Immunophenotyping for lymphoma and leukaemia: Flow cytometry and immunocytochemistry

Flow Cytometric Immunophenotyping

Flow cytometry uses a panel of antibodies to accurately differentiate the lineage of the cells in a sample (immunopheotyping). 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 the same day that the sample is submitted to us.

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 origin.

Please do not hesitate to contact the laboratory (01223 337625/337635) 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.

Sample requirements for flow cytometry

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

Multiple aspirates should be taken from the affected lymph node or organ (e.g. spleen, liver) and these should be squirted directly into separated serum taken from the patient prior to aspiration (at least 0.5mls, ideally placed into an EDTA tube). As a guide, sufficient aspirates should be taken to achieve a cloudy appearance to the serum. Alternatively, a special fixative solution (RPMI) can be used (please call 01223 337625 to request an RPMI tube). **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 and CSF if there are a high number of cells present. 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 count is <$5x10^9$ cells/L.

If possible, 5ml of **peripheral blood in normal EDTA tubes** should be submitted. However, 2ml is typically sufficient and in smaller patients (e.g. cats) or when blood collection is difficult, this should be more than adequate. **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.