

# **Use of Histopathology and Microbiology in Veterinary Dermatology**

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## **Introduction**

The skin is the largest of the organ systems and easily visible to pet owners and livestock producers alike. Therefore, any visible changes in the skin are likely to be presented to the clinical veterinarian for examination. In dermatology, pathological examination of the skin is far more commonly an ante mortal procedure on surgical biopsies rather than a post mortal one.

- Histopathological evaluation of skin biopsies forms a crucial and essential part of any dermatological evaluation and is one of the most powerful tools in dermatology - if used correctly!
- With a close relationship between the clinician and pathologist as regards clinical dermatological features, specimen collection and evaluation, skin biopsies can correctly reflect the dermatological diagnosis in very large % of cases.
- Skin histopathology has a limited range of responses, so if read in isolation it has limitations. However, if combined with clinical features of age, breed, lesion distribution, seasonality, environment, diet etc – histopathology becomes highly diagnostic.

## **When should histopathology be performed?**

1. As an integral part of the clinical workup of the allergic patient.
2. Possible neoplastic lesions – histopathology enables distinction between inflammatory and neoplastic lesions. If neoplastic, then identification as a benign or malignant neoplastic process is possible. If surgical margins are submitted in 10% buffered formalin, then assessment of whether the mass has been locally excised can be made. Invasion of vessels (lymphatics, blood vessels) can be visualized and so assessment of metastatic status is possible.
3. Ulcerative skin conditions – when the protective barrier of the epidermis is breached the clinical outcome is likely to be more serious and therapeutic intervention more urgent. Infectious aetiologies, immune mediated conditions (including drug reactions) and vasculopathies (including injected venoms) can be thoroughly investigated.
4. Where a disease condition that is definitively diagnosed by histopathology is suspected.

5. When dermatoses are not responding to therapy. In cases with severe pyoderma it is always a good idea to place the animal on a 10 day course of antibiotics which knocks back the deep pyoderma features in sections, and unmasks underlying pathology.
6. In all vesicular dermatoses as histopathology is highly diagnostic with these conditions.
7. Where the condition suspected requires expensive or dangerous therapy.
8. Any unusual skin conditions.

### **Which lesions should be biopsied?**

- Primary lesions (nodules, pustules, papules, bullae, alopecia, depigmentation, scaling, erythema) and these primary lesions should be included together with a few selected secondary lesions (crusts, lichenification, exudation, ulceration).
- If the distribution of lesions for a suspected condition is unusual they should be biopsied, for example drug associated pemphigus, where the distribution of lesions strays from that of classical pemphigus conditions.
- Any obvious neoplastic lesion.
- Collect skin biopsies from at least 6 different sites to ensure the pathologist receives a variety of lesions so that the full spectrum of lesions can be examined to facilitate more meaningful interpretive comment on the report.

### **The Submission Form**

This document is vital for a successful diagnostic outcome and in the field of dermatopathology, like no other field in veterinary science, a large % of the diagnosis rests with the clinical history and signalment, as this determines how the pathologist interprets the significance of the histological findings.

For your dermatopathology submissions only use generic laboratory submission forms to facilitate the flow of your sample through the laboratory courier network. Always ensure that a fully completed, specific, well designed, dermatopathology submission form is included in the sample envelope with your specimen. Such specialized dermatopathology forms are designed with various tick box options, small notes sections and an animal graphic, to ensure that all the critical clinical, dermatological and lesion distribution data are available to the pathologists prior to him reading out the case.

Breed associated conditions – there are a wide array of breed associated conditions where the histopathology needs to be linked to the breed and distribution of lesions to arrive at the diagnosis. The histopathology alone merely represents a pathological pattern and unless the

pathologist is provided with the critical information of the breed involved, distribution of lesions etc, he/she cannot make the diagnosis.

Lesion distribution – this can be easily and simply demonstrated on the animal graphic provided, yet logarithmically increases the chances of achieving a confirmed diagnosis.

Age of onset - when combining age of onset, distribution of lesions, presence or absence of pruritis, then histopathology can allude to underlying atopic dermatitis. Atopic animals exhibit suggestive histological changes at “non-lesional” sites, while lesional sites commonly have complicating conditions (due to immune dysregulation), and so it important that both lesional and non-lesional skin are included in your sample set.

Seasonality / Environment / Diet - histologically hypersensitivity can appear very similar across the board of potential triggers, so correlating the histopathology to lesion distribution, seasonality, environmental factors and diet composition, helps distinguish between ectoparasitic, environmental or food associated skin conditions.

Other previous or current systemic conditions – there are a plethora of systemic diseases which have cutaneous manifestations as part of their pathology. If the pathologist is not alerted to these conditions the potential link for a definitive diagnosis may be inadvertently missed.

## **The Biopsy Procedure**

### The Punch Biopsy

- Most frequently used technique due to the ease of collection.
- They can be collected under local anaesthesia.
- Enables collection of multiple specimens with small surgical wounds.
- The punch biopsy produces a standardized sample and so standardized interpretation is possible.
- Punch biopsies must include abnormal and normal skin tissue as separate biopsies, due to the manner in which the tissue is prepared.

