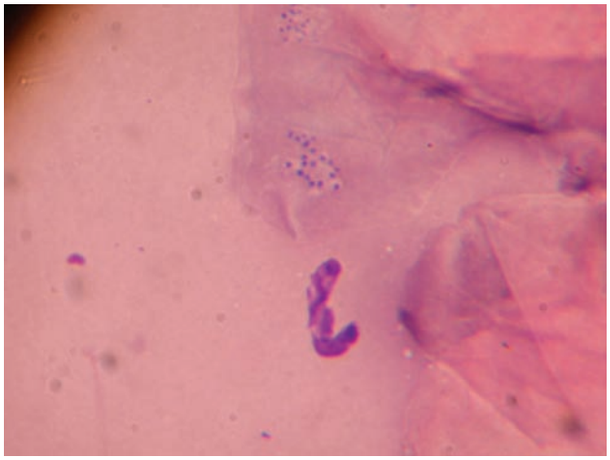


Figure 1.19E Cytological analysis reveals keratinocytes with scattered neutrophils and cocci (100×).

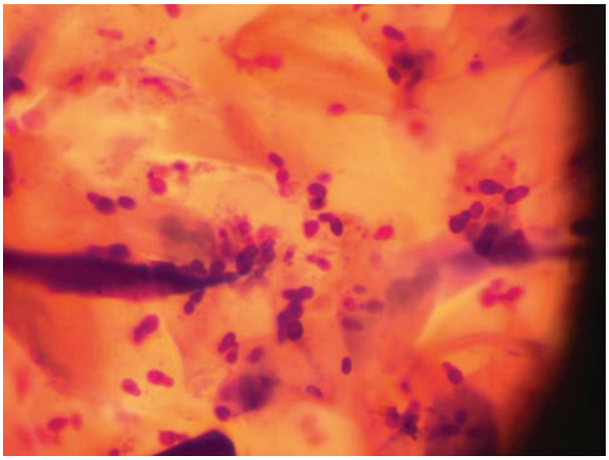


Figure 1.19F In a different case, numerous *Malassezia* are found on tape prep (100×).

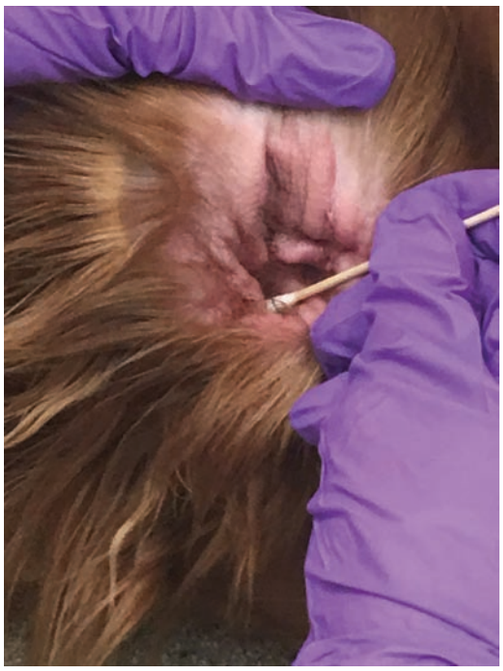


Figure 1.20 To obtain otic cytology, the cotton swab is inserted into the ear canal, no further than the vertical canal.

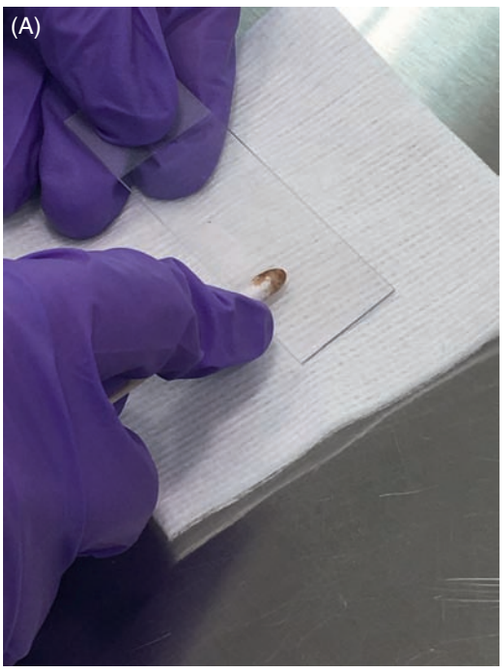


Figure 1.21A and B The swabs are then rolled onto one slide, left and right ears are labeled.

b) We need biopsy +/– staging lesions: mast cell tumor (Figure 1.35), lymphoma (Figure 1.36), plasmacytoma, melanoma (Figure 1.37).
c) We need biopsy/special stains/tissue cultures: Pyogranulomatous inflammation (Figure 1.13).
d) We need biopsy: everything else.

1.4 Trichograms

- Indications for trichogram/microscopic hair shaft evaluation when faced with cases of localized or generalized alopecia include:
  – To evaluate anagen/telogen ratio (Figures 1.38 and 1.39):
    a) Anagen: Growing stage of the hair follicle, during which the follicle is actively producing hair.

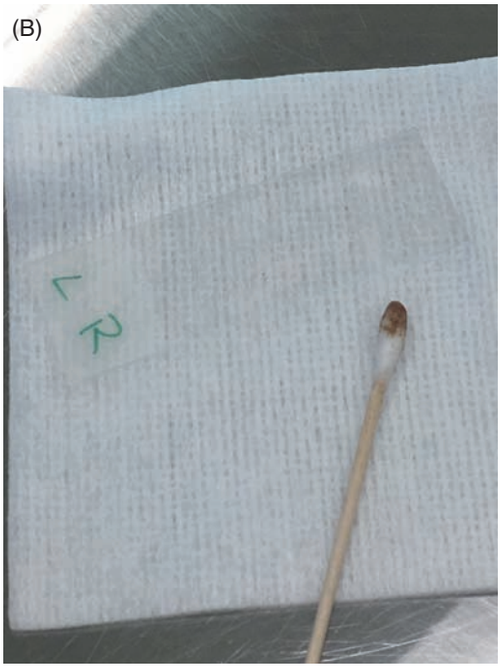


Figure 1.21A and B (Continued)

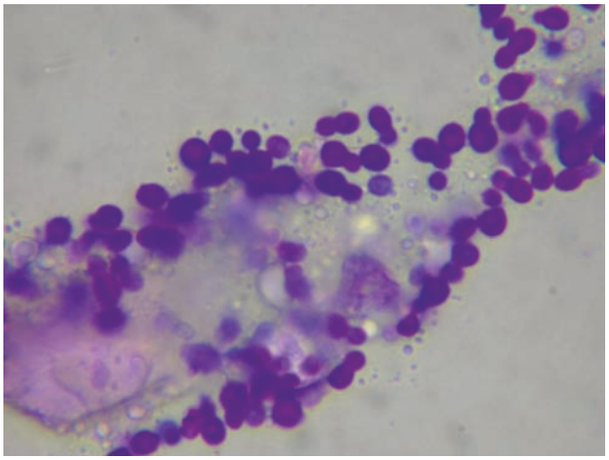


Figure 1.22 Cytology of yeast otitis (100×).

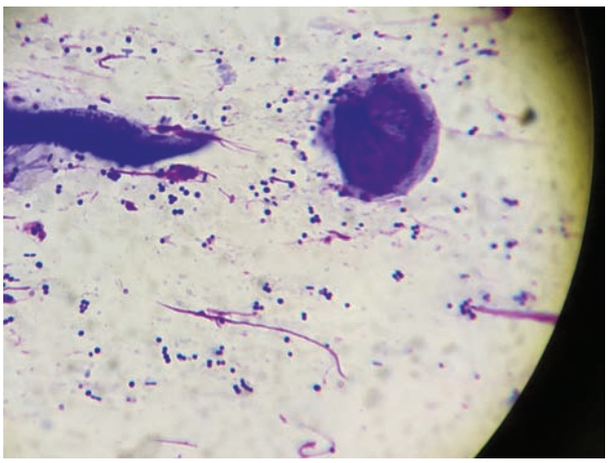


Figure 1.23 Cytology of bacterial otitis caused by cocci bacteria (100×).

