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 method of analyzing physical and chemical characteristics of cells. This technology is used widely in haematology, however is now routinely available for assessment of haematological neoplasms.

A flow cytometer analyses cells that are suspended in fluid and passed through a laser. All leukocytes express antigens on their surface. These antigens can be detected using labelled antibodies, allowing a flow cytometer to identify the lineage of cells in the sample.

Immunophenotyping abnormal cell populations in peripheral blood can help to differentiate between:

1. B and T cell lymphoid neoplasia.
2. Acute lymphocytic leukaemia and lymphoma.
3. Myeloid and lymphoid leukaemia.
4. A reactive (heterogeneous) and neoplastic (homogenous) population of lymphocytes (particularly when used in conjunction with PARR).

The cell population to be immunophenotyped must be well preserved for accurate analysis. Blood samples must be submitted to Vetpath early in the week to arrive in Sydney as soon as possible. Delays over the weekend can result in cell degradation and less meaningful results.

The following case study displays how flow cytometry can be used to characterize a leukaemia of granular lymphocytes in a dog. The patient had a lymphocytosis of 60.0 x 10^9/L which was found to be composed of cytotoxic T cells. Immunophenotyping the cell population was useful prognostically as leukaemia of cytotoxic T cells is usually indolent in dogs.

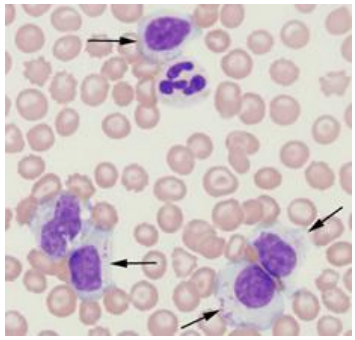

Figure 1: Marked lymphocytosis in a dog composed of small granular lymphocytes.

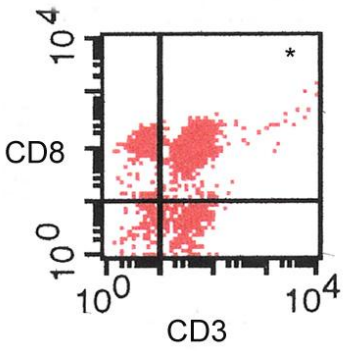

Figure 2: Dotplot of fluorescence staining for the cell population in figure 1. Most of the cells are double positive (*) for CD3 (T cell marker) and CD8 (cytotoxic T cell marker) confirming a leukaemia of cytotoxic T cells.

Source:https://ahdc.vet.cornell.edu/sects/clinpath/news/immunophenotyping_tests.cfm#Flow

Which blood tubes do I use?

There are multiple tubes available for diagnostic testing, and keeping track of which tube is for which test can be tricky. Here is a helpful guide:

EDTA – Purple

EDTA tubes are used for haematology and for cytology of fluid samples. The tubes must be inverted several times and try to obtain the correct ratio of anti-coagulant to blood ratio to optimize cell morphology. EDTA is bacteriostatic and these tubes are therefore not suitable for culture. EDTA plasma is also used for measurement of endogenous ACTH.

Serum – Red

Serum from a red top tube is used for all biochemistry tests, some endocrine tests, serological tests and therapeutic drug monitoring. The serum can be removed from the erythrocyte clot and frozen to minimize haemolysis.

Lithium Heparin – Green

Lithium heparin samples can be used for some biochemistry tests and is the preferred sample for some toxicological tests. Lithium heparin cannot be used for fibrinogen measurement or endocrine testing. Lithium heparin samples are often preferred in avian and reptilian patients because the blood can be used for both haematology and biochemistry.

Fluoride – Grey

Fluoride oxalate tubes are used for accurate determination of glucose concentration. The fluoride prevents enzymatic degradation of glucose during samples transport. The tube must be filled and mixed correctly, otherwise an artifactual hypoglycaemia can occur.

Citrate – Blue

Sodium citrate tubes are used for assessment of coagulation (PT and PTT). The tube should be filled to the appropriate capacity so that the correct ratio of citrate to blood is obtained. Under-filling the tube can cause prolonged PT and PTT. EDTA blood sample should also be submitted if a CBC is required as the sodium citrate will cause a dilution effect on the CBC results.

It is also important to collect blood into the tubes in the correct order. This helps avoid cross-contamination of additive between tubes (EDTA can interfere with a number of biochemical tests including electrolytes and calcium) and minimize clotting in anticoagulated samples. The suggested order is:

1. Sodium Citrate.
2. EDTA.
3. Plain clotted.
4. Fluoride oxalate.

The tubes should be stored in the refrigerator until transport to the laboratory. A delay in processing can result in haemolysis and cell degeneration, and so submission of a fresh smear is recommended for assessment of erythrocyte morphology.

NATA
NATA Accredited
Laboratory Number 14776

Vetpath Laboratory Services
RECEPTION DIRECT +61 8 9259 3600
LOCAL COURIER PICK-UPS +61 8 9259 3666
AFTER HOURS MOBILE 0418 916 436
FACSIMILE +61 8 9259 3627
EMAIL enquiries@vetpath.com.au
WEBSITE www.vetpath.com.au

VETERINARY PATHOLOGISTS
Jenny Hill BVSc (Hons) Dip ACVP
John Jardine BVSc MMedVet (Path) Dip ACVP MRCVS
Jon Meyer BVSc DVSc Dip ACVP
Jason Stayt BSc BVSc Dip ACVP
Leanne Twomey BSc BVMS (Hons) PhD Dip ACVP