



UNIVERSITY OF
CALGARY



*Department of Pathology and Laboratory Medicine
Division of Hematology and Transfusion Medicine*

Hematopathology Training Program

FLOW CYTOMETRY Goals & Objectives and Training Schedule

**For:
Contact:**

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GOALS AND OBJECTIVES

GENERAL OBJECTIVES:

A week-long Flow Cytometry portion of the Specialized Laboratories module is designed to familiarize the trainee with the flow-cytometry based clinical testing. Flow cytometry constitutes a key element in the diagnosis/sub-classification and monitoring of various hematolymphoid disorders. Applications of immunophenotypic testing in the diagnosis/sub-classification of hematolymphoid disorders, detection of minimal residual disease (MRD), clinical management of BMT transplant and/or immunocompromised patient will be outlined through both technical and interpretive aspects/phase of immunophenotyping.

During the **technical phase** of the rotation the trainee will learn the principles of:

- flow cytometry instrumentation,
- sample processing,
- data analysis,
- selection of the appropriate staining panel is performed,
- limitations of the techniques as imposed by artefacts/suboptimal viability etc.

During the **interpretive phase** the trainee will learn to interpret the histogram plots, and under the supervision of the hematopathologist will correlate them with the clinical history and put in the context of morphologic findings (PB smears, BM biopsies, LN biopsies/touch preps,). Under the supervision of the pathologist preceptor, the trainee will review the daily assortment of cases which may include:

- pancytopenia and acute leukemia
- malignant lymphoma,
- minimal residual disease
- ploidy assessment
- other specialized testing including, but not limited to:
 - paroxysmal nocturnal hemoglobinuria,
 - fetal-maternal haemorrhage,
 - oxidative burst,
 - perforin assessment,
 - mitogen stimulation evaluation and
 - immunodeficiency disorder work-up

In addition, an independent, concurrent and systematic self-review of immunophenotypic features of reactive and neoplastic haematopoietic and lymphoid tissue tumours is expected during this rotation.

A well organized study set flow cytometric reports/talks and articles are available on a G drive to aid the trainee in this process.

General expectations:

1. Daily material review with the technologists and service hematopathologist
2. Designated rounds attendance.
3. Pager availability for stat lymphoma/leukemia cases
4. Self study; regular daily review of teaching slides, teaching sets, G drive with CD ROM talks/lectures, relevant papers, etc.
5. Pro-active selection of subspecialty relevant cases (pediatric/neuropathology) for a review with the hematopathologist to enhance one's specialty experience
6. Writing end-of-rotation test, which will include short answer questions and histogram/plot evaluation/ discussion (last Friday of the rotation).
7. Participate in module/preceptor evaluation.
8. No scheduled vacation/absences; any unforeseen absences must be communicated to the Hematopathology Program Training Co-ordinator and individual hematopathology preceptor.

SPECIFIC OBJECTIVES:

At the completion of the flow cytometry training the trainee will have adequate knowledge of principles and clinical application of flow-cytometric techniques in the diagnosis and management of patients with hematolymphoid disorders/malignancies.

The trainee will require understanding of the following competencies:

Medical Expert/Clinical decision-Maker

Understand the basic principles in flow-cytometry including:

- Sample selection and accessioning
- Cell yield and viability testing
- Staining panel selection
- Principles of surface antigen and intracellular antigen staining preparation
- Gating strategies
- Data acquisition and reporting
- Understand the lineage specificity and association of various fluorochrome tagged monoclonal antibodies used in a clinical flow-cytometry laboratory.
- Formulate panel of various antibodies to determine the lineage/immunophenotypic diagnosis of a hematolymphoid malignancy.
- Understand and interpret the dot plots and understand the lineage/ immunophenotype of a particular hematological malignancy (ie. B- T- NK-cell neoplasm, acute myeloid leukemia).
- Suggest some additional ancillary studies to refine and modify these observations in the light of clinical information or other data.

Communicator:

General Requirements:

- Establish effective working relationships with the case hematopathologist and consulting haematologists/oncologists/pathologists.
- Establish effective working relationships with laboratory technologists, laboratory scientist and supporting staff including clerks and administrative assistants.
- Obtain and synthesize relevant clinical history from physicians, electronic and written health records.
- Listen and respond effectively.
- Discuss in a timely fashion appropriate information with the health care team.

Specific Requirements:

- Understands the role of a flow cytometrist/hematopathology consultant within the health care team.
- Act as a consultant to clinical/laboratory colleagues on the interpretation and relevance of immunophenotypic findings, with particular regard to their significance in the management of the patient.
- Understand the role of flow cytometry findings should provide in a given clinical situation and be able to communicate it effectively and in a timely fashion in an oral and written form.
- Assist in the continuing education of clinicians/pathologists and other members of the health care team.

