Skin Disease Investigation

GENERAL INFORMATION

Species: All

Collection protocol: Skin disease can be frustrating in clinical practice, but it should be remembered that the skin is one of the few organs that is easily available for examination. By careful systematic observation, the diagnoses of many dermatoses can be determined, or at least a differential list established. Specialist dermatologists will often diagnose and treat animals on the basis of the history, signalment, physical examination and a few ancillary tests (e.g. cytology).

It is important that all of the skin and the external mucous membranes are examined. Recognising the morphology of skin lesions is essential in diagnosing skin problems. Often primary lesions (e.g. vesicles) are obscured by secondary ones (e.g. crusts). Changes due to medication and self-induced trauma are also common secondary problems encountered in examination.

It is helpful for the pathologist if skin lesions and their distribution are accurately described on the accession form. It's also very helpful to indicate on the drawing from which anatomical area the biopsies were taken. The signalment, previous history and previous treatments of the case are critical in diagnosing skin disease, and we can help you much more armed with that information. Your clinical differential diagnoses are also always important. Do not worry about introducing "bias" into our process; we cannot see lesions that are not there, and rather than influencing us in the wrong direction, it is much more likely that your clinical information will allow us to provide specific and accurate interpretations.

Digital pictures are very useful as another means of submitting extra information to us. Please contact Gribbles Veterinary for the email address of a pathologist to send them to. Lastly, do not hesitate to call and ask a pathologist what they think before you undertake a test.

Comparison with other related tests: See other chapters in this section.

ENDOCRINE TESTS

Species: All.

Specimen: See specific test

Container: See specific test

Collection protocol: Thyroid hormone plays an important role in controlling metabolism of the skin. Hypothyroidism should be ruled out as the underlying cause of pyoderma. See – Thyroid function in Dogs.

Skin diseases due to cortisol abnormalities are also important – See Hyperadrenocorticism (Cushing's' Disease) in Dogs and Cats, Pituitary Pars Intermedia Dysfunction or Hyperadrenocorticism in Horses.

Dermatoses due to sex hormone abnormalities are uncommon and occur most often in dogs with testicular or adrenal tumours. These should only be considered where overall patient assessment including the results of general blood and dermatological testing support the diagnosis. These are difficult to diagnose by blood sex hormone concentrations since those fluctuate during the day and testing requires referral to an overseas laboratory.

General information about when this test is indicated: Dermatoses in older animals, particularly with non-inflammatory alopecia.

EXAMINATION FOR BACTERIA

Species: All

Specimen: Pustules or vesicle contents, or scale on microscope slide or swab; fresh (unfixed) skin biopsy.

Container: Microscope slide holder, swab, E-Swab container plain container.

Collection protocol: The normal skin is remarkably resistant to bacterial infection and even colonisation. When damaged, however, the environment becomes much more conducive to bacterial growth.

- Pustules, papules and vesicles can contain acantholytic cells, bacteria and the predominant granulocyte population. The presence of intracellular bacteria confirms their role in inflammation.
- Unruptured pustules/papules/vesicles need to be sampled. These lesions should not be surgically prepared or sterilised before sampling.
- The sample is collected via needle puncture and/or aspiration with transfer to a sterile swab for culture, then smearing for cytology.
- Secondary bacterial infection is common in eroded, ulcerated or crusted types of lesions, and culturing these is seldom helpful.
- Culture of fresh skin biopsies may be indicated for plaques, nodules, fistulous tracts, deep lesions or cellulitis. (These sites should be sterilised before surgery and the superficial epidermis excised prior to submission). It is helpful to also aspirate these for cytology at the same time.
- Sticky tape preparations of surface material are difficult to work with and NOT recommended.

The majority of primary bacterial skin infections are caused by coagulase positive staphylococci. Occasionally *Proteus*, *Escherichia coli* or *Pseudomonas* are involved, usually as secondary invaders. In cats, bacterial infections are less common and usually involve *Pasteurella*, *Rhodococcus* or ß-haemolytic *Streptococcus*.

Rare infections are caused by anaerobes or higher bacteria including *Nocardia*, *Actinomyces* and mycobacteria (e.g. feline leprosy). Unstained direct smears and fresh skin biopsies can be submitted for cytology and culture if one of these infections is suspected. Mycobacteria in particular can be very slow-growing and therefore these infections are usually initially diagnosed by cytology or histopathology. Follow-up testing to identify the species of mycobacteria is available in Melbourne by PCR.

General information about when this test is indicated: Dermatoses with prominent pustules, vesicles, exudate, cellulitis, nodules or fistulous tracts.

EXAMINATION FOR DERMATOPHYTES (RINGWORM)

Species: All mammals.