Preparation of the site for skin biopsies should retain surface crusts and exudate as these frequently provide highly diagnostic information histologically. Therefore, in short haired breeds the skin can be biopsied as is, while in long haired breeds use of standard hair clippers provides adequate preparation. Do not shave or disinfect the skin surface prior to biopsy.

If collecting punch biopsies under local anaesthetic, then 0.5 ml of local anaesthetic should be introduced into the subcutaneous space below the site where the biopsy is to be collected. If you suspect a primary subcutaneous lesion, remember the local anaesthetic will affect the histology and so in these instances it would be advisable to rather collect your skin biopsies under general anaesthetic.

Biopsy punch instruments of 6mm to 8mm diameter provide the best sample, biopsies of 4mm or less should be avoided if possible as they have a greater chance of being unrepresentative of the lesion. The biopsy punch must be driven with force into the skin to create the punch and so there is a risk of causing damage to deeper lying subcutaneous structures. Therefore, the skin should be grasped with between the forefingers and punch driven in at an angle to create the biopsy. The biopsy punch is then removed and the skin punch core, which remains in situ, should be delicately dissected out by grasping the subcutaneous fat below the skin core with plain forceps. This subcutaneous fat is then cut with curved surgical scissors to release the biopsy.

These punch biopsies are then immediately placed in 10% buffered formalin. It is important to remember to collect multiple punches (at least 6) from various sites representing different stages of clinical lesions. It is also vital to include at least a single series from apparently normal skin, especially when investigating possible atopic dermatitis.

### The Ellipse Biopsy

Ellipse skin biopsies collected in the direction of hair growth, are the preferred samples for evaluating alopecic conditions and follicular disorders.

- Following collection skin ellipses are placed on cardboard and allowed to dry for 10-20 minutes.
- The cardboard with the attached skin ellipse is then inverted and immersed skin side down into 10% buffered formalin.
- Eventually the ellipse will float off the cardboard, but by then it would have fixed in the extended state and not contorted.
- Such biopsies are then easily manageable to prepare histological sections where the hair follicles are lined up and can be evaluated from ostia to bulb.

### The Nail Bed Biopsy

The nail bed biopsy is a technique designed to effectively collect the nail bed at the site where the epithelium rolls onto the surface of the nail. This location has high diagnostic value for nailbed infections, immune mediated nailbed conditions (symmetric onychomadesis) as well as neoplastic conditions which originate from the nail bed (subungual squamous cell carcinoma, subungual melanoma).

A standard punch biopsy is utilized to collect the nail bed and the site should be prepared by clipping away of hair utilizing scissors, again do not shave the area with a razor blade. Remember, this is a painful procedure and therefore should only be attempted under general anaesthetic. The punch instrument is aligned close to and parallel to the nail and driven with force along the dorsal surface of the nail into the nail bed.

## **Expectations of The Pathology Report**

So, what can clinicians can expect to receive from their pathology report? These would include the following

- Possible diagnosis.
- Even without definitive diagnosis histopathology allows for recognition of disease patterns which guide the clinician in the right direction for further investigation.
- Exclusion of differential diagnosis or serious disease i.e. neoplasia.
- For prognostication and evaluation of efficacy of therapy in certain conditions.

## **Microbiology**

Microbiology should always form part of the routine dermatological examination in conjunction with histopathology. Limiting the use of microbiology to only those difficult cases with poor response to initial therapy, contravenes anti-microbial stewardship programs, designed to safeguard against emergence of multidrug resistant bacteria in animals. Although in many instances bacteria may be acting as secondary flare factors, they need to be addressed in the therapy program to ensure the best chances of successful outcome for the patient. Routine use of diagnostic microbiology facilitates responsible antibiotic use and increases treatment success rates.

The preferred specimens for microbiological culture include fresh skin biopsies and charcoal swabs, as aerobic and anaerobic bacterial culture as well as fungal culture can be performed on them.

- For superficial bacterial folliculitis, pustular contents and papule lesions, biopsies are the preferred sample type.
- For deep pyoderma one needs to eliminate the confusion of surface bacterial infections and to achieve this, culture should be taken from the central nonulcerated portion of the lesion. This cutaneous site should be clipped and surgically scrubbed. After removal of the biopsy the epidermis should be aseptically dissected from the dermis and dermis or charcoal swab of the dermis submitted for culture.
- For open wounds the and surface needs to be debrided and cleaned 1<sup>st</sup> before culture samples are collected. Fresh biopsy samples or charcoal swabs from the tissue below the debrided zone are then collected.

- Subcutaneous fluid samples are aspirated into a sterile syringe and then transferred into a sterile screw-top container which can be sealed to avoid leakage during transport to the laboratory.
- Fungal culture: collect charcoal swabs or fresh biopsies from the edge of the lesion to include some healthy tissue as this is the site of active replication of fungi.
- Dermatophyte culture: hair plucks or fresh biopsy samples on ice.

## References

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