Histopathology

HISTOLOGY GENERAL INFORMATION

Species: All

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

Container: Plain with 10% formalin

Collection protocol: Remember to provide as many clinical details as possible on your submission form. A picture tells a thousand words: if you can, send photos – digital photos can be emailed to your pathologist (usually firstname.lastname@gribbles.co.nz). Try to use a program that can reduce the size of your image files preferably to under 1MB. Alternatively, you can provide images on a CD or upload them to a file-sharing site (e.g. Dropbox). Pathologists will view these photos with the case slides and history provided on the submission form.

Special handling/shipping requirements: Tissues in formalin should be submitted in separate sealed plastic bags from any specimens for cytology. Formalin fumes cause artefact in the cytology specimens that reduces staining quality to a level that is often insufficient for diagnosis. Specimens should be shipped in leak-proof containers and with appropriate labelling (e.g. UN numbers).

Please be very careful not to put freshly removed tissue into narrow-necked containers. Fixed tissue becomes quite firm and warps in fixation; whilst you may have been able to squeeze it into a container, it can then conform to the shape of the container (losing its anatomic orientation) and may be impossible for us to retrieve it without cutting it or breaking the container.

The rule of thumb for tissue:fluid volume is 1:10. It is important to realise that formalin penetrates tissue slowly and might only penetrate 0.5 cm into a tissue block. This requires that the tissues placed in formalin be kept as thin as possible; having slices of only 1 cm in one dimension is optimal. If you cannot see a gross lesion, submission of a big chunk of tissue is false economy. In this situation, the centre will autolyse and it might be that only the outer, fixed part of the tissue will be trimmed anyway.

The standard all-purpose fixative is 10% buffered formalin. This is made by adding 9 volumes of water to one volume of commercial formalin (available as 40% formaldehyde) and buffering it to pH 7. Formalin that is incorrectly buffered has a deleterious effect on nuclear staining and causes yellow-brown deposits to form in the tissues (acid haematin). Neutralised (buffered) formalin (NBF) is easily made by adding 5 g of limestone chips to every 2 l of 10% solution. Otherwise, the following formula may be useful:

- Formalin (40% commercial): 100 mL
- Distilled water: 900 mL
- Na2HPO4: 6.5 g
- NaH2PO4.H2O: 4.0 g

Formalin vapour is an irritant and potentially carcinogenic. It is proven to cause dermatitis in susceptible individuals. Formalin should only be handled in well-ventilated areas and care taken at all times to avoid direct contact with the skin and mucous membranes; gloves are strongly recommended.

Formalin fixation makes most pathogens inert once complete, with the known exception of prion proteins.

BIOPSY / MASS

Species: All

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

Container: Plain with 10% formalin

Collection protocol: Skin tumours and other specimens in which surgical margins are to be evaluated should be kept as intact as possible. While we understand that you may wish to incise through the deep or lateral margin in order to assess the adequacy of excision at the time of surgery, this has the disadvantage of causing the margin to retract during fixation, making assessment of the margins difficult and sometimes impossible.

- If masses are very large, accept that there may be some delay in processing while we wait for the specimen to fix.
- Certain sites require special approaches. For example, with splenic masses the most diagnostic site to sample is the margin between the mass and apparently normal spleen, since the centre is usually composed of blood. On the converse, the centre of lytic bone lesions should be sampled rather than the periphery, which might just be reactive bone.
- Be careful not to crush the specimen with either your fingers or forceps.
- You may use sutures or non-water-soluble inks to identify the margins. Allow the specimen to dry slightly or blot with tissue paper, gently apply ink sparingly over the entire surgical margins (avoiding any area you have sliced into) and blot the ink dry before placing the sample in formalin.
- Under no circumstances should needles be used to identify specimens or to fix specimens to cardboard to keep them straight. If you wish to orientate a specimen, use sutures to tie to cardboard if required, or just allow the specimen to dry slightly on cardboard over 30-60 seconds before putting it all in formalin.
- When submitting several lumps, place them in separate jars and label appropriately according to site; or alternatively, make sure they are clearly marked in some other way (e.g. sutures/ink) so that any specimens that have dirty margins can be identified. Otherwise if one is incompletely excised, you may not know at which site to perform further surgery.
- Do not squeeze samples into containers. Once fixed, they become firm and may be impossible to remove without cutting the specimen or the container.