Collaborator:

General Requirements

- Consult effectively with other pathologists/clinicians and other health care professionals.
- Contribute effectively to interdisciplinary team activities.

Specific Requirements:

- Must have experience in neoplastic hematopathology sufficient to achieve a sound understanding of the effects/role of immunophenotypic analysis in the diagnosis and clinical management.
- Demonstrate the ability to advise on the appropriateness of obtaining additional ancillary studies and following examination of these, to advise on further appropriate investigations and management

Manager:

General Requirements:

- Ability to utilize health care resources effectively to balance patient care, turn around time, and educational/research needs.
- Allocate finite health care resources wisely.
- Work effectively and efficiently in a health care organization.
- Utilize information technology to optimize patient care, life-long learning and other activities.

Specific Requirements

- Demonstrate knowledge of the principles of laboratory management and administration.
- Demonstrate knowledge of the methods of quality control and assurance in the field of immunophenotypic testing.
- Demonstrate competence in basic computer skills with emphasis on automated electronic reporting, electronic communication and search strategies

Health Advocate

General Requirements:

- Contribute effectively to improved health of patients and communities.

Specific Requirement

- Recognize and respond to those issues where advocacy is appropriate i.e educate family practitioners, general surgeons, interventional radiologists re. appropriateness of immunophenotypic testing and its limitations.
- Understand the role of immunophenotypic testing in primary and specialized care of patient with hematolymphoid disorder.

Scholar:

General Requirements:

- Effectively develop, implement and monitor a personal self-education strategy during this week-long rotation
- Critically appraise sources of medical information, as it pertains to clinical cases.
- Facilitate learning of faculty, fellow trainees, medical students- observerships, and other health professionals i.e. laboratory technologists/nurses.

Specific Requirements:

- on completion of the rotation, the resident will be able to propose a real life/simulated research question relevant to immunophenotypic testing.
- Contribute to development of new knowledge by involvement in on-going or new/original research project/s within the Flow Cytometry Laboratory

Professional:

General Requirement:

- Deliver highest quality patient care.
- Exhibit appropriate personal and interpersonal professional behaviours.
- Practise medicine ethnically consistent with obligations of a physician.
- Demonstrate the knowledge, skills and attitudes relating to gender, culture, and ethnicity pertinent to anatomical pathology.

Specific Requirements

- Act as an appropriate role model for other trainees, students and laboratory personnel
- Demonstrate a professional attitude to peers, colleagues, laboratory and administrative staff.
- Have an appreciation of the crucial role of the hematopathologist in providing quality patient care. This will include knowledge of an individual professional limitations and the necessity of seeking appropriate second opinions.

Suggested Reading:

- Nguyen, D. (2003). Flow Cytometry in Hematopathology: A Visual Approach to Data Analysis and Interpretation. Totowa, NJ, U.S.A.: Humana Press Inc.
- Keren, D. (2001). Flow Cytometry in Clinical Diagnosis (3rd ed.). Chicago, Illinois, U.S.A.: American Society of Clinical Pathologist.
- Ortolani, C (2011). Flow Cytometry of Hematological Malignancies. Oxford, UK: Wiley-Blackwell.
- Ochs, H.D., Smith C.I.E., Puck, J.M. (2007). Primary Immunodeficiency Diseases: A Molecular and Genetic Approach. Oxford, UK: Oxford University Press.
- Geha, R., Rosen, F. (2008). Case Studies in Immunology: A Clinical Companion (5th ed.). NewYork, U.S.A.: Garland Science.
- Sun, T. (2008). Flow Cytometry and Immunohistochemistry for Hematologic Neoplasms. Philadelphia, PA, U.S.A.: Lippincott

Flow Cytometry Training Schedule

Date	Time	Description of Activities	Assigned To
Monday	09:00	Flow Cytometry Theory Laminar Flow Hydrodynamic focussing Flow Cell vs. Jet in Air Electrostatic Sorting Light sources: gas lasers, diode lasers, arc lamps Principle of fluorescence Stokes Shift Filter Theory: dichroic mirror, bandpass, long pass Optical set up of FC500 Parameters: forward scatter, side scatter, fluorescence, time, ratio Electronics: photons, analog, digital processing Histograms: log vs linear Fluorescent probes: conjugated to antibodies Tandem dye theory Fluorescent probes: DNA, RNA, DHR123, 7-AAD, PI, TO, Fura Red Spectral Overlap Determination of Voltages/Gains Determination of Compensation Regions: rectangle, polygonal, linear, prism, quadrant Gates, Boolean logic	Joanne Luider, Lab Scientist
	10:30	Staining Techniques Observe and/or perform CD4 counts (single platform, no wash technique) Observe/perform leukemia/lymphoma sample preparation and staining	Tech II
	12:00 – 13:00	Break	
	13:00	Samples and Test Selection List of available tests / Specimen rejection criteria Requisitions: Flow, Bone Marrow, using other dept reqs (cytology, pathology etc) Booking procedure On-line Guide to Services Transportation requirements/Courier/Referred in samples Acceptable sample types Sample size: PB, FNA's, CSF's, tissues Anticoagulants: Pros and cons of EDTA, Heparin, and ACD-A BM media, Tissue media Paraffin sections for DNA Ploidy/Fresh/Frozen Sample storage: PB, BM TI, serums TAT Age and viability of specimens Multi-department sample distribution: CSF's, BM, Tissues, BAL Reflex testing Choosing panels, clinical history	Tech II
	14:15	Tour of Flow Cytometry	Joanne Luider, Lab Scientist 6 th Floor FMC, Room C630 (Reporting Area)

