FLOW CYTOMETRY

SAMPLE/S REQUIRED:

May be performed on blood, bone marrow, body cavity fluids, and aspirates of solid tissues placed into liquid media (see below)

Flow cytometry is a versatile and powerful technique using laser-based technology that allows for qualitative and quantitative assessment of multiple parameters of individual cells and particles. It may be used for cell counting, cell sorting and cellular marker detection (i.e. immunophenotyping).
Flow cytometers detect and analyse multiple physical characteristics of single particles (cells) as they flow in a fluid stream through a beam of light. The properties analysed include a particle's relative size, relative granularity or internal complexity and relative fluroescence intensity. Fluorescently labelled antibodies may also be applied to detect expression of specific cell markers, thereby assisting with identification and quantification of specific cell types, cell subsets, and cells expressing aberrant antigens.

Indications:

- To allow differentiation and further classification between acute lymphoid and acute myeloid leukaemias.
- To help differentiate between reactive and neoplastic expansions of lymphocytes.
- To differentiate between B and T cell lymphoid neoplasms (lymphoma, chronic lymphocytic leukaemia etc.).

General sample collection requirements:

- The sample should be collected early in the week and sent to the laboratory as soon as possible. Samples must be received at the laboratory by Wednesday. Samples collected later in the week or on the weekend may deteriorate and will therefore not be suitable for analysis.
- Refrigerate samples immediately after collection. Keep refrigerated until courier collection.
- Ensure a thorough history accompanies the submission. This should include:
    - Signalment
    - General clinical history
    - Results of any laboratory testing, including CBC/biochemistry, cytology reports, histopathology reports, serological test results etc.
    - A specific reason for why the test has been requested (e.g. to classify a leukaemia, to differentiate between a reactive and neoplastic lymphoid population, to classify a lymphoid neoplasm as B or T-cell etc).

Specific sample collection requirements:

Further investigation of an acute leukaemia or persistent lymphocytosis

- Refer to general sample collection requirements above.
- Minimum sample requirements are 2 tubes containing at least 2 mLs of fresh, chilled, non-clotted EDTA blood and an air dried blood smear (made at the time of blood collection).
- Submit a copy of recent haematology reports, including pathologist interpretation comments (if performed at a different laboratory).

Further investigation of a lymphocyte-rich body cavity effusion

- Refer to general sample collection requirements above.
- Collect the cavity fluid into an EDTA (purple top) tube and gently invert 8-10 times, ensuring that no clot is present.

Lymph node and other organ aspirates for flow cytometry

- Refer to general sample collection requirements above.
- Place 1 mL of saline (0.9% NaCl solution but not Hartmann's solution) in a 2 mL EDTA tube and add 0.1 to 0.2 mL of serum from the patient or another animal of the same species to the tube.
- Aspirate the lymph node or other organ mass *using suction* and squirt the contents of the needle and syringe into the tube.
- Draw up saline through the needle and gently squirt back into tube to obtain more cells.
- Carry out this process several times if possible – the saline should be cloudy.
- It is also recommended that 3-4 well made smears be made by direct FNA of the lymph node or mass in question for simultaneous cytological examination.

Notes

- Animals should ideally not have been treated with any chemotherapeutic agents, including corticosteroids, prior to the first immunophenotyping test when using flow cytometry. Antigens can be downregulated on lymphocytes after treatment.
- Samples will not be diagnostic if there are insufficient numbers of the neoplastic cells in question or the cells have deteriorated or are lysed. Samples of cerebrospinal fluid are usually of too low cellularity to perform immunophenotyping using flow cytometry.
- In some cases, immunophenotyping by flow cytometry may not be diagnostic due to downregulation or aberrant expression of cell antigens in neoplastic cells.
- In rare cases, certain infectious diseases can cause clonal or restricted expansions of lymphocytes, which can mimic lymphoid neoplasia.