Special handling/shipping requirements: All samples must be shipped in leak-proof, sealed containers with appropriate labelling (i.e. UN number). Sample containers and shipping materials can be ordered from your local Gribbles Veterinary laboratory. For more information, see Histopathology – General Information

General information about the disease: Not applicable.

General information about when this test is indicated: Not applicable.

Comparison with other related tests: See – Skin Disease Investigations - Histopathology.

FIXING AND SUBMITTING LARGE ORGANS

Species: All

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

Container: Plain with 10% formalin

Collection protocol: Whole organs can be submitted where necessary. This is often best so that the pathologist can interpret gross findings, and/or assess the best samples for histopathology; e.g. splenic tumours, whole abnormal uteri, amputation specimens. Brains for TSE evaluation should be submitted whole, and not dissected before submission.

- If your practice is close to a Gribbles Veterinary laboratory and the sample will definitely arrive at the lab on the same day it was collected, it may be sent fresh and unfixed in double plastic bags or a pottle.
- If there may be some delay, it is best to completely fix large organs before shipping. Use some type of large wide-lidded receptacle (e.g. a bucket), to fix the sample. Change the formalin after 24 hours and leave it at least 2 days. It often helps to partially transect large organs (brain excepted) like slicing a loaf

of bread; this enables the formalin to penetrate adequately. Try to make the slices 1 cm apart and not to cut completely through the organ in order that anatomical detail can still be recognised.

- If you prefer to submit partially fixed tissue, be aware that there may be a delay in reporting while we wait for the sample to fix.
- Once fixed, the pink soft texture of the centre of the cut tissue will be gone. It can then be shipped in minimal formalin. Pour most of the formalin off, then wrap the organ in several layers of plastic within sealed bags (Zip lock are good) to ensure there is no leakage. It is best to put the organ within a firm rigid container to protect it in transit.

Special handling/shipping requirements: All samples must be shipped in leak-proof, sealed containers with appropriate labelling (i.e. UN number). Sample containers and shipping materials can be ordered from your local Gribbles Veterinary laboratory. For more information, see Histopathology – General Information

General information about the disease: Not applicable.

General information about when this test is indicated: Not applicable.

Comparison with other related tests: See – Necropsy, Histopathology – Fixing of Special Organs

FIXING OF SPECIAL ORGANS

Species: All

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

Container: Plain with 10% formalin

Collection protocol: Tissues such as central nervous system and eyes require special care upon removal. Artefact induced by rough handling and poor fixation can greatly impede interpretation of histological changes in these organs.

Lesions in the brain can be localised to focal areas, and the entire intact brain should always be submitted. It is very important to preserve the mid-brain and hind-brain in cases submitted for TSE evaluation.

Eyeballs need to be removed immediately after death, as autolytic change can affect the retina within 10 minutes of anoxia. Good retinal morphology is critical for diagnosis of most cases of ocular blindness. Eyeballs need to be trimmed of extraocular tissue and muscle before fixing, but leave the optic nerve in place. Intra-vitreous injection of formalin (0.25 ml in a dog or cat, 2 ml in a horse) can be used to optimise retinal histopathology; alternatively a specialist fixation protocol (e.g. use of Bouin's or Davidson's fixative) can be pursued.

Special handling/shipping requirements: All samples must be shipped in leak-proof, sealed containers with appropriate labelling (i.e. UN number). Sample containers and shipping materials can be ordered from your local Gribbles Veterinary laboratory. For more information, see Histopathology – General Information

General information about the disease: Not applicable.

General information about when this test is indicated: Not applicable.

Comparison with other related tests: See – Necropsy, Histopathology – Fixing and Submitting Organs.

HISTOPATHOLOGY – NECROPSY SPECIMENS

Species: All

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

Container: Plain with 10% formalin

Collection protocol: Histopathology samples should be collected as soon as possible after death to avoid artefact and loss of diagnostic information due to autolysis and putrefaction. Some tissues autolyse very

quickly, and in some circumstances (e.g. scouring calves with intestinal lesions) it is actually best to sacrifice a moribund animal for immediate post mortem and fixation, rather than to sample an animal that has been dead for hours.