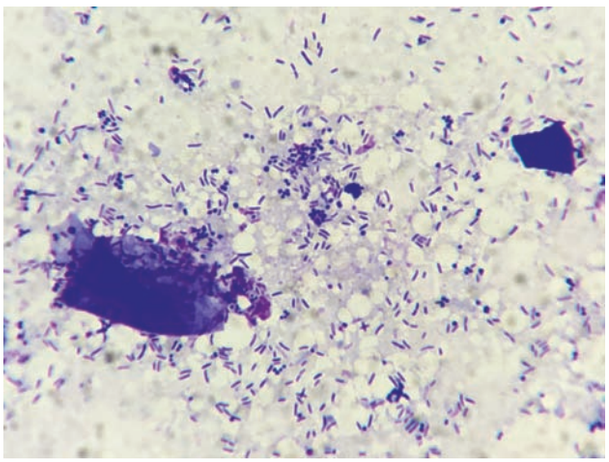


Figure 1.24 Cytology of a mixed bacterial otitis (100×).

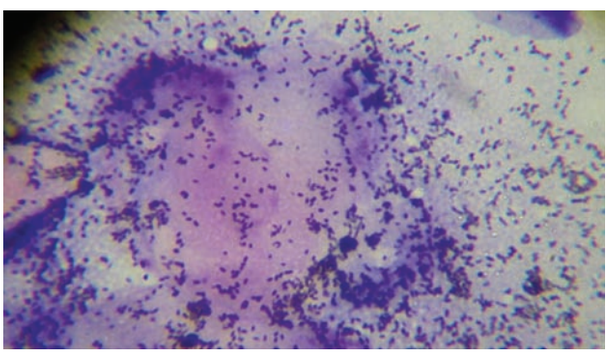


Figure 1.25A Melanin pigment granules found on cytology of a dry skin scraping of a black dog (100×).

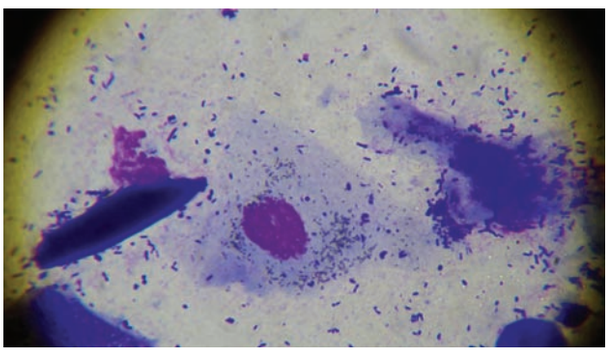


Figure 1.25B Cytology demonstrating melanin granules on an epithelial cell, surrounded by cocci and rod bacteria (100×).

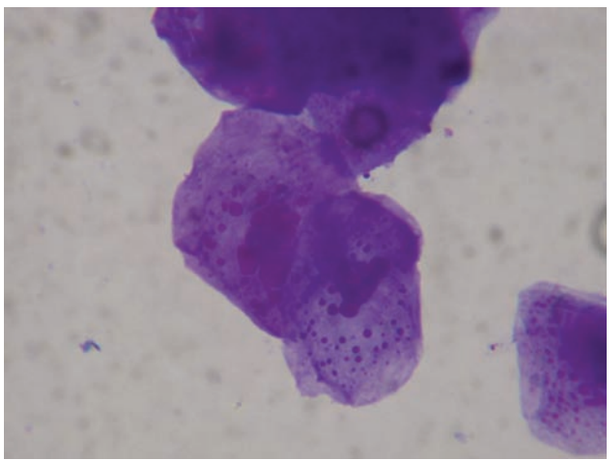


Figure 1.26 Cytology of an epithelial cell with large pink intracellular keratohyaline granules which vary in shape and size (100×).

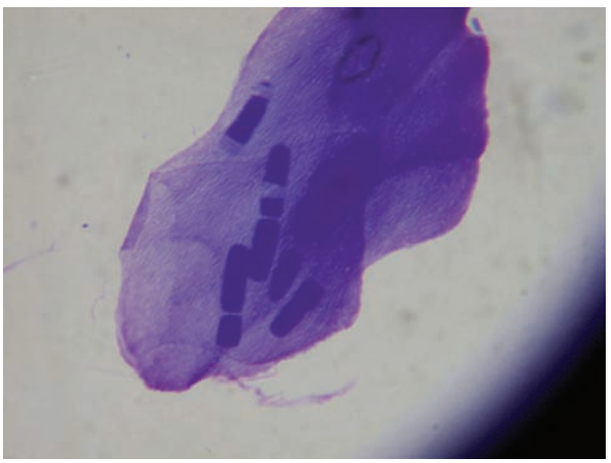


Figure 1.27 An epithelial cell from a lip fold impression, colonized by very large *Simonsiella* oral bacteria (100×).

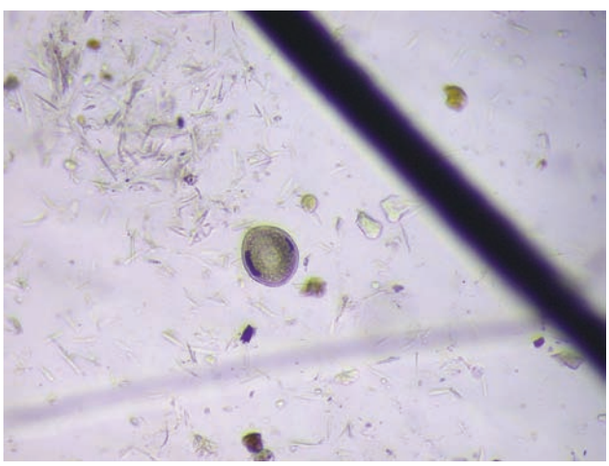
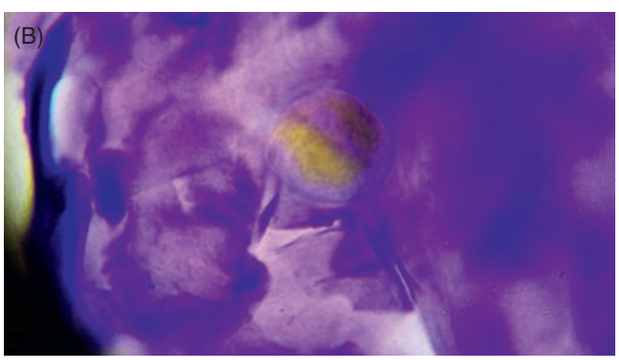
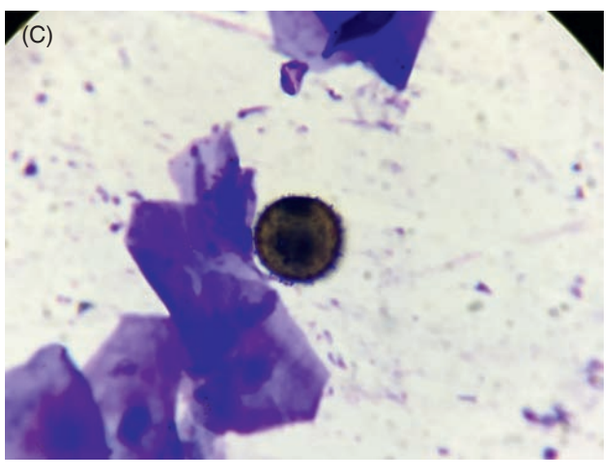


Figure 1.28A Pollen on a skin scraping (10×), which could potentially be mistaken for a parasite egg.





Figures 1.28B and C Pollen on skin cytology obtained by tape prep (100×).

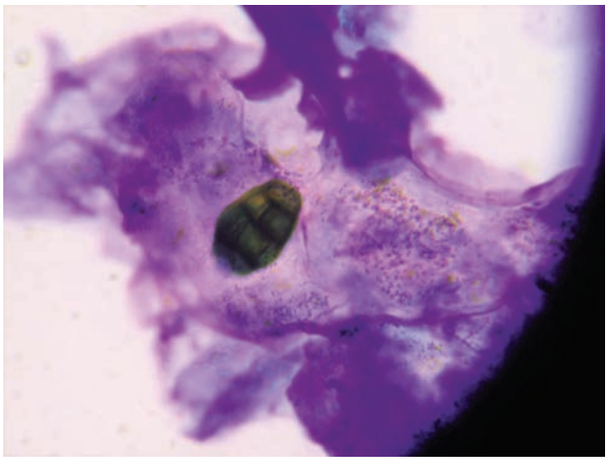


Figure 1.29 In this skin cytology, lightly pigmented epithelial cells are seen as well as a pigmented environmental mold spore, likely *Alternaria* (100×).