Specimen: Hairs, skin exudate on microscope slide, skin scrapings or nails.

Container: Microscope slide holder, envelope or plain container.

Collection protocol: Wood's lamp is an ultraviolet light instrument, often with an incorporated magnifying glass. When exposed to the UV light, hairs invaded by *Microsporum canis* or *Microsporum equinum* can give a yellow-green fluorescence (30-80% of isolates). This is due to tryptophan metabolites produced by the organisms. Fluorescence is not present in the scale or crusts, or in cultures of *M. canis*. There are other, less common dermatophytes that may also fluoresce. Medication can interfere with the test, for example, iodine washes can destroy the fluorescence and some bacteria and chemicals can give false positive results. Lack of fluorescence is inconclusive, and it should also be noted that most dermatophytes of horses do not fluoresce.

Hair is the specimen most commonly collected for the isolation of fungi. Using forceps, select hairs that fluoresce under the Wood's lamp.

Alternatively the toothbrush method is effective in selecting specimens.

- Use a new toothbrush and gently comb the hair onto paper.
- Submit the brushings and toothbrush in an envelope or non-air tight sealed container for fungal isolation.
- Choice of container is important to prevent moisture build up, which allows bacterial overgrowth.
- The toothbrush comb technique is recommended in cats that are suspected carriers of *M. canis*.

Besides fungal isolation, which can take some time, KOH digest of hair is a rapid diagnostic screening technique for dermatophytes, which can aid in light microscope diagnosis. If negative, dermatophytosis still cannot be ruled out.

Skin samples can be submitted for fungal isolation.

- Clean the skin gently of extraneous debris with gauze soaked in 70% alcohol.
- Scrape from the periphery of the lesion (ideally a new one) and adjacent skin.
- **Do not** use a paraffin-coated scalpel to collect samples for microbiology.

Samples of nail and paw pad can also be cultured for dermatophytes. Since these are often heavily contaminated with micro-organisms, they should be cleaned with 70% alcohol before sampling. Samples should be selected from the concave side of the claw, or from within the claw.

Skin impression smears can be quite useful in the diagnosis of yeast infections. Simply press the slide directly onto the lesion and allow to air dry. Sticky tape preparations are difficult to work with and not recommended.

General information about when this test is indicated: Dermatoses with prominent alopecia, follicular lesions, crusting or scaling.

EXAMINATION FOR PARASITES

Species: All

Specimen: Microscope slide

Container: Slide holder

Collection protocol: Skin scrapings are an often under-utilised diagnostic tool and can be very useful if correctly done (do not forget about those cases of localised demodicosis!).

Use a paraffin-coated scalpel and gently scrape the area whilst squeezing the skin to help extrude *Demodex* mites from hair follicles. (Hairs may need to be plucked to find mites in some cases of demodicosis). *Sarcoptes* live on the skin surface, but while very hard to find on skin scrapings, the finding of even one mite is significant. *Cheyletiella* mites, *Chorioptes* mites and lice should be found easily in coat brushings or Sellotape preparations of the coat. *Otodectes* may be found by scrapings or Sellotape preparations.

Don't forget faecal egg count as part of the work up in suspected parasitic dermatitis cases. Hookworm penetration of the skin can cause inflammation and the only way to confirm this is by testing faeces for eggs.

General information about when this test is indicated: Dermatoses with prominent alopecia, pyoderma, follicular disease, crusting or scaling.

HISTOPATHOLOGY

Species: All

Specimen: Fixed tissue (1:10 tissue:formalin)

Container: Pottle

Collection protocol: Skin biopsies are often an effective diagnostic tool. The results should be able to give you an idea of what the disease process is (e.g. inflammatory vs. neoplastic), and sometimes what agent is causing it. Even if a specific cause is not identified, by knowing if the lesion is follicular or deep dermal, neutrophil-rich or granulomatous, you can effectively reduce your differential list and focus on the most likely aetiologies. Some diseases can only be diagnosed by skin biopsy, however it is important to also recognise that biopsies are not always indicated especially for primary allergic skin disease.

- Biopsies often show non-specific changes in syndromes of allergic skin disease. Carefully consider the history, clinical signs and results of other tests before proceeding to biopsy.
- Select primary lesions to biopsy (e.g. papules, pustules, vesicles, nodules) before secondary lesions (e.g.

lichenification, excoriation).