Always include tissues with gross changes, even if you are doubtful of the significance. If the change occupies a large area of the tissue (>2 cm), a few small pieces from the edge and some from the deeper areas are recommended.

It is crucial to sample a range of organs, even if the cause of death or gross lesions seem obvious, since apparent lesions may not prove significant and it is rare to have another opportunity. The samples can be held in formalin for later analysis if required. For cases of sudden death in all species, we recommend at a minimum collecting the brain, heart, lungs, liver and kidney (known as the “Big Five”), with lymph node, gastrointestinal tract (rumen, abomasum, small intestine, large intestine), urinary bladder, skeletal muscle and spleen also often containing useful information.

Handle tissues gently, especially delicate endocrine organs (e.g. adrenals). Mucosal surfaces (e.g. intestine) should not be rubbed or washed before fixation.

Provide sections that include all the relevant architecture, e.g. for lung, include portions of large and small airways, and avoid extremities of lobes; don’t just sample areas of ventral consolidation but also a few areas from several lobes. For liver include a bile duct or two; for kidney ensure both cortex and medulla with pelvis are included.

Do not freeze tissues before or after fixing. Freezing does not absolutely preclude histopathology and sometimes frozen tissues can remain diagnostically useful, but generally speaking freezing induces artefact that can make histopathology very difficult.

Tissues fix best at warmer temperatures (up to 30°C, the warmer the temperature, the faster the fixation). On cold winter days, fixation can be aided by keeping the samples in a heated area.

Special handling/shipping requirements: All samples must be shipped in leak-proof, sealed containers with appropriate labelling (i.e. UN number). Sample containers and shipping materials can be ordered from your local Gribbles Veterinary laboratory. For more information, see Histopathology – General Information

General information about the disease: Not applicable.

General information about when this test is indicated: Not applicable.

Comparison with other related tests: See – Necropsy.

IMMUNOHISTOCHEMISTRY

Specimen: Fixed tissue processed to a paraffin block.

Container: Not applicable.

Collection protocol: Tissues are fixed after collection at biopsy or necropsy.

Special handling/shipping requirements: As apply generally to shipping of fixed tissue.

General information about the disease: Not applicable.

General information about when this test is indicated: In large part, diagnostic pathology by light microscopy relies upon recognising patterns of disease at the architectural and cellular level. However, any particular disease can have a wide range of microscopic expression, and while most cases will fall into the centre of the “bell curve”, there are always one or two at the tail ends of the curve that overlap with the tail of another diagnostic “bell curve”, and consequently defy interpretation. In other words, sometimes it’s impossible to say just from light microscopy what this or that cell is; whether the lesion is lymphoma or another type of tumour; or even whether the lesion is inflammatory or neoplastic! Furthermore, there are some cases where a

lesion can be recognised easily, but predicting it's behaviour based on light microscopy is difficult (e.g. mast cell tumours in dogs).

This problem has led to the development of a wide range of complementary diagnostic techniques including electron microscopy, karyotyping, in-situ hybridisation, PCR, flow cytometry and immunohistochemistry. In human medicine, improved diagnosis, characterisation and sub-classification of disease by multiple modalities has allowed refinement of prognosis and the best treatment for each individual patient. Medicine is becoming personalised with specific targeting of biochemical pathways promoting various diseases. In veterinary medicine we are now moving in the same direction, with better characterised diseases providing more useful prognostic information to veterinarians and the opportunity to treat with targeted therapies.

Immunohistochemistry and immunocytochemistry use immune reactions in order to identify cells or other targets (e.g. receptors or pathogens). The basic principle in most immunodiagnostics is that antibody-antigen binding between a specific antigen and a diagnostic antibody raised against it (the primary antibody), triggers a change that can be recognised in a tissue section or smear by light microscopy. In diagnostic laboratories this is usually a colour change achieved through an enzymatic reaction, with the catalysing enzyme tagged to the site by a secondary antibody that recognises the primary antibody.

Your pathologist is likely to review the stained sample alongside a control (external and/or internal), and then conclude the overall diagnosis and prognosis, or whether more testing is required to determine this. It should be understood that immunohistochemistry and immunocytochemistry are not always the "last word", since like any test they can have false or uninterpretable results (e.g. over-fixation impairs antigen detection; very poorly differentiated tumours may not express some antigens; background staining can impede recognition of positive cells). Nevertheless they are very useful tools in the classification of disease by light microscopy.

