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 is 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 reasearch 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:
## Flow Cytometry Training Schedule

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<th>Date</th>
<th>Time</th>
<th>Description of Activities</th>
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| Monday | 09:00  | **Flow Cytometry Theory**  
Laminar Flow  
Hydrodynamic focusing  
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  
6th Floor FMC, Room C630 (Reporting Area) |
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| Tuesday    | 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  
6th 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 |
| Tuesday    | 10:00 – 12:00 | **Bone Marrow Rounds**  
6th Floor Multi-headed Microscope Lab |  |
| Tuesday    | 12:00      | Break  |  |
| Tuesday    | 12:30      | Self-review of current cases  | Resident  |
| Tuesday    | 14:30 – 16:00 | Current case review  | Dr on Flowcytometry service  
6th 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) |
| Wednesday  | 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) |
| Wednesday  | 10:30      | **Reporting Current Cases** | Joanne Luider, Lab Scientist  
Patricia Johnson, Tech III  
Tech II  
Flow Cytometry Lab, Room C630 (Reporting Area) |
| Wednesday  | 12:00      | Break  |  |
| Wednesday  | 13:00      | Self-review of current cases  | Resident  |
| Wednesday  | 14:30      | Current case review  | Dr on Flowcytometry service  
6th Floor Multi-headed Microscope Lab, Room C613  |
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| 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 | 6th 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  
6th Floor FMC Lab Library |
|      | 10:00 | Break | |
|      | 13:00 | Q&A and Evaluation  
End-of-rotation test | Dr on Flowcytometry service  
6th Floor Multi-headed Microscope Lab, Room C613 |