



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

# Hematopathology Training Program

## **Molecular Hematology Goals & Objectives and Training Schedule**

**For:  
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## **GOALS & OBJECTIVES**

### **General Objectives:**

Molecular Hematology laboratory plays a key role in the diagnosis and management of various malignant and non-malignant disorders. This laboratory also provides critical information regarding the clinical management of bone marrow transplant patients. Trainees will have one week rotation in the laboratory to observe the molecular techniques currently being carried out in the laboratory that employ immunologic assays (ELISA, binding assays, Western blotting), or polymerase chain reaction (whole blood PCR, reverse transcription directed PCR, real-time PCR and inverse PCR) for: 1) detection of mutations and carrier determination in disorders of hemostasis and thrombosis, 2) the molecular detection and monitoring of abnormal fusion gene transcripts in leukemias, 3) detection of mutations in signalling proteins with prognostic value in AML, 4) JAK-2 mutations in chronic MPDs and 5) the determination of blood cell chimerism after bone marrow/PBSC transplantation. For those assays not currently running in the laboratory, the resident will review assay theory, procedures, and previous results with the laboratory director. This rotation will provide opportunity to have a broader understanding of technical aspects, clinical application, and appropriate use/ limitations of molecular techniques in the diagnosis and clinical management of various hematologic disorders.

The resident/fellow will also participate in a sign out session of Molecular Hematology. During this session a hematopathologist//hematologist and laboratory director will review all the molecular hematology results of the week. The clinical application, appropriate subsequent testing and advice to clinician will be discussed and the report will be finalized in the light of available clinical information and additional assay performed in various other laboratories.

### **General expectations:**

1. Daily meeting with laboratory section head
2. Daily visit to molecular hematology laboratory and observation of advance molecular testing protocols
3. Participation in at least one molecular sign-out session with consulting hematopathologists
4. Self study, regular review of supplied review papers and powerpoint presentations or other publications/learning materials
5. Write a final quiz consisting of 25 multiple choice and short answer questions relating to molecular protocols and role of molecular testing in diagnosis and monitoring of hematologic disease
6. Participate in module preceptor evaluation

### **Specific Objectives:**

At the conclusion of the training week, the trainee will be familiar with and will acquire an understanding of the following competencies:

## **Medical expert/Clinical decision-maker**

### **General Requirements:**

1. Demonstrate understanding of the value of molecular technologies in diagnosis and therapeutic monitoring of patients with hematologic diseases
2. Demonstrate a knowledge of principles of DNA and RNA based molecular assays
3. Demonstrate a knowledge of principles of advanced immunologic based assays

### **Specific Requirements:**

1. Understand the concepts and distinct features of:
  - a. von Willebrand disease and its classification by vWF multimer gel analysis
  - b. inherited risk factors for thrombosis and corresponding clotting factor gene mutation/polymorphisms
  - c. HITTS associated antibodies and their detection by immunologic and functional assays
  - d. type 2N vWD vs. mild hemophilia A
  - e. carrier determination in hemophilia A and B by immunologic (probabilistic) and gene mutation (direct) analyses.
  - f. molecular abnormalities in leukemias and chronic myeloproliferative disorders such as PV, ET and IMF; and residual disease detection by molecular methods
  - g. cell signalling pathway gene mutations as prognostic indicators in acute leukemias such as AML
  - h. hematopoietic cell chimerism after BM/PBSC transplantation and its detection by PCR STR and real-time PCR InDel analyses
2. Awareness of appropriate use of molecular testing and ethical and medical/legal implications of molecular DNA screening assays in a family setting
3. Appreciate the important role of quality assurance in the molecular laboratory
4. Understand the limitations of molecular data

## **Communicator**

### **General Requirements:**

1. Establish effective working relationships with consulting hematologists/oncologists/pathologists and staff in other specialized laboratories
2. Obtain and synthesize relevant clinical history from physicians, electronic and written health records.
3. Listen and respond effectively.
4. Discuss, in a timely fashion, appropriate information with the health care team.

### **Specific Requirements:**

1. Understand the role of a pathology consultant with respect to molecular testing
2. Act as a consultant to clinical colleagues on the interpretation and relevance of molecular data, with particular regard to their significance in the management of the patient.
3. Understand the role molecular data 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.
4. Assist in the continuing education of clinicians/pathologists and other members of the health care team particularly with respect to appropriate use/ordering of advanced molecular testing.

### **Collaborator**

#### **General Requirements:**

1. Consult effectively with other hematopathologists/clinicians and health care professionals.
2. Contribute effectively to other interdisciplinary team activities including BMT group, adult and pediatric bleeding disorders clinics, and other CLS specialized laboratories.

