Flow cytometry for immunophenotyping of lymphoma and leukaemia

Flow cytometry uses a panel of antibodies to accurately differentiate the lineage of the cells in a sample (immunophenotyping). This allows us to differentiate between lymphoid (including distinguishing T or B cell) and myeloid cells, as well as between acute and chronic leukaemias. There is growing evidence that the immunophenotype can also be useful for predicting prognosis. This service is available for dogs and cats and we report the results by the end of the following working day after receipt of the sample.

In cases with an equivocal cytological diagnosis of lymphoma or leukaemia, flow cytometry can also be helpful to determine if an antigenically homogeneous (likely clonal) population of cells is present, or whether there is an abnormal (aberrant) immunophenotype, which may be supportive of a neoplastic process (especially useful in T zone lymphoma).

Please do not hesitate to contact the laboratory (01223 337625) if you have any questions relating to flow cytometry or you would like to discuss whether it is suitable for your patient.

Samples submitted for flow cytometry include a cell count on the sample (machine count in leukaemia cases or fluid cell count for lymphoma cases) and a brief cytological description of the cells, however if a full cytology report or CBC is required, this should be requested separately. We evaluate the suitability of all samples prior to setting up the flow and if the sample is not appropriate, we will contact you to discuss this. A small sample evaluation fee will be applied to these samples if flow cytometry is not performed. Please note that samples must be taken from an affected tissue (i.e. lymph node or infiltrated organ aspirates in a lymphoma, blood where circulating neoplastic cells are present such as stage V lymphoma or leukaemia).

Sample requirements for flow cytometry

Lymphoma immunophenotyping (on LN or other organ aspirates, effusions)

Aspirates from solid tissues such as lymph nodes or infiltrated organs (obtained in the same was as for FNA cytology) must be placed immediately into an appropriate fluid medium. This can be prepared in house by obtaining 0.5ml of EDTA plasma (or separated serum, ideally obtained from the patient), placing this into a fresh 1ml EDTA tube and adding 0.5ml saline. Alternatively, a pre-prepared solution (RPMI) can be used (please call 01223 337625 to request an RPMI tube). The fluid can be drawn back into the syringe and re-injected to the tube in order to maximise cellularity and as an approximate guide, sufficient aspirates should be taken to achieve a cloudy appearance to the fluid.

Samples should be submitted to the laboratory within 24 hours and **next day delivery (stipulating delivery between 9am and 1pm)** is recommended. Normal **cytology smears** (at least 2, ideally unstained) and recent clinical history (including any relevant results where possible) should also be submitted with the aspirates.

Immunophenotyping can also be performed on body fluids such as pleural and abdominal effusions or CSF if there are a high number of cells present. Fluid should be placed into an EDTA tube (as for normal fluid analysis). Please contact the laboratory to discuss this if required.

Leukaemia immunophenotyping (on blood)

Leukaemia immunophenotyping can only be attempted if there is evidence of a lymphocytosis (lymphocyte count > $5x10^9$ cells/L) on haematology and/or circulating atypical cells. Please contact the laboratory for advice if the peripheral lymphocyte (or atypical cell) count is <$5x10^9$ cells/L.

Peripheral blood in normal EDTA tubes should be submitted. 1-2ml is typically sufficient, but please contact the laboratory if in doubt. **Fresh blood smears** should also be submitted, along with any related history or results if possible. **Samples should be submitted to the laboratory within 24 hours** and **next day delivery (stipulating delivery between 9am and 1pm)** is recommended.

Flow cytometry can also be performed on aspirated bone marrow material. Please contact the laboratory to discuss the case with one of our pathologists prior to submission.