Does your patient have long-standing lymphocytosis, lymphadenopathy, organomegaly?

Flow cytometry can provide a definitive diagnosis by excluding or confirming a lymphoproliferative disorder

a) Clinpath’s data show:
   – 23-30% of patients with an asymptomatic lymphocytosis are found to have a lymphoproliferative disorder
   – 7-10% of patients found to have a lymphoproliferative disorder have a normal full blood count
   – 14-20% of patients with a lymphocytosis do not have a lymphoproliferative disorder

b) Flow reports are always generated by a Consultant Haematologist in correlation with clinical history and morphology
   – this provides the opportunity to offer clinical advice on further investigation and management.

Are you following up patients with HIV, chronic infections, immunodeficiency or inflammatory disorders?

a) T cell subsets – CD4, CD8, Natural killer cells, B cell counts can assist in monitoring the underlying immune status
What is a Flow Cytometer?

Clinpath Laboratories is the only private pathology provider offering an in-house flow cytometry service. With the expertise of an on-site haematologist, Dr Lakshmi Nath, and specialist lymphoma pathologists, A/Profs David Ellis and John Miliauskas, we are able to offer a complete and integrated approach to the diagnosis of lymphoproliferative disorders with a comprehensive report.

A flow cytometer (FCM) is an instrument that can analyse cells in a column of fluid which ‘flows’ through a laser beam at a rate of up to 1000 cells per second. The laser beam strikes each cell, resulting in light being reflected from the cell. The cells are analysed initially without fluorescence, then further classified using fluorescence.

What can the flow cytometer tell us about a cell and how are these characteristics detected?

- Its relative size (by forward scatter of the incident light)
- Its relative complexity (by the side scatter of the incident light)
- Its surface antigenicity (by specific monoclonal labels which fluoresce)

Without fluorescence - using light scatter

- Sensors within the FCM detect light reflected at low angle (forward to the path of the cell) and side-angle (at 90 degrees to the cell’s path), corresponding respectively to the cell’s size and internal complexity.
- This allows the FCM to resolve lymphocytes, monocytes and granulocytes into three distinct populations by virtue of their size and granularity and can identify the presence of unusual cell populations (e.g. blasts or plasma cells).

Using fluorescent labelled monoclonal antibodies (MABs)

- Simultaneously, the MABs can be coupled with specific fluorescent labels which are detected as they fluoresce at different wavelengths and the intensity of light captured determines the cell’s surface antigenic profile.
- Cell populations may be considered abnormal if they show an unusual antigenic profile for a particular cell line or if ‘clonality’ can be demonstrated by kappa or lambda light chain restriction on B cells.

The result can be visualised as a graphical representation in two dimensions.
Applications of Flow Cytometry

Blood, bone marrow and lymph node cells have traditionally been classified by morphology, using light microscopy. More recently, combinations of antigens have been identified on the cell membranes, that enable us to identify their lineage by immunoperoxidase stains in tissue sections or by flow cytometry in cell samples.

Monoclonal antibodies (MABs) have been developed that recognise these antigens and have been grouped so that those that reacted similarly were allocated a specific CD (Cluster of Designation) number.

However, no single cellular antigen is tumour-specific.

To diagnose haematological malignancies, we use a panel of antibodies to differentiate normal from abnormal cells and allow specific sub-classification of the various leukaemias and lymphomas.

These cell surface antigens can be assessed by flow cytometry using combinations of MABs. The ‘normal’ cells are defined by established antigen combinations. The abnormal (malignant) cells show aberrant combinations, loss of the usually found antigens or over-representation of a population normally found in low numbers.

How is flow cytometry used in clinical haematology?

Assessment of persistent lymphocytosis

Persistent lymphocytosis may be morphologically unremarkable and it is difficult to be sure if it is reactive (e.g. due to viral illness, hyposplenism, autoimmune or drugs, etc.) or if it is a chronic lymphoproliferative disorder.

A lymphocyte surface marker (LSM) study is able to determine whether a lymphocytosis is reactive or neoplastic. In reactive proliferations, the B cells will be shown to be polyclonal (i.e. there will be a normal ratio of cells which express kappa and lambda light chains – roughly 1:1 in humans, but not in all species). Often the T cells and T cell subsets, as well as the natural killer and B cells, will be elevated and/or may show increased levels of activation antigens.

Note the examples of polyclonal and monoclonal populations shown on the opposite page.

Diagnosis, sub-classification and monitoring of haematological malignancies

Lymphocytosis is one only indication for flow cytometry studies. Lymphadenopathy, splenomegaly and/or the laboratory findings of lymphocytosis, pancytopenia, isolated cytopenias or a leucoerythroblastic blood film may alert the physician to the possibility of a haematological malignancy and the evaluation of LSMs may be indicated in blood or bone marrow.

Chronic lymphoproliferative disorders are most commonly of B cell origin.

LSM studies can diagnose a malignant proliferation if clonality is demonstrated (that is, excess kappa or lambda light chain expression is demonstrated) or if the B cells show aberrant loss of light chain expression (for example, as in B cell chronic lymphocytic leukaemia).

Sub-classification of the various B cell lymphoproliferative disorders depends on the morphological findings and the combination of antigens expressed on the cells (for example, co-expression of CD23 and aberrant expression of the T cell antigen CD5 on a monoclonal B cell population suggest B cell chronic lymphocytic leukaemia).