Date	Time	Description of Activities	Assigned To
	14:30 – 16:00	Flow Cytometric Analysis – Principles Tour of FCS Express application software. Listmode file standards: FCS 2.0 vs FCS 3.0 Types of 2 parameter plots: dot, contour, density Regions: linear, polygonal, rectangle, prism, auto Gates/gating strategies/Boolean logic Scaling of histograms Stops Colour tracking Overlay and subtraction plots of publication Creating/modifying protocols and panels Standardization, Compensation Exporting data High Level Multicolour Sorting	Joanne Luider, Lab Scientist 6 th Floor FMC, Room C630 (Reporting Area)
Tuesday	08:30	Hematopoietic Stem cell transplant Review stem cell markers: CD34, 90, 38, 33, Dr, ALDH, side population Overview of role of FC: CD34 PB, BM, PHER, CMV IEA, MRD CD34 protocols: ISHAGE vs Calgary Follow CD34 PHER from pneumatic tube to reporting (TAT) CD3 Counts (T Cell Add Back/BMT Program) Positive Stem Cell Collection - Clinimac	Joanne Luider, Lab Scientist
	10:00 – 12:00	Bone Marrow Rounds	6 th Floor Multi-headed Microscope Lab
	12:00	Break	
	12:30	Self-review of current cases	Resident
	14:30 – 16:00	Current case review	Dr on Flowcytometry service 6 th Floor Multi-headed Microscope Lab, Room C613
Wednesday	08:30	Data Acquisition/Instrumentation Observe and/or perform data acquisition on 5 colour FC500 instruments. Review instrument QC (Alignment, Standardization, Linearity, Compensation Verification)	Patricia Johnson, Tech III Flow Cytometry Lab, Room C629 (Instrument Room)
	10:00	Sorting Review the principles and operation of a cell sorter Understand principles of high level multicolour flow Review current clinical/research applications for cell sorting	Rhonda Bailey, Sorter Tech Flow Cytometry Lab, Room C629 (Instrument Room)
	10:30	Reporting Current Cases	Joanne Luider, Lab Scientist Patricia Johnson, Tech III Tech II Flow Cytometry Lab, Room C630 (Reporting Area)
	12:00	Break	
	13:00	Self-review of current cases	Resident
	14:30	Current case review	Dr on Flowcytometry service 6 th Floor Multi-headed Microscope Lab, Room C613

Date	Time	Description of Activities	Assigned To
Thursday	09:00	Non-neoplastic Applications of Flow Cytometry – Part I Fetomaternal Hemorrhage Paroxysmal Nocturnal Hemoglobinuria NRBC Enumeration RBC Retics Red Cell Associated Immunoglobulin Platelet Counts Platelet Antibodies Platelet Surface Markers Platelet Reticulocytes Platelet Storage Pool Deficiency Platelet Microparticles PLA-1 Phenotyping Neutrophil Antibodies Hereditary Spherocytosis	Patricia Johnson, Tech III
	10:00 – 12:00	Lymphoma Review Session	6 th Floor Multi-headed Microscope Lab
	12:00	Hematology Education Rounds	TBCC Rm CC104
	14:00	Non-neoplastic Applications of Flow Cytometry – Part II CD4 Counts HLA-B27 DNA Ploidy/POC Interstitial Lung Disease CD3 Counts (Solid Organ Transplant) Lymphocyte Subsets/Rituximab Panel Neuroblastoma Immunophenotyping	Tech II
Friday	08:30	Immunodeficiency: Theory and Case Review Immunophenotyping Screen Mitogen Stimulation Assay SCID Leukocyte Adhesion Deficiency CGD/Neutrophil Function Wiskott Aldrich/CD43 Hyper IgM Syndrome ALPS HLH TCR Vb Repertoire	Joanne Luider, Lab Scientist 6 th Floor FMC Lab Library
	10:00	Break	
	13:00	Q&A and Evaluation End-of-rotation test	Dr on Flowcytometry service 6 th Floor Multi-headed Microscope Lab, Room C613