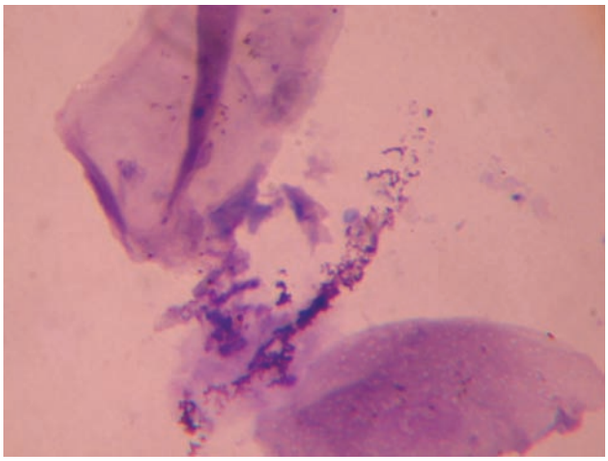


Figure 1.30 This skin cytology shows epithelial cells and amorphous purple debris which is stain precipitate (100×).

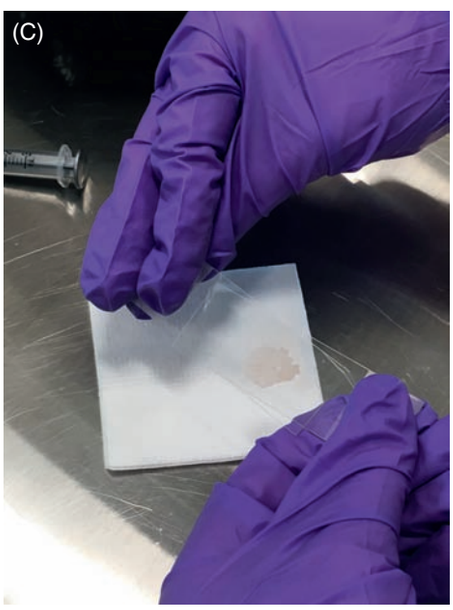


Figure 1.31A–C Aspiration of a dermal mass for cytology.

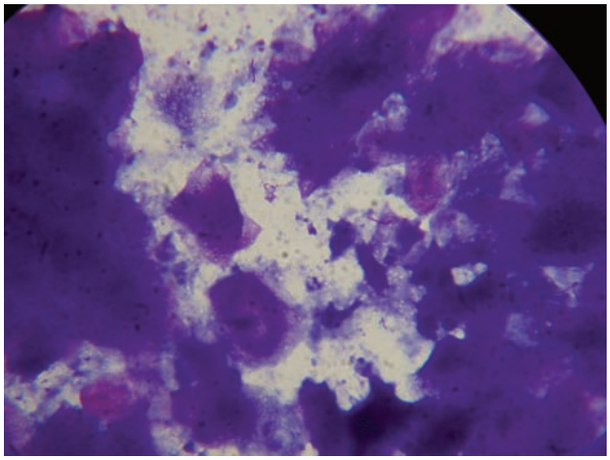


Figure 1.32 This skin mass aspirate cytology shows amorphous keratinaceous debris consistent with a benign follicular cyst/tumor (100×).

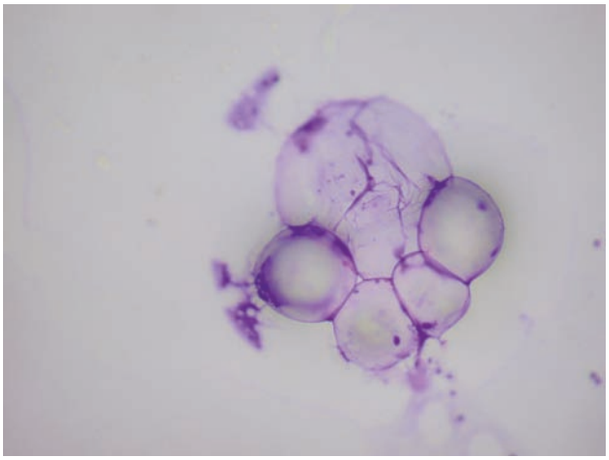


Figure 1.33 Most cells and lipid from lipoma aspirates will dissolve in the methanol stain, but sometimes the delicate adipocyte cells can be found (100×).

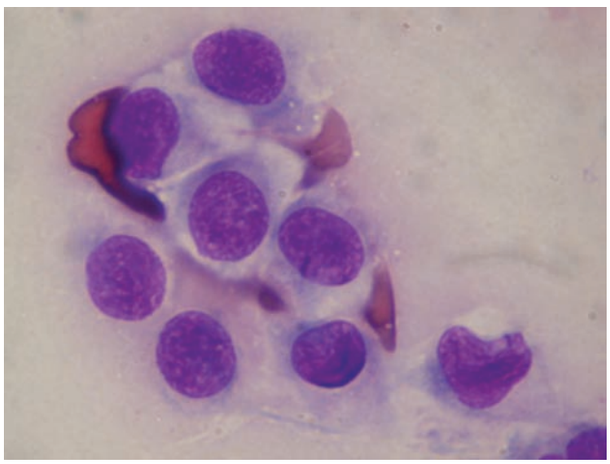


Figure 1.34 Cytology of a histiocytoma shows round cells with moderate light blue, wispy cytoplasm, occasional small vacuoles, and some nuclei can be indented in a "kidney bean" shape (100×).

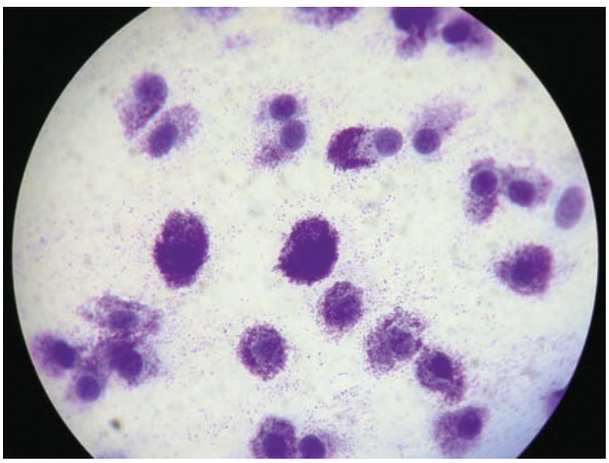


Figure 1.35 Cytology of a mast cell tumor demonstrating round cells with purple granules (100×).

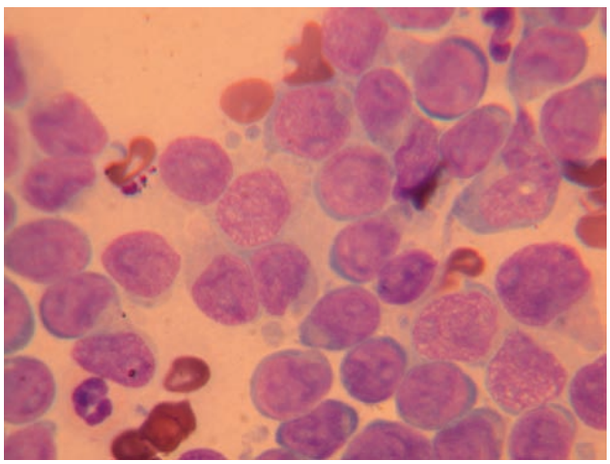


Figure 1.36 Impression smear cytology of cutaneous lymphoma showing round cells (lymphoblasts) with large nuclei and scant dark blue cytoplasm (100×).

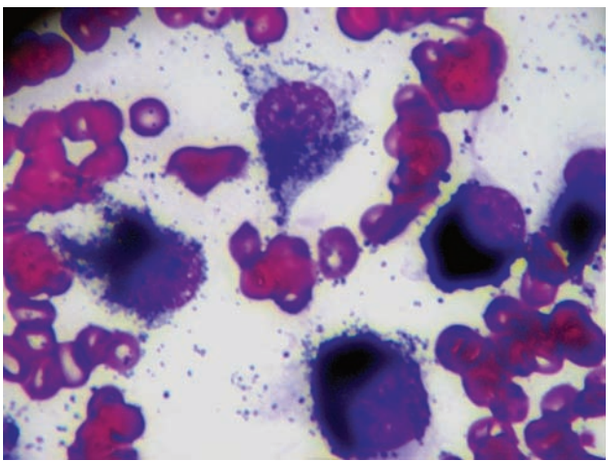


Figure 1.37 Cytology of a cutaneous melanocytoma, showing round to stellate cells with dark pigment granules; numerous red blood cells are also present (100×).

Figure 1.38 A typical club shaped anagen hair root (10×).

