

A guide to diagnosing skin infections in practice



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Introduction

Due to the fact that 25% of dogs and 18% of cats presented in practice suffer from skin infections or skin disorders, dermatology is one of the routine challenges the veterinarian faces.* To find the cause of the disorder and to achieve a successful therapy, it is important to perform a detailed diagnosis.

There are a number of dermatologic tests to determine the cause of infection, and not all require a specialist laboratory. Some of these tests can easily and quickly be performed in practice. They do not require specialist equipment and can in most cases deliver a definite result while the patient is still in the practice.

This booklet looks at the six most commonly used dermatological tests that can easily be performed in any practice. It describes – in an easy-to-use format – when and why a test should be performed, what it will show up, what utensils are required and how it is conducted, with a large selection of images to help visualise individual steps.

This booklet is provided by Pfizer Animal Health to support everyday practice and enhance dermatology services.



Equipment needed for “in-practice” dermatological tests

* Bio'sat study, Pfizer Animal Health, July 2009

Cytology

When do I do it?

- When bacterial or fungal infection is suspected (inflammatory alopecia, seborrhoea, scales, papules, pustules, crusts, erosions, ulcers)
- In patients with nodules/tumours - do cytology on every nodule/tumour
- In patients with suspected pemphigoid diseases (erosions, pustules, crusts)
- In every patient with otitis externa

What can I find?

- Cocci (most likely *Staphylococcus sp.*)
- Rods → culture and sensitivity advisable
- Inflammatory cells with intracellular bacteria → clinically relevant infection that requires systemic antibiotic treatment
- Eosinophils → can point to ectoparasites or allergies
- Macrophages → chronic, sterile and infectious processes!
- *Malassezia spp.* → one or more *Malassezia sp.* per High Power Field (x 1000 magnification) may be clinically relevant, basal numbers vary with the climate. In cases of *Malassezia* hypersensitivity a much lower number of *Malassezia* (e.g. one in every two or three HPFs) can cause clinical disease. A topical or systemic treatment should be considered.
- Neoplastic cells

What do I need?

- Slides, DiffQuick® or similar stain, mineral oil, adhesive tape, microscope, needle and syringe

How do I do it?

- Impression smear
 - Rub or impress a slide on moist, exuding or greasy surface of infected skin.
 - Role a cotton bud on the skin surface or insert it in the ears and role cotton bud on the slide.
 - Insert needle (25 - 27 ga.) into the pustule holding the needle parallel to the skin so that only the pustule is punctured, no deeper cells or blood are required, top is lifted off and slide impressed onto the ruptured pustule.
 - Use the sticky surface of the adhesive tape to collect cells and surface organisms from dry and / or scaly skin and then place this (sticky side down) onto a glass slide with a drop of DiffQuick®. The tape acts as its own coverslip.
- Aspiration smear
 - Insert needle into nodules or abscesses and re-insert a number of times without leaving the skin. Withdraw the needle. A syringe with the plunger pulled back is attached to the needle and contents is blown onto a slide and air dried.
- Stain the air dried slides (e.g. DiffQuick®)
- Put the slides under a microscope, condenser up

Tip

- In case of dry skin or in the interdigital area:
 - Moisten a cotton bud with saline solution or carefully rub the edge of a slide on the skin and then rub the material on the slide
 - Press clear sticky tape (sticky side down) onto the skin. Stain the tape like a slide, let it air dry and press it onto a slide or put a drop of the blue stain of DiffQuick® on a slide and press the tape sticky side down on the drop. Evaluate under a microscope.



Superficial pyoderma*



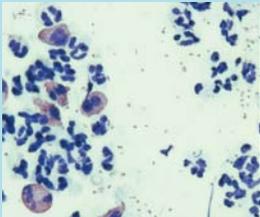
Use the adhesive tape technique on dry skin or in the interdigital area*



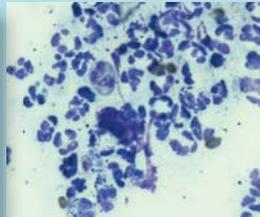
Impression smear: slide pressed on skin*



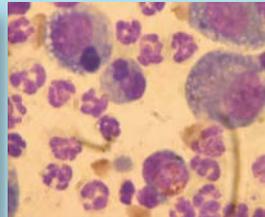
Aspiration smear: insert needle into nodule*



