

# Diagnostic solution to all myeloid and lymphoproliferative disorders

Including the leukaemias, multiple myeloma & MDS

Clinpath is the only SA private pathology provider able to offer a complete on-site diagnostic flow cytometry service.

- a) Bone marrow biopsy - expertise available - haematologist for reporting and performing bone marrows anywhere; both local and under GA
- b) Flow cytometry – All samples reported by a specialist haematologist
  - Always correlated with clinical history and morphology
  - Comprehensive and relevant reporting with clinical advice on further investigations and management
  - All samples, fluids and tissues can be tested and reported
  - Fast turn-around time of 24-48 hours with complete diagnosis
- c) Histopathology of lymph nodes and tissues – reported by two well-known lymphoma specialists – Associate Professor John Miliauskas and Associate Professor David Ellis

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 cell membranes. These 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 (for example, due to viral illness, hyposplenism, autoimmune or endocrine disorders, etcetera) 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 (that is, there will be a normal ratio of cells which express kappa and lambda light chains - roughly 1:1 in man but not in all species). Often, the T cells and T cell sub-sets, as well as the natural killer and B cells, will be elevated and/or may show increased levels of activation antigens.

### Diagnosis, sub-classification and monitoring of haematological malignancies

Lymphocytosis is only one 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.

### Sub-typing of lymphoproliferative disorders into B, T or NK cell origin

LSM studies can diagnose a malignant proliferation if clonality is demonstrated (that is, excess kappa or lambda light chain expression is detected) 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, coexpression of CD23 and aberrant expression of the T cell antigen CD5 on a monoclonal B cell population suggests 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/CD5 or dual expression of CD4 and CD8).

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

### Important diagnostic tool in identifying aberrant plasma cell populations

Flow cytometry can confirm monoclonality in plasma cell neoplasms, especially where morphological assessment is difficult because of low cell numbers.

### Diagnosis and sub-classification of leukaemias

#### Chronic myeloid leukaemia 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 the response to therapy. 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 provide early warning of 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 soft tissues.

### Blood, bone marrow

A fresh anticoagulated specimen (in EDTA or a lithium heparin tube) is preferred. Keep at room temperature. Send to the laboratory within 24 hours.

### Body fluids (including CSF)

Send fresh. There is no need to add a preservative. Do not add formalin. Refrigerate and send within 24 hours.

### Fine needle aspirates of lymph nodes, lymphoid tissue and all other fresh tissue

Place into Hank's solution or normal saline to preserve the cells. Do not add formalin. Refrigerate and send within 24 hours. Clinical information and relevant laboratory information are imperative so that we can select the most appropriate antibody combination to use in testing. In this way, it is more likely that a small abnormal population will be identified.

For any enquiries about flow cytometry on all specimens, please contact Dr Lakshmi Nath on 8366 2057 or the haematology supervisor on 8366 2056.

## Flow Cytometry – Adjunct to 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.

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.

### When is flow cytometry most useful in the assessment of lymphoid lesions?

#### 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 of lymphomas. 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.

### Flow Cytometry on FNAs

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, guided by the initial assessment of cytological and/or histopathological findings.

### What are the limitations of flow cytometry as applied to lymphoid lesions?

The sensitivity of flow cytometry is highest in low-grade B cell lymphomas (which are often the most difficult to diagnose cytologically). However, in other situations, flow cytometry may not be so useful.

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.

T cell and Hodgkin's lymphomas generally remain outside the diagnostic capability of flow cytometry, although occasionally there are some clues to the diagnosis.

For any enquiries about histological specimens, please contact Dr Suchitra Somers on 8366 2030 or the histology supervisor on 8366 2040.