- If you are sampling an animal with inflammatory skin disease, try to sample as early in the course of the disease as possible, before the development of chronic, secondary non-specific changes. Try to sample entire fresh lesions (if small, e.g. vesicles) or the edge of larger fresh lesions.
- Conversely, if the animal has a non-inflammatory skin disease (e.g. suspected endocrine alopecia, a mass), avoid sampling the edge of the lesion or fresh lesions, and instead select the centre of the well-developed areas, since this is most likely to have classical histological changes. It should be noted it is uncommon for a skin biopsy to have changes specific to a particular endocrine disorder.
- It is important to note on the accession form any treatment the animal has received in recent weeks. Corticosteroid drugs should optimally be withheld for 2-3 weeks prior to the biopsy. (Depot steroids might need a 6-8 week withdrawal). Antibiotics before biopsy may be helpful unless a primary infection is suspected, and culture of the biopsy required. These reduce changes due to secondary infection, important since infections can obliterate the primary lesion and sometimes mimic immune-mediated disease (e.g. pemphigus foliaceus-like dermatophytosis).
- For skin disease investigations, multiple biopsies are always advisable.
- Generally, a 6 to 8 mm biopsy punch provides the best specimen. Smaller biopsies may be taken from the nose, pinnae and feet. Make sure the instrument is sharp to avoid distortion of the tissue, and take the punch in a continuous rotating motion rather than a “back and forth” twist.
- Ensure if possible that the lesion is in the centre of the punch biopsy, since when processed these are generally sectioned in half by default. An eccentric lesion may therefore be missed if it is not obvious at the time of processing.
- When biopsying alopecic areas, using a permanent marker, please draw a line to indicate the direction of hair growth to enable accurate trimming and processing of the tissue.
- If the lesion is suspected to be deeper in the subcutaneous tissue, a punch biopsy may not penetrate deep enough. Excisional biopsy with a scalpel is indicated in this situation, or where the shearing action of the punch may damage lesions, or where there are larger vesicular and pustular lesions. These biopsies should be elliptical and a minimum of 5 x 15 mm.
- Incisional biopsies (i.e. sampling part of a mass) are often useful in cases of suspected neoplasia, where the lesion may need to be graded or staged before curative surgery is undertaken.
- In horses, full-thickness biopsies of the coronary band result in permanent hoof wall defects and therefore superficial “shave” biopsies are preferred from that area.
- It is usually not necessary to biopsy normal skin for a “control”, unless the species of animal is unusual or exotic.
- Biopsies can usually be taken with sedation, systemic pain relief and local anaesthesia of the site. If a deep dermal or subcutaneous disease is suspected it is best to avoid local injection, and to use general anaesthesia or ring blocks to prevent damage to the area. It is also important to avoid local anaesthesia if the biopsy will be cultured (lidocaine can inhibit bacterial growth).
- It is best to do as little as possible to clean the biopsy site since disinfectants, scrubbing and clipping will damage the skin and potentially remove the epidermis and valuable information (e.g. acantholytic cells in crusts, vesicles). Gently dabbing the skin with alcohol, and scissor cutting of long hair in the area is the most interference there should be.
- Care is needed in handling the biopsy specimen. It is easy to crush and distort the sample with forceps. The specimen should be gently blotted to remove excess blood. It may then be placed into a tissue cassette if very small (this reduces trauma to the sample floating in formalin during transit, and also reduces handling at the laboratory before processing).
- Punch biopsies do not curl and can be placed directly in formalin. Biopsies taken with a scalpel often need to be attached to cardboard to prevent curling. Adherence of the deep margin should occur within 60

seconds, then place the cardboard and attached sample into 10% buffered formalin. (Do not wait longer than 1-2 minutes, in order to prevent autolysis).

- Unless identification of individual sites is important all the biopsies can be placed in the same container.

General information about when this test is indicated:

Any potential neoplasm, persistent ulceration, suspected disease that can only be diagnosed by biopsy (e.g. sebaceous adenitis), any unusual dermatosis, vesicular dermatitis, any suspected disease with an expensive/difficult/dangerous treatment, any dermatosis not responding to apparently rational therapy.

IMMUNOLOGIC TESTS

See - Anti-nuclear antibody test

PCR TESTS

Species: Cat, dog

Specimen: Paraffin-embedded formalin-fixed tissue, fixed tissue or fresh tissue.

Container: Fixed tissue (1:10 tissue:formalin) or fresh in plain container.

Collection protocol: Surgical or necropsy collection of tissue samples.

General information about the disease: Mycobacterial skin disease in cats and dogs is often nodular or diffuse and can be due to *Mycobacterium lepraemurium* or opportunistic mycobacteria. Rarely, more serious disease is caused by other species such as *M. bovis* and *M. avium*.

Gribbles Australia offers PCR for *Mycobacterium* testing and speciation. (Speciation may require referral to a reference laboratory in some circumstances).

General information about when this test is indicated: Cats and Dogs with pyogranulomatous dermatitis or panniculitis and acid-fast bacteria consistent with mycobacteria.