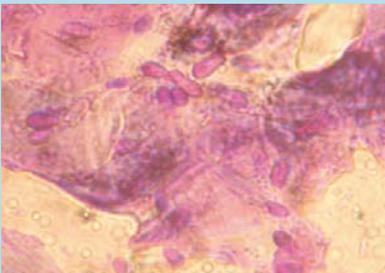
Eosinophils, neutrophils and bacteria*



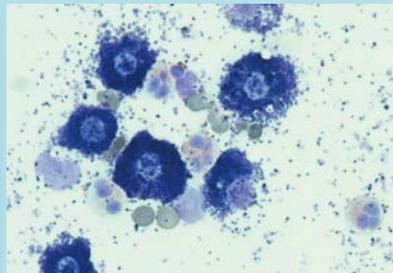
Superficial pyoderma: neutrophils with intracellular cocci*



Pyogranulomatous inflammation (deep pyoderma): many neutrophils and macrophages, few bacteria†



Malassezia and bacteria†



Grade 1 mast cell tumour with eosinophils*

*Courtesy of Sonya Bettenay
†Courtesy of Stefanie Peters

Superficial Skin Scraping

When do I do it?

- On every patient with pruritic or scaly skin

What can I find?

- *Cheyletiella* sp., *Scabies* sp., *Notoedres cati*, *Otodectes cynotis* → A finding of one of these mites or their eggs is diagnostic
- Dermatophyte spore infested hairshafts

What do I need?

- Slides, cover slips, scalpel blade, mineral oil, microscope.

How do I do it?

- Gently clip the affected area(s) leaving 2-3 mm of hair so that the scales and crusts are not dislodged. Put mineral oil on your scalpel blade and several drops directly onto the skin.
- The oil is gently scraped off with the scalpel blade and the material is put on one or more slides. These mites live on or within the surface layers; there is no need to draw blood with a superficial skin scrape.
- Examine slides under microscope with the condenser down

Tip

- Sample the areas where the mites you are looking for are most likely to be found. For *Sarcoptes* spp. this would be ear margins, elbows, hocks and ventral body.
- Mites may be hard to find. The bigger the surface area scraped, the better the chance of a positive result. In the case of a negative result and *Sarcoptes* mites are still suspected a diagnostic therapeutic trial over a 6 week period is the most definitive next step.
- A serum antibody test is highly sensitive, but the antibodies take 2-5 weeks (up to 4 months) after infestation to rise, and cross reactions with dust mites are possible.
- Some people prefer to use adhesive tape to collect *Cheyletiella* mites. With this technique, the tape is pressed over multiple sites of scaling and is also dragged across the hair shafts. The tape is placed directly onto a glass slide without any oil or staining and is again viewed with the condenser down.
- When Dermatophytosis is suspected, use only the minimum of oil necessary to “fix” or “hold” the hairs, surface scale and debris.



Scaly dog skin*



Scaly dog skin caused by *Cheyletiella* mites†



Put several drops of mineral oil directly onto clipped areas†



Place oil and debris onto a slide. Scrape several large areas and prepare various slides for evaluation*



Sarcoptes †



Cheyletiella mites†



Cheyletiella blakei †



Otodectes cynotis †

*Courtesy of Sonya Bettenay

†Courtesy of Francesco Albanese and Frederico Leone

Deep Skin Scraping

When do I do it?

- In cases of suspected demodicosis (non-inflammatory alopecia, comedones, pustules, crusts, inflammatory alopecia)

What can I find?

- *Demodex* mites → more than one mite is diagnostic

What do I need?

- Slides, cover slips, scalpel blade, mineral oil, microscope

How do I do it?

- If necessary, clip 1cm² of the area you want to scrape to remove hairs
- Squeeze the skin prior to scraping to push the mites out from the depths of the hair follicles
- Scrape the skin in the direction of hair growth with a blade covered with mineral oil until capillary bleeding is observed
- Put the material on the slide
- Place the slide under a microscope with the condenser down and use the x 400 magnification to look for adults, larvae/nymphs and eggs

Tip

- Feet and face are hard to scrape → Trichogram may give equivalent results if 1cm² of hairs are sampled.
- Some breeds (e.g. Shar Pei) are hard to scrape and may have to be biopsied for diagnosis.



