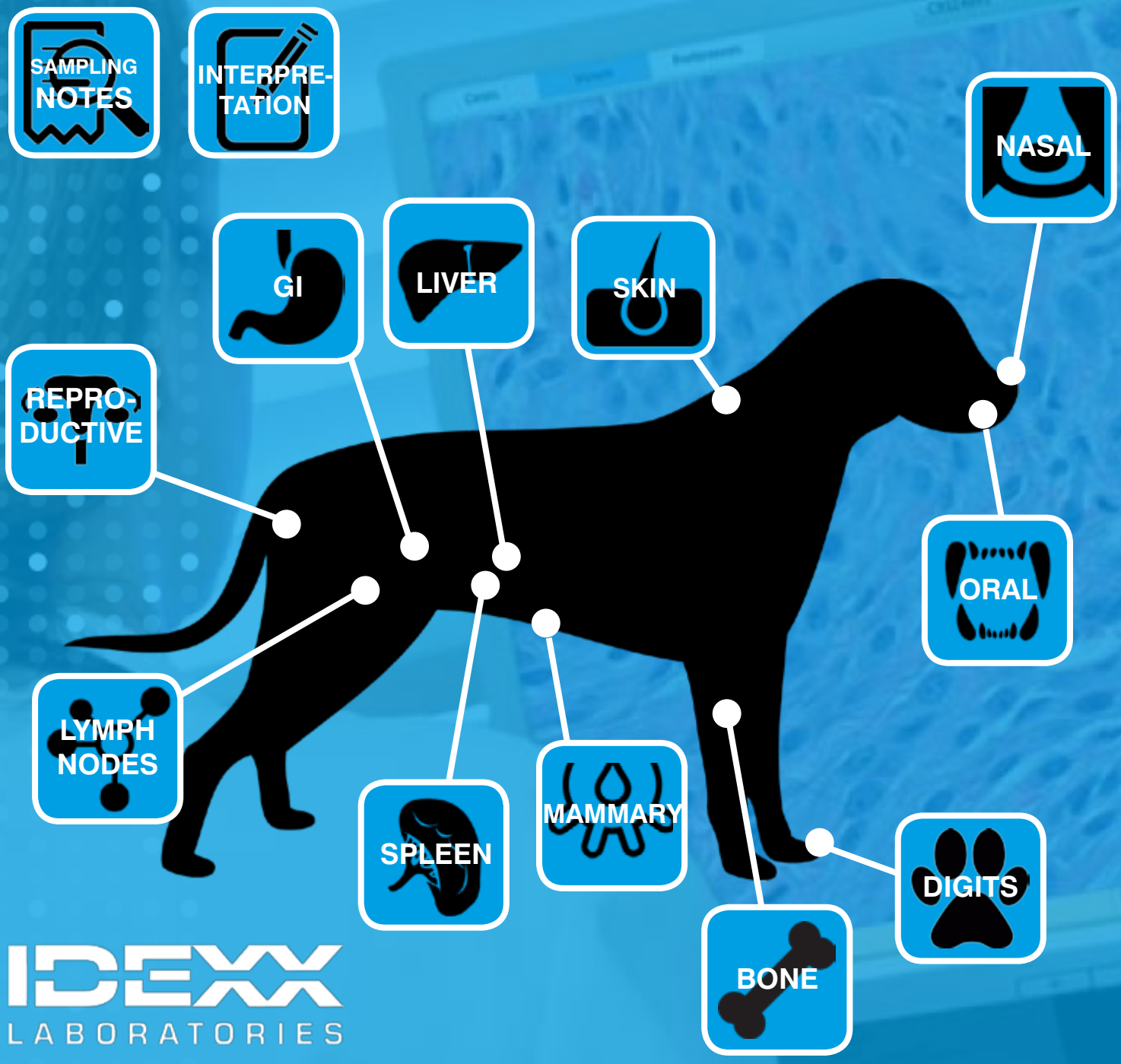
GUIDE TO HISTOLOGY SAMPLE SUBMISSION

Pathologists fully understand the frustration that clinicians can feel when receiving a report that is non-diagnostic or non-committal. These guidelines are to try and help you submit samples that have the best chance of allowing the pathologist to reach a definitive diagnosis. Pathologists are here to help you and your patient and we also find it frustrating when we are not able to provide you with the most helpful report. Please bear in mind, however, that the nature of pathology is such that there will always be a small number of cases with non-specific or equivocal findings, even with excellent quality samples and thorough accompanying history. It can be helpful to warn owners prior to sampling about this possibility.