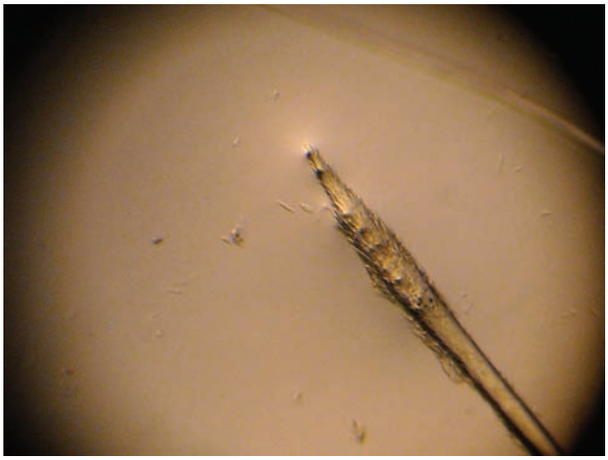


Figure 1.39 The telogen hair root has a spear shape (10×).

Figure 1.40 Trichogram of an overgrooming cat shows hairs sheared off mid-shaft (10×).

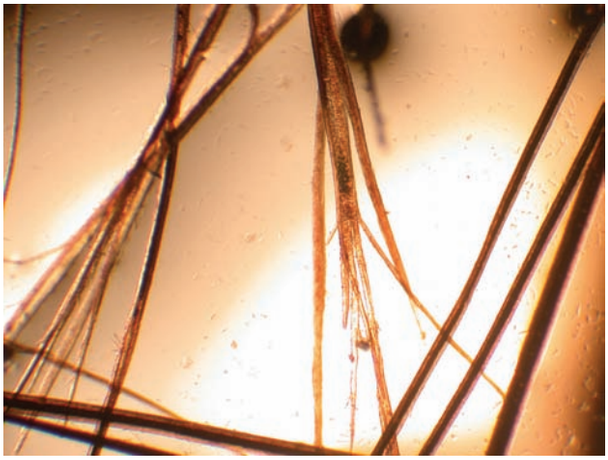


Figure 1.41 This trichogram demonstrates hair damage due to aggressive brushing (4×).

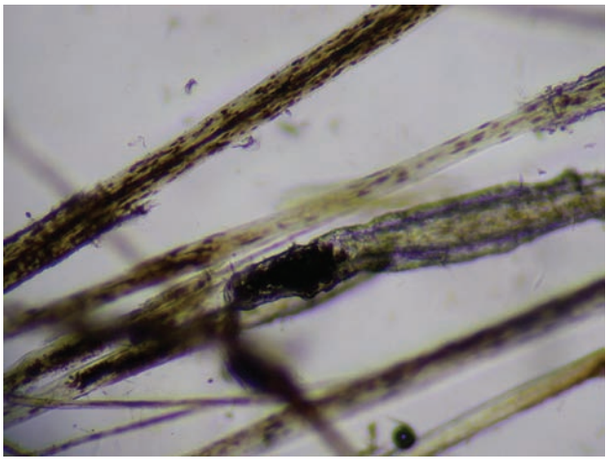


Figure 1.42 Trichogram of a dog with color dilution alopecia; note the clumps of pigment causing distortion of the hair cuticles as well as the hair root (10×).

b) Telogen: Resting stage of the hair follicle, in which the non-growing hair is retained in the follicle and subsequently lost.

- To investigate barbering potential in cases in which the animal is not witnessed to be licking or chewing at the fur (Figure 1.40).
- To evaluate hair shaft integrity in cases of suspected chemical or physical injury to the fur such as aggressive brushing (Figure 1.41).
- To evaluate hair shaft pigment distribution, in suspected cases of color dilution alopecia (Figure 1.42).
- To evaluate potential hair shaft infection such as dermatophytosis (Figure 1.43).

- The hair cycle can vary with breed, age, gender, season, and health status:
  - Beagles were reported to have approx. 50 : 50 anagen : telogen ratio in one study (Al-Bagdadi, Titkemeyer, and Lovell, 1977), though another reference states 20 : 80.

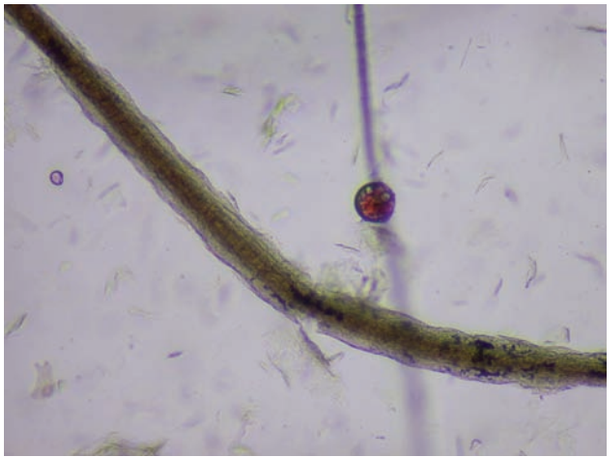


Figure 1.43 Trichogram of a cat with dermatophytosis showing the dermatophyte organisms invading the walls of the hair shaft, creating a "rotten log" appearance. The hair was obtained in this case via skin scrapings, causing the micelle of red blood cells within mineral oil above the affected hair (10×).

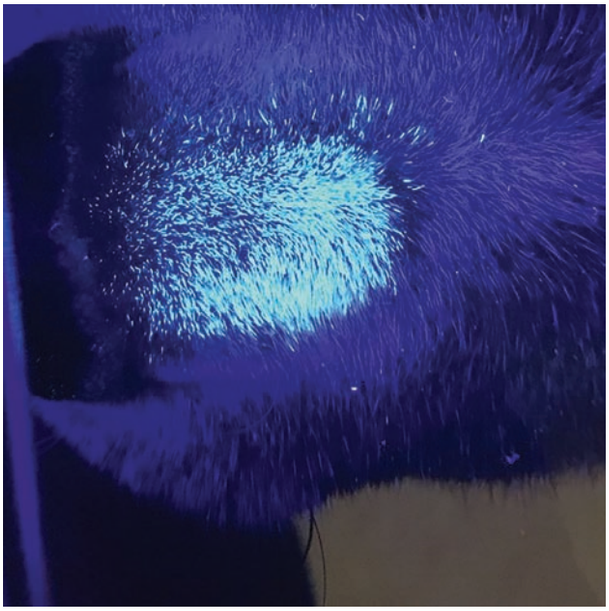


Figure 1.44 Wood's lamp examination of a dog with *Microsporum canis*; the infected hair shafts fluoresce apple green.

Figure 1.45A Toothbrush samples are obtained with a new toothbrush vigorously brushed across affected areas.

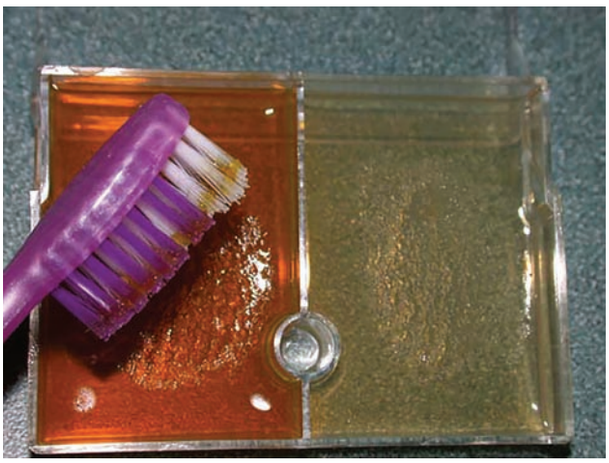


Figure 1.45B The toothbrush bristles are then gently and partially embedded into the dermatophyte culture media.

- Nordic breeds are thought to have a telogen predominant cycle (10 : 90) (Dunstan et al. 2001); Welle and Wiener, 2016), though one study on normal Huskies showed a 50 : 50 anagen/telogen ratio (Diaz et al. 2006).
- There is no exact "normal ratio" but observing trends in anagen and telogen hairs can be helpful.
- In a study of Boxers and Labradors, more telogen hairs were found during the hottest months of the year, and an increase in anagen hairs during the coldest months (Favarato and Conceição, 2008).
- The mean percentage of telogen hairs was 93, 90, and 55.3% for Boxer, Labrador, and Schnauzer.
- Adult Labradors housed indoors had >80% telogen hairs in all seasons.
- Dogs with continuously growing haircoats (i.e. Poodles) have approx. 90% anagen hair bulbs (similar to humans).
  - Trichogram of a Poodle with only 50% anagen roots may increase suspicion for possible endocrinopathy.