Dog with demodicosis: inflammatory alopecia and hyperpigmentation*



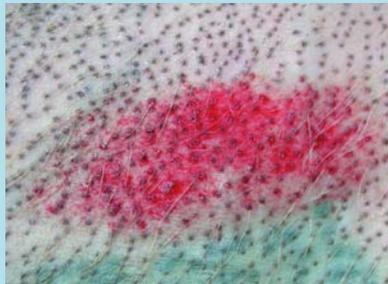
Puppy: focal alopecia[†]



Squeeze skin prior to scraping*



Scrape until light capillary bleeding is observed*



Excoriation after deep skin scraping[†]



Demodex mites[†]



*Demodex canis**

*Courtesy of Sonya Bettenay

[†]Courtesy of Francesco Albanese and Frederico Leone

Wood's Lamp Examination

When do I do it?

- In every patient with possible *Microsporum canis* infection (inflammatory and non-inflammatory alopecia)

What can I find?

- Fluorescing hairshafts

What do I need?

- Wood's lamp, which has been "warmed up" for 5 minutes prior to use

How do I do it?

- Illuminate the affected area in a darkened room. In 50 - 60 % of *Microsporum canis* infections there will be greenish fluorescence which runs along the hair shafts.

Be careful: A lack of fluorescent areas does not rule out dermatophytosis as only half of them show fluorescence.

In the case of negative results → perform fungal culture using McKenzie toothbrush technique (see: Fungal Culture, Tip).

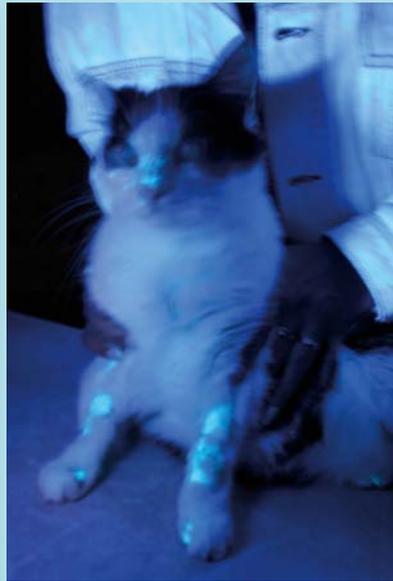
- Pluck hairs with fluorescence along hair shafts and use them for trichoscopy and/or fungal culture.

Tip

- For better results warm the lamp up for 5 minutes before use because the stability of the wave length and intensity is temperature-dependent.
- Drugs, soaps and bacteria (*Pseudomonas sp.*) or occasional individual scales can fluoresce as well, but they should not be associated with the hair shafts.



Wood's Lamp[†]



Positive fluorescence in feline dermatophytosis[†]



Yorkshire Terrier with a mixed *Microsporum canis* and *Microsporum gypseum* infection*



Microsporum canis: fluorescence running along hair shaft[▲]

[†]Courtesy of Stefanie Peters

^{*}Courtesy of Tim Nuttall

[▲]Courtesy of Teton New Media

Fungal Culture

When do I do it?

- In every patient with suspected fungal infection

What can I find?

- *Microsporum canis*
 - White, woolly colonies with a yellowish reverse pigment
 - Abundant thick walled, spindle-shaped macroconidia with knobs at the ends and typically more than six internal compartments
- *Microsporum gypseum*
 - Granular, beige cultures with yellowish reverse pigment and thin-walled macroconidia with less than six internal compartments
- *Trichophyton mentagrophytes*
 - Variable colonies with very few, cigar-shaped macroconidia and large numbers of small, round microconidia

What do I need?

- Dermatophyte test medium (DTM), clear sticky tape, slides, microscope, methylene blue or DiffQuick® blue

How do I do it?

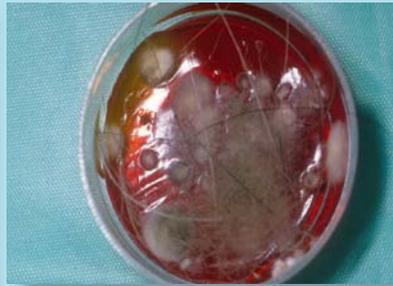
- Use a scalpel without oil to scrape and haemostats to pluck; take hairs and scale from the edge of a lesion (preferably the ones fluorescing under the Wood's light)
- Impress hairs and scale gently on DTM; do not screw the lid tight
- Incubate the agar at 20-25°C (a warm, dark space with moisture)
- Check the agar **daily** over a 3 week period
- Colour change (pH change) that occurs when the colony is still small and then spreads as the colony grows, is indicative of dermatophytes
- When the colony is 10-14 days old, impress clear sticky tape (sticky side down) gently on the suspicious colonies and lay it down onto a drop of methylene blue on a slide.
- Evaluate the sample under a microscope with the condenser up. The sticky tape acts as its own cover slip.

