

Dermatology: importance of correct sample selection and techniques

MANY dermatological patients have a primary or underlying disease that causes secondary skin manifestations, which prompt their owner to seek veterinary advice.

It is therefore crucial for the veterinary surgeon to investigate not only the obvious problems, but to search for the aetiology that must be treated or controlled, or the dermatosis will recur.

Key points and indications of the procedures will be discussed in this article.

There are five major categories that cause dermatological problems in small animals:

- infections;
- allergies;
- endocrinological problems;
- immune mediated processes; and
- neoplastic processes.

The most common skin diseases can be clinically distinguished by recognising their basic patterns. A practical approach during clinical examination is, therefore, advisable.

The skin and its adnexa are easily accessible and most investigations can easily be carried out within the surgery. Accurate sampling techniques are crucial for acquiring maximum information. Some tests will have to be carried out by external laboratories. These samples have to be prepared and packaged correctly (transport media etc.), or the requested tests cannot be run or may produce erroneous results.

It is also most important to label all samples individually, request the correct test and provide a thorough clinical history for the pathologist. If

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in doubt, contact the laboratory beforehand. Consider the zoonotic potential of certain diseases. Under health and safety regulations, it is your responsibility to educate staff and owners and inform the laboratory of your suspicions.

If you plan to carry out simple procedures at the surgery it is important that good lightning is provided throughout the consultation room. This is crucial to carry out a detailed clinical examination and enables the veterinary surgeon to sample correctly. Adequately trained support staff should be on hand to assist when needed.

The internal laboratory should be equipped with a good light microscope. This should, preferably, be binocular and should be serviced on a regular basis to give best results.

Qualified staff should manage the practice laboratory.

The following equipment should also be to hand:

- magnifying glass;
- otoscope;
- Wood's lamp;
- scissors and electric clippers for hair removal;
- forceps (plain and rubber-coated tips);

- liquid paraffin;
- KOH (potassium hydroxide);
- scalpel blades of various shapes and sizes;
- cotton buds, alcoholic wipes and swabs;
- microscope slides and cover slips;
- stain (for example, Diff-Quik and lactophenol blue);
- swabs for bacteriology (charcoal and dry);
- toothbrush;
- dermatophyte test medium and Sellotape;
- fine comb (flea/lice comb);
- biopsy punches and surgical kit;
- containers with 10 per cent formalin;
- sterile universal containers; and
- syringes and blood containers.

Staining in laboratory

When preparing slides for further clinical examination and staining, the following should be borne in mind: most smears and tape strips can be air-dried prior to staining, but, where waxy or oily preparations are involved, it is more rewarding to heat fix them or they will disappear during the fixing process. The stain should be changed frequently, depending on use, and be free from contamination from floating debris

that will obscure the results. Stain intensity diminishes in old and repeatedly used stains, and vital structures may no longer be recognised. When not in use, the stains should always be covered with lids in order to eliminate evaporation.

Coat brushings

Coat brushings are mainly used to detect ectoparasites, such as fleas,



Figure 1. *Staphylococcus intermedius* on a stained agar plate.



Figure 2. *Microsporum canis* from a ringworm case

lice, mites and their eggs. They can be found by fine combing the hairs and collecting the falling debris onto a light paper (flea dirt stains reddish-brown on slightly wet paper) or into a petri dish. Hairs can be examined under the microscope for evidence of

particular damage. Debris can be examined for mites. Sticky tape may also be used by repeatedly pressing it onto the skin before mounting it on slides to detect ectoparasites.

Hair plucks

Hair plucks are best taken along the hairline with rubber-coated forceps in order to prevent damage to hairs. They are immersed in liquid paraffin before being mounted on a slide with a cover-slip.

Microscopic examination should always start with low power and questionable structures should be investigated, in detail, under higher magnification. Mites are often better detected if the sample is treated with KOH and left for 24 hours before microscopy.

Hair plucks can also be used for dermatophyte cultures.

Skin scrapings

These are used to detect mites in particular, but also hyphae and spores of some dermatophytes such as *Microsporum* and *Trichophyton* spp. Different mite species inhabit various depths of the skin and it is, therefore, advisable to consider this before sampling. Skin scrapings

range from superficial to deep skin scrapings, which go down to the superficial dermis. Irrespective of depth, a No. 10 scalpel blade (No. 15 for difficult to reach areas) should be used. Every animal requires a new, sterile blade. The tip

should be immersed in paraffin before scraping in the direction of the hair growth.