Figure 1.46A and B Use a high-quality Wood's lamps ideally with magnification.

1.5 Dermatophyte culture technique

- To obtain samples for dermatophyte culture, use sterile hemostats to pluck hairs from around the periphery of a newly formed or expanding skin lesion, and avoid areas which may have been recently medicated.
- Ideal hairs to select are those in areas of active crusting, and hairs which appear damaged or misshapen and/or fluoresce under a Wood's lamp (Figure 1.44).
- In addition to hair plucks (which can potentially miss infected hairs and may not sample infected epithelium adequately), it is ideal to also obtain samples using the MacKenzie toothbrush technique:
  - In this technique, a new toothbrush is removed from its packaging and the toothbrush is rubbed gently over the suspect area, including the skin and haired margins of alopecic or scaly lesions (Figure 1.45A).
  - If culturing a suspected asymptomatic dermatophyte carrier, use the toothbrush to sample the fur over the entire body, ending on the face and paws.
  - The toothbrush bristles are then gently embedded into the fungal culture media, taking care not to embed the bristles too deeply (which risks displacement of culture media when the bristles are removed (Figure 1.45B).
  - Hair and debris which are caught within the bristles can be removed with sterile hemostats and then placed on the fungal culture surface.
  - Toothbrushes can be obtained inexpensively in bulk from dollar stores or from online distributors.
  - The MacKenzie toothbrush technique is helpful to screen asymptomatic carriers, and to obtain samples from animals undergoing antifungal treatment in which skin lesions have clinically resolved. In these cases, the toothbrush is stroked over the entire body, concentrating especially in areas with prior lesions and, in cats, on the face, ears, and paws.
  - In cases of suspected onychomycosis, the toothbrush can be used on the affected nail bed; additionally, samples of nail bed fur can be obtained with sterile hemostats, and the proximal affected nail can be sampled using a scalpel blade to shave off small pieces of keratin (precleaning of the nail with alcohol is recommended to help reduce accumulated saprophytic or environmental fungal organisms). If an avulsed toenail is considered for fungal culture, the distal part of the nail should be discarded, and ideally samples for culture obtained by scraping the proximal concave aspect of the claw.

1.6 Wood's lamp examination

- Wood's lamps and black lights are different!
  - A Wood's lamp is a UV light which emits wavelength 320–450 nm (peak 365 nm), filtered through a cobalt or nickel filter.
  - A black light is composed of a clear glass that filters medium and short-wave UV light and emits a large amount of blue visible light along with long

wave UV light; It is hard to see fluorescence due to the large amount of visible light.
  - An example of a black light is the light bulb in a bug catcher.
- Use a Wood's lamp with electric plug and magnification (Figure 1.46A and 1.46B):
  - Warm up for five to ten minutes before use, light's wavelength and intensity are temperature dependent.
  - Expose fur for three to five minutes, some ringworm strains may be slow to fluoresce (or our eyes are slow to adapt to darkness).
  - Examine in dark room, hold lamp a few inches away from skin/fur.
- Pluck fluorescing hairs (Figure 1.44) for dermatophyte test medium (DTM) and direct microscopic examination.
- False negative Wood's lamp results can occur in 20–50% of *Microsporum canis* cases (usually due to user error), in all *Microsporum gypseum* and *Trichophyton mentagrophytes* cases, and after use of topical iodine.
- False positive Wood's lamp reactions can occur due to pyoderma, *Demodex*, keratin, soap, topical medications, and carpet fibers.
- The fluorescent metabolic product is a pigment that is incorporated into the hairs and will remain even when the fungus is dead.
- As infection resolves, fluorescence is lost in the proximal hairs and glowing tips may remain yet be culture negative.

1.7 Dermatophyte culture medium selection and incubation

- DTM contains Sabouraud's dextrose agar with cycloheximide, gentamycin, and chlortetracycline as antifungal and antibacterial agents to retard growth of contaminant organisms. Additionally, the pH indicator phenol red is added.
  - Dermatophytes preferentially metabolize protein in the culture medium, causing alkaline metabolites and turning the yellow fungal culture medium to a red color at exactly the same time as the dermatophyte colony appears.
  - Most other fungi initially utilize carbohydrates with resultant acidic metabolites; these saprophytic fungi can eventually consume protein and cause media color change, but this usually happens several days after fungal growth occurs.
- Daily observation and logging of fungal growth correlated with media color change is thus very important in correctly interpreting DTM culture results.
- Additionally, since some non-dermatophyte fungal organisms can cause positive media color change concurrent with colony growth and mimic dermatophytes, microscopic examination of all suspect colonies is very important to avoid misidentification.
- Microscopic examination can be done in the clinic, or the entire culture plate can be sent to a reference laboratory for fungal identification (usually at a reduced cost compared to fungal culture).
- To facilitate fungal sporulation and identification, it is helpful to use a DTM plate which also has a separate area of plain Sabouraud's agar or rapid sporulation medium (RSM) which do not contain inhibiting agents.
- According to a fungal culture manufacturer's recommendations (www.vetlab.com), culture media should be stored at 2–25°C (36–77°F) and protected from light prior to inoculation.
  - Plates should be allowed to warm to room temperature prior to inoculation.
  - Prior to and during inoculation procedures, plates should be handled in a manner that minimizes exposure of the media to the environment. Expired plates or plates that exhibit drying, cracking, discoloration, microbial contamination, or other signs of deterioration should not be used. The presence of excessive condensation may appear in plates that have been damaged by exposure to temperature extremes.
- Fungal cultures should be incubated at room temperature (25–30°C) with 30% humidity.
- Most organisms will appear within 7–10 days, however plates should be kept for 14–21 days, especially when no growth is seen initially, or when the sample has been obtained from a pet currently under therapy with antifungal medications.
  - According to one DTM manufacturer (www.vetlab.com), dermatophyte culture plates may be incubated in full light, although some recommend incubation in the dark to avoid UV light-induced inhibition of fungal growth.
  - In dry climates, culture plates should be placed in plastic bags or containers to prevent dehydration of the media which can inhibit growth of organisms.
- After 48–72 hours, begin examining the plates daily for characteristic media color changes and fungal growth.
- If optimal dermatophyte culture storage conditions, daily observation of fungal colony growth and media color change, and subsequent microscopic identification of suspect fungal organisms are not feasible in the individual clinic situation, then submission of skin and hair samples (placed in a sterile red top tube) from suspect cases to a veterinary reference laboratory for fungal culture is recommended to avoid misdiagnosis. Even some veterinary dermatologists elect this option to minimize the chance of false negative or false positive dermatophyte cultures.

1.8 Identification of dermatophytes

- Macroscopic fungal colony morphology is an important first step in determining if a dermatophyte is present.

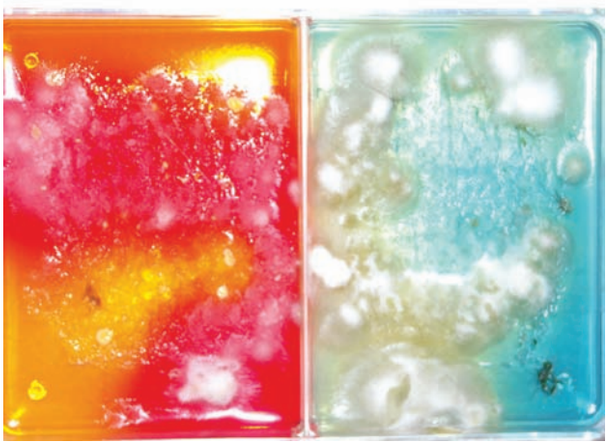


Figure 1.47 A dermatophyte culture plate growing *Microsporum canis*; white to cream cottony colonies with concurrent media color change.

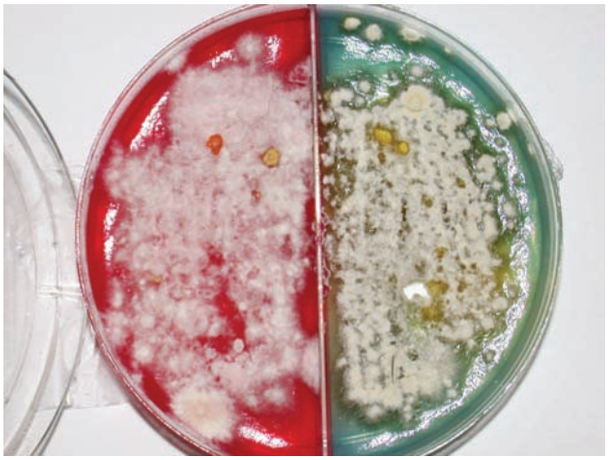


Figure 1.48 *Microsporum gypseum* has a white to buff-colored, slightly powdery appearance.

