FLOW CYTOMETRY AND CONFOCAL LASER SCANNING MICROSOCPY LABORATORY

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The laborartory is used as central facility at Institute of Pathology. The laboratory provides research oriented and diagnostic services to educational institutes, colleges, universities, national scientific institutes and large scale industries as well. The laboratory has Confocal Laser Scanning Microscope from Ziess and FACS Calibur Flow Cytometer from Becton and Dickinson. The Flow Cytometer is capable of sorting cells from heterogenous samples.

FLOW CYTOMETRY LABORATORY

ROUTINE APPLICATIONS:

A. Research projects

1. To study the role of cyclooxygenases (Cox-1 and Cox-2) in cytokines dysfunction of invasive and non-invasive TCC of human bladder.

2. To study the expression and relevance of tissue transglutaminase in superficial and invasive human bladder Transitional Cell Carcinoma (TCC).

3. Immunomodulatory responses on urinary bladder cancer patients.

Current pathological and clinical parameters provide important prognostic information which still have limited ability to predict the true malignant, recurrence and invasive potential of most bladder tumours.

We are involved in investigating the basic mechanism involved in carcinogenesis and tumour progression. Molecular biology has provided a host of markers which are of potential value of bladder carcinoma. These markers may serve as tools for early and accurate prediction of tumor recurrence, progression and development of metastases and for prediction of response to therapy. The precise prediction of tumor biological behavior would facilitate treatment selection for patients who may benefit from radical surgical treatment or adjuvant therapy.

B. Diagnostic Services

1. Leukemia and Lymphomas:

Analysis of multiple characteristics by flow cytometry involves the immunophenotyping of leukemias and lymphomas. Immunophenotyping as part of the diagnostic work-up of hematologic malignancies offers a rapid and effective means of providing a diagnosis. The ability to analyze multiple cellular characteristics, alongwith new antibodies and gating strategies, has substantially enhanced the utility of flow cytometry in the diagnosis of leukemias and lymphomas. Different leukemias and lymphomas often have subtle differences in their antigen profiles that make them ideal for analysis by flow cytometry. Diagnostic interpretations depend on a combination of antigen patterns and fluorescence intensity. Flow cytometry is very effective in distinguishing myeloid and lymphoid lineages in acute leukemias and minimally differentiated leukemias. Although most acute myeloid leukemias are difficult to classify by phenotype alone, flow cytometry can be useful in distinguishing certain acute myeloid leukemias, such as acute promyelocytic leukemia. Flow cytometry can also be used to identify leukemias that may be resistant to therapy .

2. Leukocyte Analysis:

As HIV disease progresses, CD4-positive T lymphocytes decrease in total number. The absolute CD4 count provides a powerful laboratory measurement for predicting, staging, and monitoring disease progression and response to treatment in HIV-infected individuals.

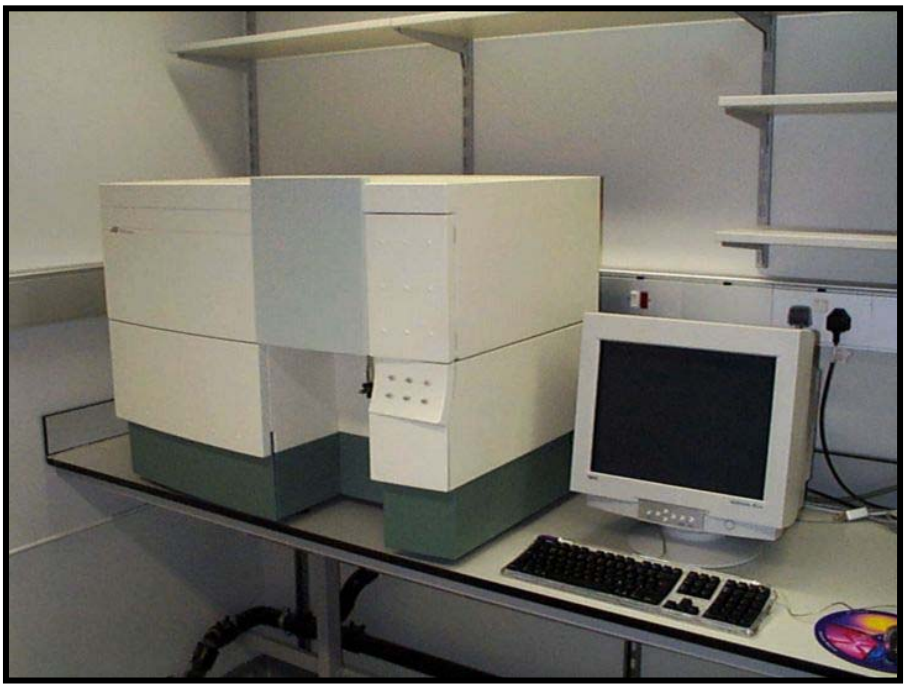
3. DNA analysis:

Abnormal DNA content, also known as "DNA content aneuploidy" is determined in a tumor cell population. DNA aneuploidy generally is associated with malignancy; however, certain benign conditions may appear aneuploid. DNA aneuploidy correlates with a worse prognosis in many types of cancer but is associated with improved survival in multiple myeloma, and childhood acute lymphoblastic leukemia (ALL). Although conventional cytogenetics can detect smaller DNA content differences, flow cytometry allows more rapid analysis of a larger number of cells.

4. Immunophenotyping Applications in Hematology:

The distributed nature of the hematopoietic system makes it amenable to flow cytometric analysis. Many surface proteins and glycoproteins on erythrocytes, leukocytes, and platelets have been studied. The availability of monoclonal antibodies directed against these surface proteins permits flow cytometric analysis of erythrocytes, leukocytes and platelets. Antibodies against intracellular proteins like interleukins are also commercially available and permit analysis of an increasing number of intracellular markers.

The B-cell lymphoproliferative disorders often have specific antigen patterns. The use of a wide range of antibodies allows clinicians to make specific diagnoses based on patterns of antigen expression. Not only is the presence or absence of antigens useful in making specific diagnoses, the strength of antigen expression can also aid in diagnosis.



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CONFOCAL LASER SCANNING MICROSCOPY:

Confocal microscopy offers several advantages over conventional optical microscopy, including controllable depth of field, the elimination of image degrading out-of-focus information, and the ability to collect serial optical sections from thick specimens. The key to the confocal approach is the use of spatial filtering to eliminate out-of-focus light or flare in specimens that are thicker than the plane of focus with which extremely high-quality images can be obtained from specimens prepared for conventional optical microscopy and in its great number of applications in many areas of current research interest.

Choosing Fluorophore Combinations for Confocal Microscopy - In planning multiple label fluorescence staining protocols for widefield and laser scanning confocal

fluorescence microscopy experiments, the choice of probes is important in obtaining the best target signal while simultaneously minimizing bleed-through artifacts.

Three Color Imaging for Confocal Microscopy - The laser scanning confocal microscope is routinely used to produce digital images of single-, double-, and triple-labeled fluorescent samples. The use of red, green and blue (RGB) color is most informative for displaying the distribution of up to three fluorescent probes labeling in a cell, where any colocalization is observed as a different additive color when the images are colorized and merged into a single three-color image.

Applications in Confocal Microscopy - The broad range of applications available in laser scanning confocal microscopy includes a wide variety of studies in neuroanatomy and neurophysiology, as well as morphological studies of a wide spectrum of cells and tissues. In addition, the growing use of new fluorescent proteins is rapidly expanding the number of original research reports coupling these useful tools to modern microscopic investigations. Other applications include resonance energy transfer, stem cell research, photobleaching studies, multiphoton microscopy, DNA hybridization, membrane and ion probes, bioluminescent proteins and epitope tagging.

Co-localization of Fluoroephores in Confocal Microsocpy- Two or more fluorescence emission signals can often overlap in digital images recorded by confocal microscopy due to their close proximity within the specimen. This effect is known as colocalization and usually occurs when fluorescently labeled molecules bind to targets that lie in very close or identical spatial positions.

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