We have created this guide to try an allow you to gain more insight from your histopathology submissions

Click on an Icon to find out more



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Interpretation

Please also remember that the histopathological diagnosis should be interpreted in the context of the clinical presentation, clinical exam findings, other laboratory tests, and response to treatment. If the diagnosis does not fit the clinical picture, please let us know. Contact details are on the reports you receive, just below the pathologists' signatures. It may be that in some cases, additional tests or repeat sampling are required. Follow-up information is also very helpful to pathologists when there is a question over the definitive diagnosis. In many cases, pathologists will seek a second opinion from colleagues but it may be that a consensus is not able to be reached. Common reasons why a definitive diagnosis may not be provided include:

- **Inadequate history**
- **Insufficient samples (too few, too small, too superficial)**
- **Too much sampling artefact**
- **Concurrent processes**

  (e.g. inflamed tumour where inflammation may be masking neoplastic cells)
- **Too much secondary inflammation, ulceration, necrosis**
- **Undifferentiated or poorly differentiated malignant tumours**

Sampling Notes

The sensitivity and specificity of histopathology is influenced by sample quality. Please try and avoid or minimise, as far as possible, the following:

- **Crushing of tissue with forceps**
- **Stretching of tissue by manipulation during sampling**
- **Drying out tissue under surgical lamps**
- **Coagulation of tissue with electrocautery/diathermy**
- **Delaying fixation prior to submission**

Remember that larger samples are more likely to be representative and therefore diagnostic compared to smaller samples. Smaller samples are also much more prone to collection artefact. When submitting incisional biopsies, submitting a portion of the junction between mass and adjacent tissue might help demonstrate whether an individual mass is benign or malignant, as the presence or absence of invasion might be the sole distinguishing criterion for some lesions. Succinct and relevant history is essential for appropriate interpretation. It is documented for a number of conditions that a diagnosis cannot be made on histopathology alone and results of relevant adjunctive testing (this may include imaging studies, clinicopathological testing or response to trial treatment) may be necessary to make the distinction. If the relevant tests are not performed before sample submission, then a report might not be definitive, but details of further work-up will be discussed in the comment. Additionally, even if the diagnosis is not absolutely definitive, it may be that some differential diagnoses can be excluded. It is important to tell us where samples are from but providing a list of sites alone is not a sufficient history. We appreciate the time constraints in practice but providing the correct and relevant history saves phone calls and amendment of reports at a later date.

Adequate fixation is essential for good quality samples. If submitting multiple samples from the same patient, ensure separate containers are used and are labelled, to ensure each diagnosis correlates with each site sampled. Please don't rely on features such as colour or size for sample distinction, as fixation markedly distorts tissue and samples often cannot be differentiated when received in the lab. If margins are of particular concern, it can be helpful to mark or orientate the sample using small suture tags or ink.

Organ Specific Sampling Advice

Nasal

- Detail whether a mass is present on imaging/rhinoscopy and if there is turbinate lysis
- Deep samples required – often samples are too superficial. Consider submitting samples for culture also
- Case example – nasal planum crusting – mucocutaneous pyoderma and discoid lupus erythematosus with secondary nasal pyoderma are identical on histopathology, and clinical response to treatment usually clinches the diagnosis. In general, it is advisable to trial antibiotic treatment prior to sampling

Organ Specific Sampling Advice

Oral

- Exact location essential – are lesions gingival, buccal or lingual?
- Correlation with imaging studies very useful for mass lesions, especially if malignancy is suspected
- Case example – an inflamed, well differentiated fibrosarcoma can be impossible to differentiated from a focus of inflamed gingival hyperplasia and the presence or absence of bone invasion on imaging may be the sole distinguishing factor
- Avoid using cautery for excision, as this severely impedes histological assessment of margins
- Superficial changes (ulceration, inflammation) can obscure deeper underlying changes
- Case example – epithelial dysplasia secondary to inflammation can be impossible to differentiate from an inflamed early squamous cell carcinoma if biopsies are superficial. The presence of deeper invasive behaviour may be the only method of differentiation and this may not be able to be evaluated in small, superficial samples

Organ Specific Sampling Advice

Digits

- Mass lesions often have surface ulceration, granulation and inflammation – incisional biopsies may be too superficial but excisional biopsy may mean amputation of a digit which is understandably less feasible in some cases
- For cases with nail pathology, nailbed epithelium is needed for a diagnosis. Nail clippings may not be sufficient for a diagnosis. Ideally, biopsy through amputation of P3, or a dewclaw can be sacrificed if present. Up to 50% of cases will have no histological changes so clinical information is essential. Many will have secondary bacterial infection but it is helpful to rule out fungal disease with culture. Leishmania can also cause nailbed pathology so adjunctive testing may be indicated

Organ Specific Sampling Advice

Bone

- Reactive bone and well-differentiated neoplastic bone are extremely similar on histopathology
- Tell us if there is a history of trauma, a mass lesion, a proliferative or lytic lesion on radiographs
- A definitive diagnosis of osteosarcoma can only be made when there is evidence of osteoid and/or bone production by malignant mesenchymal cells. Commonly, tiny biopsies may not be representative of the entirety of bone tumours and examination of several sections from different areas of the tumour may be necessary before evidence of osteoid production is detected. Multiple biopsy samples collected following close examination of the radiograph, that include areas of medullary lysis and/or sclerosis are mostly diagnostic
- Reactive bone formation may be present in regions adjacent to neoplasia, e.g. invasion of mandibular bone by a feline oral squamous cell carcinoma