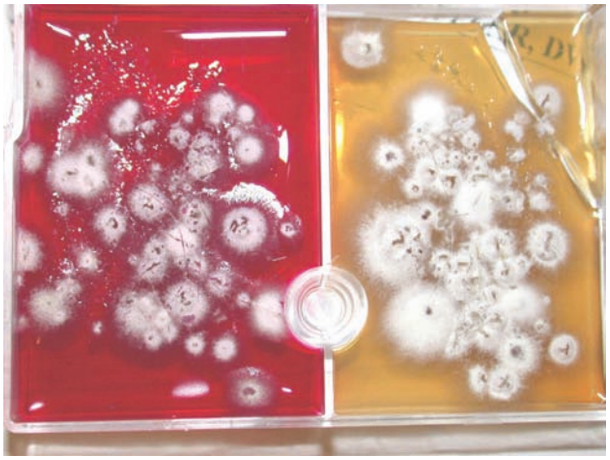


Figure 1.49 *Trichophyton mentagrophytes* colonies are white to cream in color with a powdery to granular texture.

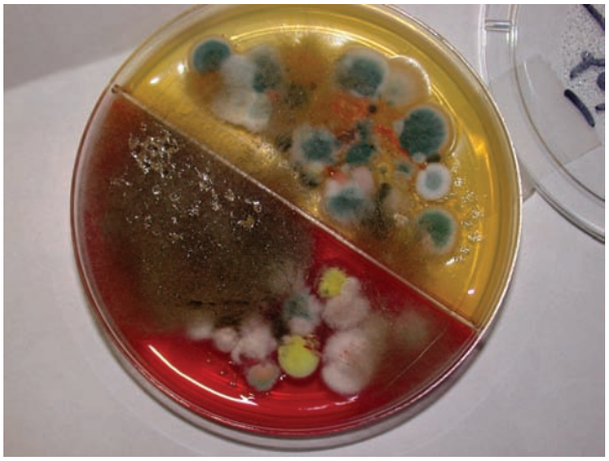


Figure 1.50 Dermatophytes are never black, green, or gray; this dermatophyte culture has numerous contaminant colonies, and any colonies of interest should be recultured onto a new plate. Contaminant fungi can also cause media color change usually after several days of incubation.

- *Microsporum* and *Trichophyton*, the most important dermatophytes in dogs and cats, appear as white, light yellow, tan, or buff-colored, cottony to powdery appearing colonies (Figures 1.47–1.49).
- Dermatophytes are never black, green, or gray (Figure 1.50).
- Microscopic evaluation of suspect fungal growth is also important, since some environmental fungi can mimic dermatophytes in gross colony morphology and ability to turn the media red.
- Gloves should be worn to avoid transmission of dermatophyte spores to the hands. A small piece of clear acetate tape is gently touched to the surface of the fungal colony and then the tape is applied to a glass slide over a drop of blue stain such as methylene blue, or the blue Diff-Quik solution (basophilic thiazine dye).
- The slide is examined under 10–40× for the characteristic dermatophyte macroconidia.
- In early cultures, only fungal hyphae with no macroconidia may be seen (especially in cases of *Trichophyton*), and these cultures should be incubated longer to allow for spore development.
- *M. canis* has numerous large spindle-shaped, thick-walled spores with a terminal knob and six or more internal cells (Figure 1.51).
- *M. gypseum* produces numerous large spindle-shaped spores with thin walls, no terminal knob, and six or less internal cells (Figure 1.52).

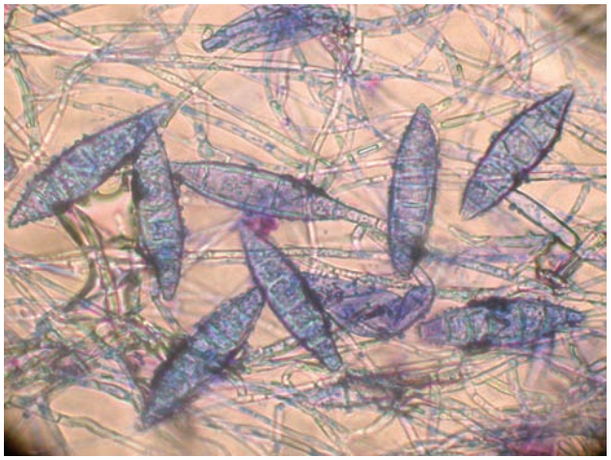


Figure 1.51 *Microsporum canis* has numerous large spindle-shaped, thick-walled spores with a terminal knob and six or more internal cells (40×).

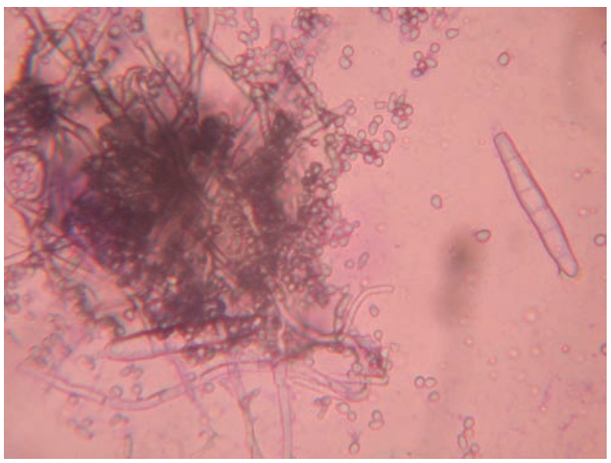


Figure 1.53 *Trichophyton mentagrophytes* produces sparse, cigar-shaped spores with thin walls; there are numerous round microconidia (40×).

- *T. mentagrophytes* produces long cigar-shaped macroconidia with thin walls; spores may be few in number; spiral-shaped hyphae are also characteristic of *Trichophyton* (Figures 1.53 and 1.54).
- In cases in which the fungal species cannot be easily identified in the clinic, then the dermatophyte culture should be submitted to a veterinary reference laboratory for fungal identification.

1.9 Dermatophyte PCR

- In recent years, dermatophyte PCR (polymerase chain reaction) has become an available screening test for dermatophyte infections in dogs and cats.
- Samples for PCR should be obtained from lesions using a toothbrush and by collecting scales and crusts.

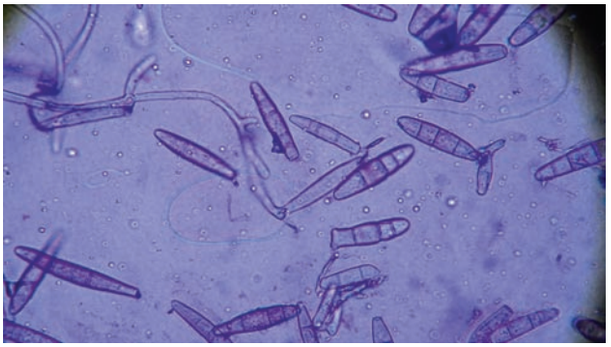


Figure 1.52 *Microsporum gypseum* produces numerous large spindle-shaped spores with thin walls, no terminal knob, and six or less internal cells (40×).

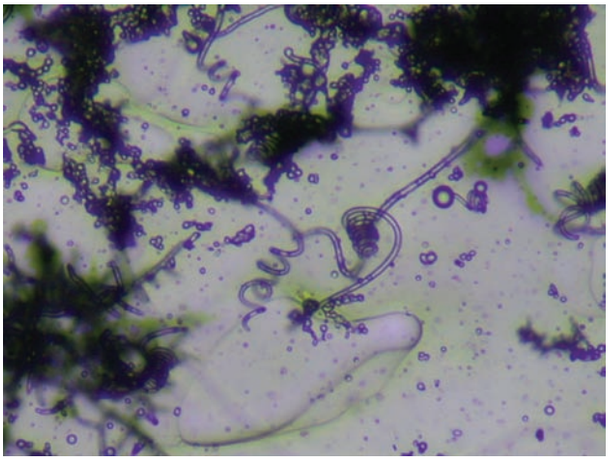


Figure 1.54 Spiral hyphae are also suggestive of *Trichophyton mentagrophytes* (40×).