Tip

- If the patient has no clearly circumscribed lesion or an asymptomatic carrier is suspected use the **McKenzie toothbrush technique**.
 - Brush the hair with a new toothbrush
 - Gently place the hairs and scale, using a sterile needle, onto the agar or cut the bristles with sterile scissors
 - Put all the material (bristles, hairs, scale) onto the agar
- Agar colour changes can also occur with large saprophyte colonies. **Daily** control of the culture is **imperative** to notice a colour change that accompanies a growing culture.



Dog with localised dermatophytosis (Kerion)[†]



DTM false positive: saprophyte colonies[†]



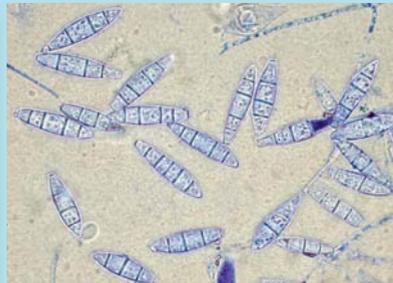
DTM positive: *Microsporium canis*^{*}



Microsporium canis macroconidia^{*}



DTM positive: *Microsporium gypseum*[▲]



Microsporium gypseum macroconidia[▲]



DTM positive: *Trichophyton mentagrophytes*[▲]



Trichophyton mentagrophytes macroconidia and microconidia^{*}

[†]Courtesy of Stefanie Peters

^{*}Courtesy of Tim Nuttall

[▲]Courtesy of Francesco Albanese

Trichogram

When do I do it?

- In every patient with alopecia
 - To look for broken hair tips when one suspects self-induced alopecia
 - To determine if hairs are in anagen or telogen phase (the interpretation of ratios of telogen hairs to anagen hairs in dogs is breed and season-dependent and exact ratios have not been established)
 - When dermatophytosis is suspected
 - As an alternative to a deep skin scraping when *Demodex* is suspected

What can I find?

- Broken off hair tips → caused by self-trauma
- Tapered hair tips → hair loss is caused by events within the follicle e.g. endocrine disorders or inflammation involving the hair follicle
- Hairs in anagen (growing) phase → roots of anagen hairs are rounded, curled, bent and often smooth and pigmented
- Hairs in telogen (resting) phase → roots of telogen hairs are lancet-shaped and lack pigmentation, although the base of the hair may show a roughened or brush-like edge
 - The presence of numerous anagen phase hairs should decrease the suspicion for an endocrinopathy
- In the case of dermatophytosis, affected hairs are covered with spores and penetrated by hyphae
- Colour dilution alopecia → melanin is clumped in the hair shaft

What do I need?

- Forceps/haemostat or rubber covered clamp, mineral oil, slide, cover slip, microscope

How do I do it?

- Pluck a small number of hairs in a partially or completely alopecic area using forceps/clamp in direction of hair growth; hold the forceps/clamp close to the skin surface and grasp all hair shafts which emerge
- Put a drop of mineral oil onto a slide, place the hairs in parallel order on the mineral oil, separate them to evaluate roots and tips adequately
- Cover hairs with a cover slip and evaluate under microscope

Tip

- Cover the tips of your forceps or clamp with rubber or silicon sleeves to avoid crushing or breaking the hair shafts
- You can also use the trichogram technique to look for *Demodex* mites in affected areas that are difficult to scrape (e.g. close to the eye, pododermatitis), ideally a 1-2 cm² area will be plucked, the same area as with a skin scraping
 - You might find *Demodex* mites hanging on the hairs or sometimes hiding behind them. Only positive results are diagnostic.
 - You can also find lice, *Cheyletiella* mites and their eggs



Sampling hairs for a trichogram*



Trichogram with pointy hair tips*



Trichogram with broken off hair tips*



Hair bulbs in anagen phase*



Hair bulbs in telogen phase*



Demodex canis: two adult mites and one larvae on a hair bulb†



Colour dilution alopecia - macromelanosomes▲

*Courtesy of Sonya Bettenay

†Courtesy of Francesco Albanese and Federico Leone

▲Courtesy of Francesco Albanese



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