Vetpath is a specialist veterinary laboratory dedicated to providing our clients with the finest laboratory diagnostic service. A team of veterinary pathologists and medical scientists with extensive experience in veterinary diagnostic pathology forms the core of the Vetpath team.

VNews

VETPATH LABORATORY SERVICES

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Flow cytometry

Flow cytometry is a diagnostic technique used to rapidly characterize cell populations in fluid suspensions. Labelled antibodies bind to cell surface antigens to allow specific identification and quantification of cells.

Flow cytometry is can be useful to differentiate between a reactive and neoplastic lymphocyte population, and to identify an atypical cell type (B lymphocyte, T lymphocyte or myeloid cell). Lymphocytic effusions and suspected lymphoma in lymph nodes or organs can also be evaluated.

Viable intact cells are required for flow cytometry and the sample must travel from Vetpath to a reference laboratory in Sydney within 48 hours. We recommend that samples be submitted early in the week (Monday or Tuesday) to ensure they arrive in Sydney within the required time frame.

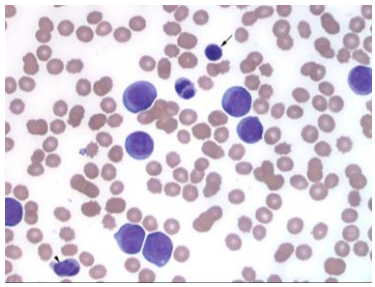
Peripheral blood and effusion fluid should be submitted in EDTA tubes and a minimum of 2ml is required. The following protocol can be used for lymph node and organ aspirates:

1. Place 1 ml of normal saline (0.9% NaCl Solution, not Hartmann's solution) in a 2ml EDTA tube and add 0.1 to 0.2 ml of cell free serum from the patient or another animal of the same species.
2. Aspirate the lymph node or other organ mass using suction and squirt the contents of the needle and syringe into the EDTA tube with saline and serum.
3. Draw up saline through the needle and gently squirt back into tube to obtain more cells.
4. Carry out this process several times if possible – the saline should be cloudy.

Samples should be refrigerated until submission.

Source:
http://www.vetnostics.com.au/globalassets/directory-sites/vetnostics/test-information-pdf/flow-cytometry.pdf

Case example - AML
The image below is from a dog with acute myeloid leukaemia (AML). A severe leukocytosis composed predominantly of large blast cells is present. Morphological identification of the cells is difficult and they could be lymphoid or myeloid.



Flow cytometric analysis confirmed an AML, with the cells being positive for CD34 (stem cell antigen), CD11b and CD11c (myeloid antigens) and CD4 (can be expressed on some AML, neutrophils or T lymphocytes).

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What is the difference between histochemical and immunohisto-chemical staining?

Histochemical staining is the use of chemical stains to identify and determine the distribution of various substances in cells and biological tissues. Staining relies on the chemical characteristic of the cells and their contents.

Different cell types contain different compounds, and these can be detected using histochemical stains with specific characteristics. In the case of mast cells, the mast cell granules stain metachromatically (purple to red) with toluidine blue, a basic dye, making their identification easier in formalin-fixed tissues than in H&E stained sections. The amount of staining with toluidine blue depends on the amount of heparin present in the mast cell granules. Mast cell granules also tend to stain better in cytology samples using automated Romanowsky (e.g. Wright - Giemsa) compared to fast Romanowsky (e.g. Diff Quik) stains.

Immunohistochemistry is the process of detecting target antigens in sections of intact biological tissue using specific antibodies. c-KIT (CD117 antibody) and Ki67 are immunohistochemical markers used at Vetpath to provide prognostic information for cutaneous and subcutaneous mast cell tumours. Mast cells express a KIT receptor which is involved in mast cell differentiation and regulation. On immunohistochemical staining, c-KIT (CD117 antibody) recognises and stains antigen on the cell surface of cells, whereas in more malignant mast cells cytoplasmic staining may occur. This KIT staining pattern can be used for prognostic evaluation of canine mast cell tumours, with a poorer prognosis and survival time associated with cytoplasmic KIT-staining (patterns II and III).

Ki-67 is a nuclear protein expressed in all phases of the mitotic cell cycle, but not expressed in non-cycling cells. Ki-67 expression in high grade mast cell tumours is used to determine a proliferation index, or relative number of cells that are actively proliferating. An increased proliferation index together with increased c-KIT cytoplasmic staining is associated with a poorer prognosis, increased rate of recurrence, metastasis and lower survival time.

Immunohistochemistry is recommended for high grade cutaneous mast cell tumours, subcutaneous mast cell tumours with a mitotic index >4, and those tumours with an infiltrative growth pattern. c-KIT immunohistochemistry may also be used to evaluate high grade mast cell tumours for susceptibility to treatment with chemotherapeutic agents.

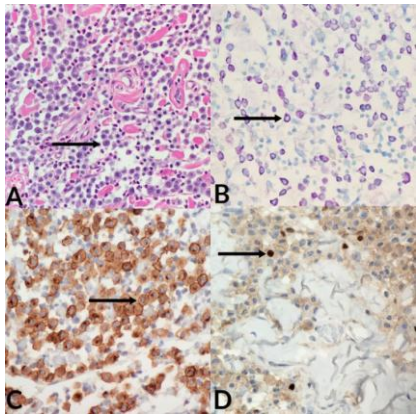

Figure:
A Hematoxylin and Eosin
B Toluidine blue – cytoplasmic staining
B CD117 (c-KIT) – membranous and cytoplasmic staining
D Ki-67 – nuclear staining

References:
Int Arch All Imm 2018; 176: 55-60.
JVIM 2006; 20: 377–381
Vet Path 44:3, 2007, 41:4, 2004, 48: 1
2011

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