  - If toothbrushes are submitted, wrap the head of the sample in a plastic bag (do not tape shut) and then place this into a second bag.
  - If crusts or scales are submitted, use a sterile red top tube. Dry skin scraping samples can be collected using a skin scraping spatula or forceps.
- Because dermatophyte PCR is very sensitive, false negative results are rare, but can occur if not enough material is submitted for analysis; it is important to submit adequate hairs with intact roots.
- Because PCR testing accurately identifies fungal DNA but does not discriminate between viable and nonviable fungal DNA, discordant test results between PCR and dermatophyte culture can occur when dead dermatophyte DNA is detected by PCR in successfully treated animals in which the dermatophyte culture is negative.
  - Rarely, a positive PCR may be misinterpreted as clinical infection if a single environmental dermatophyte spore such as *M. gypseum* or *Trichophyton* is coincidentally picked up.

- Fungal PCR is reported as positive or negative. Unlike follow-up dermatophyte cultures in which decreasing colony counts can be assessed as a measure of treatment efficacy, PCR cannot accurately determine number of dermatophyte spores present.
- Ideally, both dermatophyte PCR and dermatophyte culture should be used concurrently for optimal diagnosis of dermatophytosis, then follow-up dermatophyte cultures are obtained by toothbrush technique every one to two weeks to determine when treatment may be discontinued (after two negative cultures).

1.10 Bacterial culture

- Culture is indicated if there is poor response to empiric antibiotics, deep pyoderma, or rod bacteria on skin cytology.
- In superficial infections, aerobic bacterial culture is the only culture needed.
- In animals with draining tracts or nodules, samples may also be needed for anaerobic culture, fungal culture, or mycobacterial culture.
  - In these cases, a deep tissue biopsy rather than a swab is the best sample.
- Prior to culture, stop topical and systemic antimicrobials for 48 hours if possible (however, if numerous bacteria are found on cytology despite antibiotic treatment, culture delay may not be necessary).
- To obtain the culture sample, options include using a sterile culturette to swab a freshly ruptured pustule (Figure 1.55) or lift a crust and use the swab to sample the exudate under crust. In dry lesions, use a saline moistened swab to rub under rim of epidermal collarette or to vigorously rub several scaly areas (Figure 1.56).

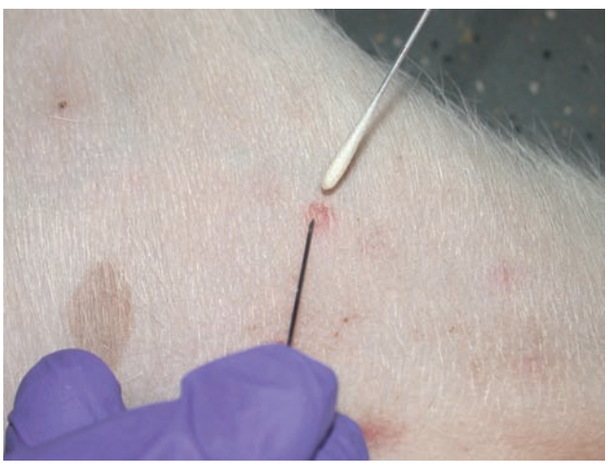


Figure 1.55 A sterile culturette for aerobic culture is used to sample a pustule ruptured by a sterile needle.

- Alternatively, bacterial skin cultures can be performed by obtaining a 4–6 mm punch biopsy of papule, pustule, or crusted lesion, which is then placed in a sterile red top tube (+/– with 0.25–0.5 cc sterile/not bacteriostatic saline to keep it moist; contact the laboratory you are using to determine their preferred submission protocol), then submit for macerated tissue culture.
  - Lidocaine has antibacterial properties, so ideally use sedation to obtain biopsies for culture.
- It is important to always perform skin (or ear) cytology at the time of culture in order to be able to interpret culture results, for example, if cocci bacteria were seen on cytology, but only rods were cultured, this indicates that the culture should be rechecked by the lab or resubmitted. Or if only yeast are found on cytology of skin or ears, then this would mean bacterial culture is not indicated.

1.11 Skin biopsies (*See videos on companion website*)

- Skin biopsies should be performed in cases of suspected neoplasia, vesicular or ulcerative diseases, unusual or atypical cases, and in cases which have not responded to conventional trial therapy.

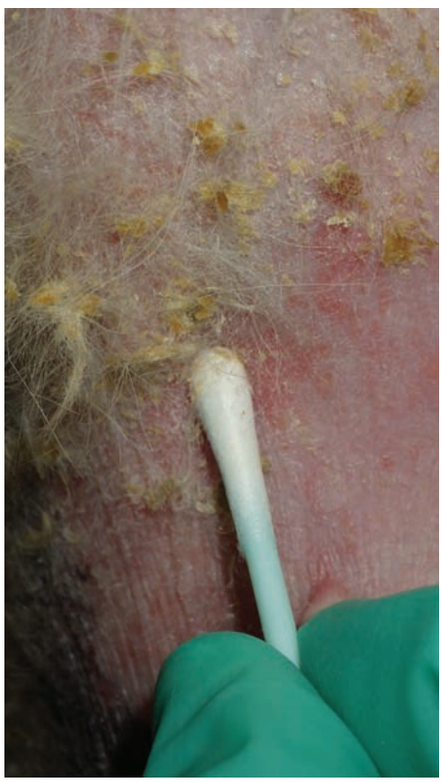


Figure 1.56 When pustules are not present, use a saline moistened culturette to sample under multiple crusts and submit for aerobic culture.

  - In general, skin biopsies should be performed within three to four weeks of the onset of the disease, since more chronic lesions can be difficult to interpret due to changes secondary to infection, scarring, or steroid therapy.
- Check cytology of superficial or crusty lesions prior to biopsy; since secondary infection can alter histopathologic findings (e.g. discoid lupus and mucocutaneous pyoderma appear very similar histologically), pretreatment with antibiotics for two to three weeks may be necessary.
- Steroid therapy can also change biopsy results, and ideally patients should not receive oral or topical steroids within two to three weeks and injectable long-acting steroids within six to eight weeks of performing the biopsy procedure (severe, life-threatening cases would obviously be an exception to this rule).
- If possible, choose primary lesions such as papules, pustules, vesicles, macules, or nodules. In suspected cases of discoid lupus erythematosus, select early depigmented lesions (before erosion or scarring occurs).
  - Even if primary lesions are not present, diagnostic information can be obtained from crusts, which should be carefully preserved with the skin biopsies. Lesions which are less likely to be diagnostic include excoriations, ulcers, or chronically scarred areas.
- More information is gained by performing multiple (three to five) samples, obtained from a variety of lesions.
- To obtain biopsy samples, local anesthetic (0.5–1 cc of 1–2% lidocaine injected with a 25G needle subcutaneously under the lesion) and/or mild sedation will be needed (Figure 1.57).
  - Hair overlying the lesion may be gently clipped, but the clipper blades should not touch the skin.

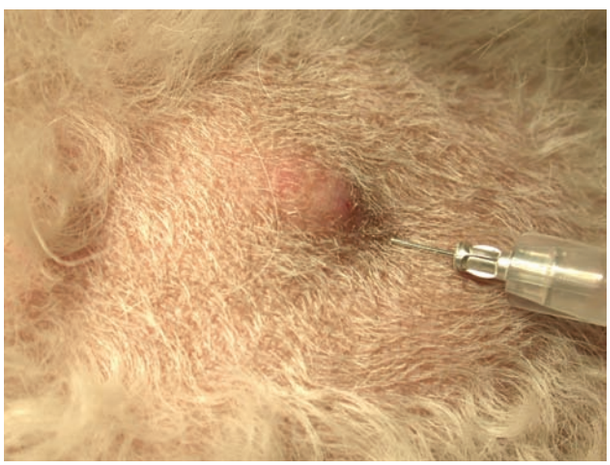
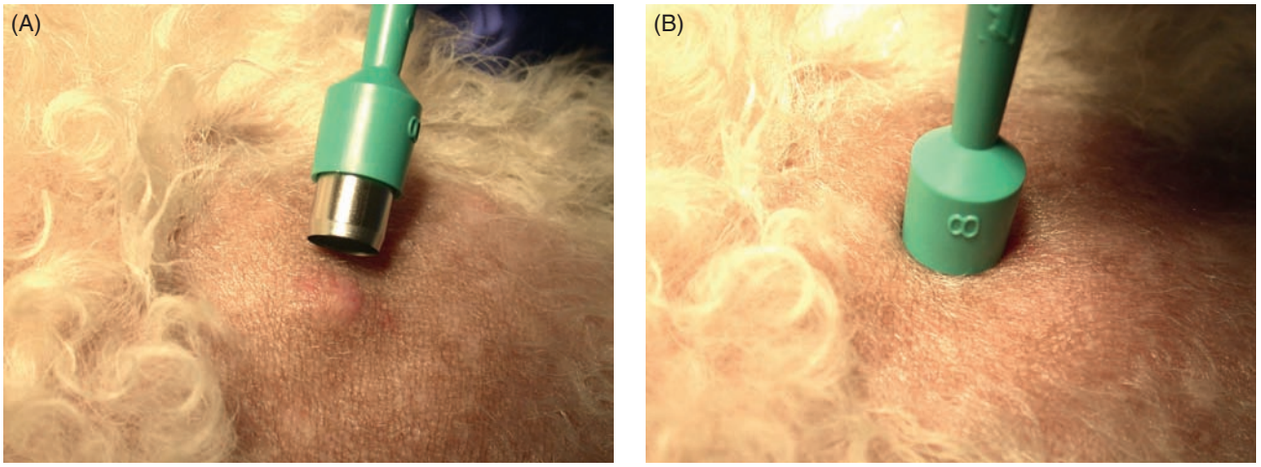


Figure 1.57 Lidocaine is injected using a 25G needle subcutaneously under the lesion to be sampled.