The tests listed here have been validated in Gribbles Veterinary for the species listed. If you want to test another species or require an antibody or test not listed here, please contact your local Gribbles Veterinary laboratory since it may be possible, or available through one of our partner medical laboratories. Those tests may work, but have not necessarily been subjected to a validation process in our network.

If you require specific information on the clone and manufacturer of primary antibody used in a certain test, please contact your local laboratory.

CD3

Species: Dog, Cat

CD3 is part of the T-cell receptor, the critical part of T-cells responsible for recognising antigens presented by other cells. Therefore, this antibody recognises lymphocytes of T-cell lineage (including CD4+ and CD8+ cells). It may also label Natural Killer cells, and cerebellar Purkinje cells (although the latter can be distinguished by location and morphology). This antibody is used to confirm and characterise diagnoses of T-cell lymphoma, and to help rule out lymphoma in cases of poorly differentiated "round cell" tumours. It is typically used in conjunction with a B-cell antibody (e.g. CD20 or CD79a).

This is helpful because the prognosis and treatment for different forms of lymphoma varies according to their classification. There are low-grade T- and B-cell lymphomas that may have a fair to good prognosis, medium-grade B-cell lymphomas that are often chemoresponsive, and high-grade T-cell lymphomas that may be poorly chemoresponsive and may have a poor prognosis.

CD18

Species: Dog

CD18 is part of an integrin molecule, essential for the adhesion of leukocytes to vessel walls as they migrate into tissues. Macrophages and granulocytes express 10x more CD18 than lymphocytes. Therefore while not specific to histiocytes, this antibody helps to identify "round" or "spindle" cells in tissue sections as histiocytic, and to rule out other possibilities (e.g. lymphoma, melanoma, soft tissue sarcoma). This sometimes requires

concomitant use of other antibodies to rule out other possibilities.

This is helpful since both benign and malignant histiocytic diseases can be difficult to distinguish from reactive processes, “round cell” tumours and “spindle cell” tumours. Histiocytic sarcomas may be cured by excision if localised, but if disseminated tend to have a worse prognosis than soft tissue sarcomas.

CD20 and CD79a

Species: Dog, Cat (CD20 only)

CD20 is a transmembrane protein found on B-lymphocytes, which plays a role in their differentiation into plasma cells. CD79a is part of the B-cell receptor, the critical part of B-cells responsible for recognising antigens. Therefore, this antibody recognises lymphocytes of B-cell lineage (including plasma cells).

These antibodies are used to confirm and characterise diagnoses of B-cell lymphoma, and to help rule out lymphoma in cases of poorly differentiated “round cell” tumours. They are typically used in conjunction with the T-cell antibody CD3. Some plasma cell tumours are also CD79a positive.

This is helpful because the prognosis and treatment for different forms of lymphoma and plasma cell neoplasia varies according to their classification. There are low-grade T- and B-cell lymphomas that may have a fair to good prognosis, medium-grade B-cell lymphomas that are often chemoresponsive, and high-grade T-cell lymphomas that may be poorly chemoresponsive and may have a poor prognosis. Plasma cell tumours tend to be benign and have a good prognosis compared to some other round cell tumours.

CD31

Species: Dog

CD31 (also known as PECAM-1) is a glycoprotein expressed strongly by endothelial cells, megakaryocytes and platelets; it can also be found on haemopoietic stem cells and leukocytes. It is used during transendothelial migration of leukocytes.

This antibody is useful to identify spindle cells as endothelial, allowing the distinction of haemangiosarcomas, angiosarcomas and lymphangiosarcomas from other “spindle cell” tumours, (e.g. spindloid melanomas and soft tissue sarcomas). This is helpful since malignant vascular tumours often have a worse prognosis (e.g. early metastasis) than soft tissue sarcomas.

c-Kit / CD117

Species: Dog

c-Kit/CD117 is a tyrosine kinase receptor for stem cell factor, found on mast cells, interstitial cells of Cajal and some other cells (e.g. bone marrow stem cells, melanocytes, basal cells of the skin, melanocytes, and germ cells). It is therefore not specific to mast cells, however mast cell tumours generally stain well with antibodies to this receptor and this test is helpful to confirm that diagnosis.