Deep skin scrapings should produce a slight capillary bleed to indicate the right depth of sampling. The recovered material should be emulsified with more liquid paraffin on a microscopic slide. A coverslip can be used to trap material and potential mites. It should be examined as soon as possible, starting with low power (x40). Any details are usually best viewed at x 100.

Dermatophytes

Wood's lamps are useful to investigate *Microsporum canis*. This dermatophyte fluoresces in an apple green colour. *Trichophyton* and other species do not fluoresce. Bear in mind that the lamp itself needs about five to 10 minutes to warm up. Examination should be done slowly and last several minutes, as fluorescence is sometimes delayed. False negatives are possible and suspicious lesions, broken hairs and scales should be further examined by microscopy and culture.

Culture material

There are different transport mediums, but the most commonly used is charcoal. If unsure, contact your external laboratory for the most appropriate. Swabs should not be refrigerated, as this may inhibit the growth of some sensitive bacteria. Ideally, the swab should reach the laboratory within 24 hours of sampling.

Fungal cultures

Use hair plucks (broken hairs from the centre and intact hairs from the margins) to isolate most dermatophytes but bear in mind that some are only found in the

Stratum corneum and require dry skin scrapings. Hairs should be removed along the direction of hair growth with rubber-tipped tweezers and placed in a sterile container. Yeasts can be sampled by using a moist, sterile swab submitted in an appropriate transport medium.

Culture is best carried out on Sabouraud medium (contact plates are available). Alternatively a DTM (dermatophyte test medium) can be used in practice for quicker results but this yields occasional false negatives. Cultures are stained with lactophenol blue and details are further examined under the microscope.

Deep fungal infections may only be diagnosed by culturing a biopsy. Swabbing the site with alcohol, prior to sampling, will reduce bacterial contamination.

Bacterial cultures

Samples should be taken prior to local or systemic antibiotic therapy. The most common transport medium used for bacteriology is a charcoal swab. Surface skin lesions should be harvested by gently rotating a swab across them. The swab should be taken from the lining of any abscess or pyogranulomatous wounds and not contain a large amount of pus which could lead to poor bacterial growth and a false negative result. The surface of intact pustules should be wiped with, for example, surgical spirit before sterile lancing. Expressed fluid should be carefully collected with a swab and submitted in transport medium before posting it. Chronic or recurrent infections often require a skin biopsy. Tissue samples should go down to a depth of four to six millimetres.

Cytology

Suspicious lesions may be touched

with sticky tape, creating an imprint of the surface.

Pustules, nodules and abscesses can be lanced and sampled carefully by collecting the material with a dry swab, which is then rolled across a microscopic slide, or the material can be aspirated and expressed carefully without too much pressure through a sterile needle. The slide can then be stained with Diff-Quik or Giemsa-based stains for investigation and indication of predominant pathogens while choosing appropriate further tests or awaiting results.

Histology

• **Biopsies.** Punches are available in different sizes, but should be at least six millimetres in diameter to give valid results. The site should be clipped prior to the procedure, but not surgically prepared, as this may remove vital information. The punch should be rotated until it reaches into the subcutaneous fat layer and then gently lifted with the help of a sterile needle.

Once the biopsy is removed, it should be placed on a small piece of cardboard and an arrow should be pointing towards the direction of hair growth. Once the sample firmly sticks to the card it can be placed into 10 per cent formalin and sent off to the pathologist.

It is always advisable to include several biopsies in order to obtain best results. Ulcerated lesions are best sampled with larger elliptical biopsies. These enable the laboratory to line-up the sample correctly when sectioning. Larger masses, as well as pustules, nodules and papules, should be removed in total if possible and sectioned into smaller pieces, no larger than one square centimetre. Whenever infectious diseases are suspected it should be mentioned to the

pathologist, as some of these may require special stains for their detection.

Blood samples

- **Hormones.** Several endocrinological disorders, for example, Cushings, hypothyroidism etc. may also cause alterations to skin and adnexa. Taking the appropriate blood sample and using the correct protocol should evaluate these problems satisfactory.
- **Allergies.** Allergies can be further investigated by using intradermal tests usually carried out by a specialist or serological testing methods at the external laboratory.
- **Other diseases.** Arthropods may cause skin irritations or allergic reactions, for example sweet-itch just at the attachment site or bite wound, but may also cause diseases such as leishmaniasis and ehrlichiosis. These usually go beyond the scope of the internal laboratory and can be investigated with various blood tests that can be discussed with a pathologist at the external laboratory.

References

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