  - Do not surgically prep or disinfect the areas, as important skin debris will be lost.
  - Do not exceed a total lidocaine dose of 5 mg/kg for dogs or 2.5 mg/kg for cats (2% lidocaine = 20 mg/ml); lidocaine may be diluted 1 : 1 with saline if needed for small patients.
- 6 mm punch biopsies are preferred for most cases; 4 mm punches may be necessary for difficult areas such as near the eye, on the ear, and on the nasal planum or footpads of smaller patients.
  - Use new, sharp biopsy punches, as older used ones tend to shear and distort tissue and create artifact.
- Excisional biopsy with a scalpel may be indicated for larger or nodular lesions or for diseases of the subcutaneous fat.
- Place the area of interest in the center of the biopsy punch and do not include a significant amount of normal skin with the biopsy (Figure 1.58A and 1.58B); the only time a lesion should be biopsied on the margin is in the case of an ulcerative skin disease.
  - If a large lesion appears different in the center and leading edges, biopsy both areas.
- Push the biopsy punch down gently in a rotational motion in one direction to avoid shearing artifact until the epidermis and dermis are penetrated and the biopsy punch rotates freely.
  - When handling the skin biopsy, avoid crush artifact by grasping only the subcutaneous tissue with thumb forceps (Figure 1.59).
  - Prior to fixation, skin biopsies can be placed fat side down on pieces of wooden tongue depressor to prevent tissue folding and aid in orientation when samples are processed.
- Biopsies from radically different lesions or nodules should be tagged with a suture or placed in individually labeled containers for differentiation.
- Within five minutes of obtaining the specimens, skin biopsies should be fixed in 10% neutral phosphate buffered formalin (minimum 10 parts formalin to 1 part tissue for adequate fixation).
- Close skin biopsy sites with 3-0 Nylon in a simple interrupted or cruciate pattern (Figure 1.60A and 1.60B). To close biopsy sites in difficult to access places for future suture removal, use absorbable suture.
- When obtaining a sample for tissue cultures, place the biopsy in a sterile red top tube (+/– with 0.25–0.5 cc sterile/not bacteriostatic saline to keep it moist; contact the laboratory you are using to determine their preferred submission protocol).
- Skin histopathology results are optimized by utilizing experienced dermatopathologists.
  - In cases with histologic inflammation suspected to be related to infectious disease, special stains to highlight organisms are indicated, and many



Figures 1.58A and B Place the biopsy punch in the center of the area of interest, then push down gently in a rotational motion in one direction to avoid shearing artifact until the biopsy punch rotates freely.

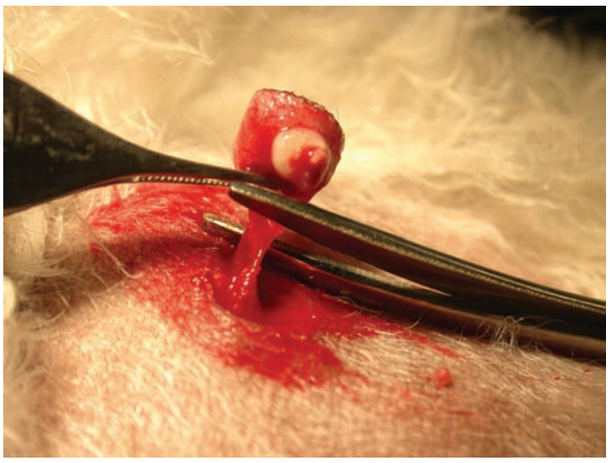
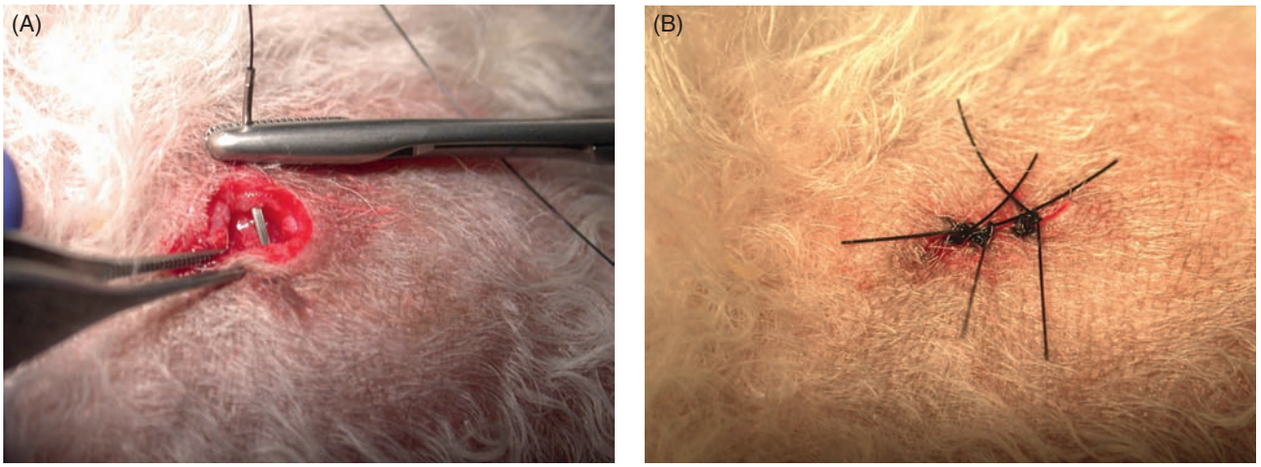


Figure 1.59 Avoid crush artifact by grasping the subcutaneous tissue/fat with the forceps.

dermatopathologists will add these stains on at no extra cost.
  - Special stains which may be needed include Gram stain for bacteria, GMS (Gomori methenamine silver)and/or PAS Periodic acid–Schiff) stains for fungal organisms and Zygomycetes such as pythiosis, and acid-fast stains for mycobacteria and filamentous bacteria.
- It is also essential to give a complete signalment and history, including description and distribution of lesions, other symptoms and results of pertinent diagnostic tests, current or past therapy/response to therapy, and the clinician's differential diagnoses. Many pathologists will accept and appreciate digital patient photographs. These elements are important to allow the pathologist to formulate an accurate diagnosis, and if there are questions, most pathologists are happy to discuss their findings with the clinician.



Figures 1.60A and B Close the biopsy site with two to three simple interrupted, or one or two cruciate sutures.

1.12 Allergy testing

- It is important to emphasize that allergy testing is not a first line screening test in the work up of dogs and cats with dermatologic disease.
- Allergy testing for environmental allergens, whether by serology or intradermal testing, is only performed when the clinical diagnosis of atopic dermatitis has been made by first ruling out all other causes of pruritus/dermatitis.
  - Rule out parasites with skin scrapings and trial treatment for parasites such as fleas and mites.
  - Rule out infection with cytology/culture and treatment of infectious organisms.
  - Rule out adverse food reaction with a carefully performed prescription or home cooked hypoallergenic diet trial for six to eight weeks with no other treats, table scraps, rawhides, milkbones, pill pockets, fish oil, chewable flavored medications, or supplements.
  - Serology for food allergy is not accurate and cannot be used as an alternative to a hypoallergenic diet trial.
- The purpose of allergy testing is to determine which allergens will be selected for a hyposensitization vaccine.
- Please see Chapter 10 for more information about allergy testing and allergen selection.

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