Less commonly, T cell chronic lymphoproliferative disorders and Natural Killer (NK) cell proliferations are diagnosed.

In these cases, the T cells may demonstrate abnormal expression of normal T cell antigens (for example, loss of CD7 or dual expression of CD4 and CD8).

If a T cell malignancy is suspected, then additional genetic studies of the T cell receptor should be performed to demonstrate clonality. For NK cells, serial flow cytometry may demonstrate a progression of the disorder to a large granular lymphocyte leukaemia.

Diagnosis and sub-classification of leukaemias

Chronic myeloid leukaemias and myelodysplasias

The chronic myeloid leukaemias and myelodysplasias are generally diagnosed morphologically, but LSMs can be a useful adjunct in assessing the percentage of blasts in the marrow and also identifying abnormal antigenic expression, which may be of prognostic significance.

The acute leukaemias

Acute leukaemias may be myeloblastic or lymphoblastic and, although the diagnosis is generally made by bone marrow morphology, immunophenotypic and cytogenetic profiles are recommended, as these will have prognostic and therapeutic significance.

Monitoring of haematological malignancies

Following therapy, flow cytometry provides a sensitive method for the monitoring of haematological malignancies. With knowledge of the primary diagnosis, the flow cytometer is able to detect tiny residual cell populations of about 1% of the total cells analysed and can confirm clinical remission or suggest a possible relapse.
Applications of Flow Cytometry

What specimen is required?
Specimens which can be tested include blood, bone marrow, body fluids (including CSF), aspirates and biopsies of lymph nodes, lymphoid tissue and all other tissue.

Blood, bone marrow
A fresh anticoagulated specimen (in EDTA or a lithium heparin tube) is preferred. Keep at room temperature and send to the laboratory immediately (preferably) or within 24 hours.

Body fluids (including CSF)
Send fresh. There is no need to add a preservative. Do not add formalin. Refrigerate and send to the laboratory immediately or, at the latest, within 24 hours.

Flow Cytometry – Adjunct to the Diagnosis of Lymphoma

How does flow cytometry apply to the diagnosis of lymphomas?
The flow cytometer identifies sub-sets of lymphocytes and other haemopoietic cell lines. The technique is very usefully applied to fine needle aspiration (FNA) and tissue specimens where there is suspicion of a lymphoproliferative disorder as there is limitation on what morphology can offer. Tissue must be sent fresh for analysis, as formalin fixation destroys cell surface antigens. Fresh tissue or material from FNA should be placed in a transport medium, such as Hank’s solution, which is available from our Stores Department.

How does flow cytometry help in the assessment of lymphoid lesions in histopathology samples like core biopsies, lymph nodes and other soft tissues?
Distinguishing reactive lymphoid hyperplasias from low-grade B cell lymphomas
Flow cytometry is very useful in this setting, as these conditions may appear very similar on cytological or histological slides. Normal and reactive B cell populations show expression of both kappa and lambda light chains, which are essential components of the immunoglobulin molecule. B cell lymphomas, on the other hand, are composed of monoclonal populations of B cells, which express only a single light chain, either kappa or lambda (‘light chain restriction’). This is the most important feature of malignancy in FNA and tissue specimens, as more than 80% of nodal lymphomas are of B cell type.

Sub-classification of lymphomas
Not only does flow cytometry help in the diagnosis of lymphoma, but the technique also assists in lymphoma sub-classification. Indeed, flow cytometric findings have been included in the new World Health Organisation (WHO) lymphoma classification. Examples where flow cytometry is especially helpful are:

Mantle cell lymphoma
One of the most difficult lymphomas to diagnose and classify accurately on morphological grounds alone is mantle cell lymphoma, an entity with a very distinctive and unique immunophenotype by flow cytometry, which is virtually always diagnostic (expression of CD5 antigen without CD23). On morphology alone, this tumour can be confused with low-grade lymphomas. The distinction is important, as the prognosis and treatment may differ.

Follicular lymphomas
Follicular lymphomas may be confused with a benign follicular proliferation. As follicular lymphomas commonly express CD10, flow cytometry can identify these lesions.

Partial replacement of a lymph node by lymphoma
If a lymph node is only partly involved by malignant lymphoma, the two populations of normal and abnormal cells may be difficult to differentiate on the histopathology or cytology slides.

Flow cytometry can detect even a small population of malignant cells admixed with normal cells.

What are the limitations of flow cytometry as applied to lymphoid lesions?
The sensitivity of flow cytometry is highest when the sample size is sufficient and contains viable cells. Flow cytometry has greater sensitivity in the diagnosis of low grade B cell lymphomas (which are often the most difficult to diagnose cytologically). However, in other situations, flow cytometry may produce a false negative result.

Large cell lymphomas, either because of their greater cell fragility or their lack of light chain expression, are the commonest B cell lymphomas associated with a false negative result. However, as the morphological findings are usually diagnostic, the practical significance of this is usually not great.

Hodgkin lymphoma generally remains outside the diagnostic capability of flow cytometry, although occasionally there are some clues to diagnosis.

Combining morphological findings and flow cytometric results leads to a greater degree of diagnostic accuracy, and has made FNA a first-line test in the investigation of enlarged lymph nodes.

Fresh material should be collected from all FNAs and biopsies of lymph nodes/ lymphoid tissue and flow cytometry requested. Flow cytometry can then be performed, if indicated by the initial assessment of cytological or histopathological findings.