In the intestinal tract, Gastrointestinal Stromal Tumours (arising from interstitial cells of Cajal) are differentiated from other “spindle cell” tumours (e.g. leiomyosarcoma) by this antibody. Many canine GIST’s have aggressive behaviour and may metastasise to local lymph nodes, the mesentery and liver.

Confirmation of a diagnosis of mast cell tumour enables the use of specific treatments such as tyrosine kinase inhibitors. These block signals stimulating cell proliferation from c-Kit and other tyrosine kinase receptors. Constitutive activation of c-Kit is seen in a percentage of canine mast cell tumours in association with mutations in the c-Kit receptor.

Cytokeratin AE1/AE3 and Cytokeratin 8/18

Species: Dog

Cytokeratins form part of the cytoskeleton in epithelial cells, and less commonly in other cell types. They are found in different combinations in different types of epithelial cells, and therefore specific cytokeratin antibodies can identify these cell lines. Cytokeratin AE1/AE3 is a “cocktail” of antibodies recognising most epithelial cells. Cytokeratin 8/18 identifies most carcinomas, but is not found in squamous cell carcinoma or

mesothelioma and so can help to diagnose these.

These antibodies are useful to differentiate carcinomas from each other and other tumour types, which may have a different prognosis and treatment regime. Cytokeratin AE1/AE3 is often used in combination with vimentin.

Ki67

Species: Dog

Ki67 is a nuclear protein that is expressed during all stages of the cell cycle, except G0 (i.e. “resting”, non-dividing cells). Since mitotic figures are sometimes hard to distinguish histologically from artefactual nuclear distortion or necrotic/apoptotic nuclear changes, antibodies to Ki67 allow more sensitive detection of proliferative activity in a population of cells.

The percentage of positive cells produces a quantitative assessment of tumour proliferation. This is useful in tumours that are difficult to prognosticate by histopathological features, such as mast cell tumours and melanomas.

Melan A

Species: Dog

Melan A is a melanocytic antigen. It is very specific for melanomas, but can also cross-react with steroid hormone-producing cells.

This antibody allows the distinction of melanomas from other “round”, “epithelioid” or “spindle cell” tumours (e.g. histiocytic sarcoma, carcinoma, and soft tissue sarcoma). This is useful because the prognosis for malignant melanoma is often more guarded than for some differential diagnoses; and also because specific treatment for melanoma (the “melanoma vaccine”) may be useful in some melanoma patients.

Smooth Muscle Actin

Species: Dog

Smooth muscle actin is part of the contractile apparatus and cytoskeleton of smooth muscle, myofibroblasts, pericytes, liver peri-sinusoidal cells and myoepithelial cells.

This antibody can be used to determine if a tumour or cell of interest arises from smooth muscle, and is used to help diagnose muscle tumours (such as leiomyosarcomas) and to sub-classify mammary tumours. This is clinically useful since spindloid muscle tumours can be indistinguishable from other “spindle cell” tumours (e.g. some forms of melanoma or GIST), and sub-classification of mammary tumours allows better prognostication.

Synaptophysin

Species: Dog

Synaptophysin is a component of the neuronal synaptic vesicle and neurosecretory granules. Antibodies to synaptophysin label neurons and neuroendocrine cells.

This antibody is mainly used to distinguish neuroendocrine tumours from other epithelial or mesenchymal tumours. Neuroendocrine carcinomas can be aggressive and may also secrete hormones.

TTF-1

Species: Dog

TTF-1 is a transcription factor regulating thyroid-specific genes, and also regulating transcription in the brain and lung (Clara cells and Type 2 pneumocytes). TTF-1 antibodies stain most follicular thyroid tumours, some C-cell tumours and some pulmonary carcinomas.

This is useful to differentiate thyroid carcinomas from other epithelial tumours (especially in small biopsies), and to differentiate pulmonary carcinomas from other lesions, including tumours that might have

metastasised to the lung.

Vimentin

Species: Dog

Vimentin is a part of the cytoskeleton in mesenchymal cells and mesoderm-derived epithelia (e.g. endometrium and ovary).

This test is used to help distinguish mesenchymal tumours from epithelial tumours, and to identify tumours co-expressing vimentin and cytokeratin (e.g. mesotheliomas).