Organ Specific Sampling Advice

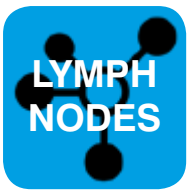
Mammary

- Incisional biopsies and FNAs are rarely conclusive, low-grade carcinomas and adenomas are often impossible to differentiate without marginal tissue in many cases, and invasion, if present, may be focal
- If submitting multiple mass lesions, clearly indicate locations – many patients have a mixture of benign and malignant tumours
- Orientate strips if submitted, can mark areas of concern with sutures if required, but bear in mind we often find additional smaller mass lesions at dissection

Organ Specific Sampling Advice

Spleen

- Submit whole organ if possible. Large splenic masses can often be very haemorrhagic and necrotic with patchy and minimal viable tumour tissue. If the pathologist is suspicious of neoplasia on initial sections, further sections can subsequently be taken if the whole organ is submitted
- If partial submission is essential, submit from margin of mass and adjacent tissue, and if multiple masses, submit sample of each



Organ Specific Sampling Advice

Lymph Nodes

- Excisional biopsy is preferred as allows full evaluation of architecture
- Many lymph nodes that contain neoplasia also have areas of reactive lymphoid hyperplasia, thus partial sampling of a lymph node can result in an incomplete diagnosis
- Architecture can help in differentiating between subtypes of lymphoma, although adjunctive testing is often needed
- Mesenteric lymph nodes should not be submitted for GI cases unless there are accompanying GI biopsies, as they provide no specific information about pathology in the GI tract

Organ Specific Sampling Advice

Reproductive Tract

- Submit both testes for male – many have bilateral tumours, sometimes the larger testis is normal and smaller testis is shrunken and abnormal, rather than the larger testis being pathologically enlarged
- For females, submit ovaries and uterus together – many cases have concurrent lesions e.g. ovarian cysts or tumours with pyometra

Organ Specific Sampling Advice

Gastrointestinal Tract

- Endoscopic biopsies allow evaluation of multiple areas of mucosa but don't show deeper changes e.g. invasion. Often too superficial to be diagnostic for gastric tumours. Submit at least 8-10 from each site for optimal interpretation due to inevitable sampling artefact from biopsy forceps
- Incisional biopsies usually better quality but fewer in number, may miss focal lesions. Allow for evaluation of invasion, which for example might help differentiate lymphocytic enteritis from lymphoma

Organ Specific Sampling Advice

Liver

- Complete history and relevant clinicopathological and imaging findings needed
- Be specific when describing changes – ‘abnormal liver’ is not helpful
- Pathology is often patchy and variable between lobes, submit multiple samples if possible
- Biopsy not indicated for e.g. single abnormal raised ALT on pre-anaesthetic bloods – needs to be repeatable and chronic
- Single toxic insult with marked enzyme elevations – liver will be histologically normal by about day 5-7 following injury

Organ Specific Sampling Advice

Skin

Skin/subcutaneous mass lesions

- Ensure location is recorded. For example, apocrine tumours can look identical to mammary tumours histologically and location is the primary method of differentiation
- Location may also influence prognosis, e.g. melanomas – nailbed lesions aggressive, skin lesions usually benign
- Case example – inflamed soft tissue sarcoma or inflamed reactive fibroplasia? Features that can help make the distinction include age of patient, history of trauma, presence or absence of additional lesions, duration of lesion and rate of growth

Dermatopathology cases

- Do not biopsy cases where there are no active primary lesions. Avoid biopsying cases where there is pyoderma as the main presentation; reconsider if signs are still present after management of infection
- Avoid biopsying secondary lesions (pyoderma, ulceration, scarring, lichenification etc.)
- The cause of pruritic skin disease often cannot be differentiated on histology (particularly atopy and flea allergy). Consider only biopsying pruritic patients where clinical signs cannot be explained by classic allergic skin disease
- Biopsy cannot differentiate between endocrine alopecias, but consider whether biopsy is needed to make the diagnosis
- At least 6 punches recommended when multiple lesions are present to sample range of lesions/chronicity; some patients may have more than one process
- Avoid clipping or scrubbing of areas to be sampled. Don't inject local anaesthetic into the dermis right at the site of biopsy as it creates artefact, inject into the subcutis
- At least 2-3 weeks withdrawal from steroid treatment unless lesions are severe and impacting quality of life
- Full history essential – distribution of lesions, presentation (pruritic, alopecic, pustular etc.), response to trial treatment, signalment (breed predispositions often important)

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