#### **Specific Requirements:**

1. Must have sufficient exposure to advanced molecular methods and use/limitations of molecular data to achieve a sound understanding of the role of molecular technologies in clinical management.
2. Demonstrate the ability to advise on the appropriateness of molecular testing and following examination of these, to advise on further appropriate investigations and management
3. Understand and communicate effectively to other health professionals the medico/legal implications of gene based screening in a family or population based setting

### **Manager**

#### **General Requirements:**

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

**Specific Requirements:**

1. Demonstrate knowledge of the principles of laboratory management and administration particularly with respect to operation of a molecular laboratory
2. Demonstrate knowledge of the methods of quality control in the field of molecular pathology, particularly with respect to PCR based methodologies
3. Demonstrate knowledge of the methods for professional quality assurance as applied to advanced immunologic and DNA/RNA based technologies and the role of external surveys, variance reporting/tracking and other QA programs
4. Demonstrate competence in basic computer skills with emphasis on automated electronic reporting, electronic communication and search strategies

**Health Advocate**

**General Requirements:**

1. Contribute effectively to improve the health of patients and communities
2. Recognize and respond to those issues where advocacy is appropriate
3. Understand the role of consult pathology in patient's care

**Specific Requirements:**

1. As members of an interdisciplinary team of professionals responsible for individual and population health care, the consult pathologist will endeavour to ensure that laboratory practices and test selection are regularly evaluated to determine that they meet these community needs
2. Reinforce to the public and to the profession the essential contribution of laboratory medicine to health

**Scholar**

**General Requirements:**

1. Develop, implement and monitor a personal continuing education strategy
2. Critically appraise sources of medical information
3. Facilitate learning of patients, house staff/students and other health professionals
4. Contribute to development of new knowledge

**Specific Requirements:**

1. Show proficiency in utilizing electronic access to medical literature in researching a given topic in molecular medicine and show familiarity with use of powerpoint or similar software for preparing a seminar presentation on that topic

## **Professional**

### **General Requirements:**

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

### **Specific Requirements:**

- 1. Act as an appropriate role model for students and others**
- 2. Demonstrate a professional attitude to colleagues and other laboratory staff**
- 3. Have an appreciation of the crucial role of the pathologist in providing quality patient care including; knowledge of an individual professional limitations and the necessity of seeking appropriate second opinions/ opinions of specialists from other disciplines**

## Molecular Hematology Rotation

Day	Time	Topic	Objectives	Details
<b>Monday</b>	8:30 – 11:00	Tour of Molecular Hematology Laboratory	Familiarity with the department's physical location and services offered  Introduction to Staff  Review of training program	Lab layout PCR/non-PCR areas Overview of molecular services and work centres Other department interactions Training schedule Planned testing vs case reviews Reference materials: Procedure manual Selected journal publications
		Laboratory Hazards and Safety	Review of the types of laboratory hazards present in the laboratory and the regulations in place to ensure visitor and staff safety including use of PPE and lab policies	Biohazards UV light Cryogenics Radioisotopes HV Equipment Working with PCR products
		Basis of Molecular Diagnosis	Review of cell biology principles as they relate to molecular testing	<b>Review:</b> Cell structure and Gene organization Properties of DNA/RNA Regulation of gene expression DNA mutations, sequence variations, identity Proteins Cell signaling
		Laboratory Test Menu	Detailed laboratory services and test menu review	Clients Procedure manual and GTS listing Hemostasis and oncology service, volumes and TAT
	<i>13:00-13:30</i>	Hemostasis Principles	Blood clotting, acquired and inherited disorders of hemostasis	<b>Review:</b> Primary/secondary hemostasis Vascular and circulating components Central role of thrombin in regulation of pro-coagulant and anti-coagulant pathways Inherited defects in coagulation Inherited and acquired risk factors for deep vein thrombosis
	<i>13:30-16:30</i>	Acquired Thrombosis Risk Assessment  Hemophilia Diagnosis and Carrier Determination	Screening for HIT associated Antibodies  ELISA for FVIII and vWF  vWD 2N FVIII binding assay	Specimen requirements, receipt and assay methodology <b>Observe:</b> assay technique and data analysis. Reporting format and Pathnet QC/QA Assay principles and set-up Reagents and methodology

Day	Time	Topic	Objectives	Details
		Mild Hemophilia A or vWD 2N?		<p><b>Case study:</b> Data analysis and interpretation Use in Hem A carrier determination QC/QA Assay principles and set-up Reagents and procedure review Appropriate use of testing</p> <p><b>Case study:</b> Interpretation and limitations QA/QC issues</p>
Tuesday	8:30- 9:30	VWD Sub-typing	vWF multimer analysis	<p><b>Review:</b> Assay principles and set-up Appropriate use of testing Discussion of technique</p> <p><b>Case study:</b> Interpretation and limitations of results QC/QA issues</p>
	9:30-10:30	DNA- based Assays	<p>Review of DNA-based Assays</p> <p>DNA biochemical properties</p> <p>Specimen requirements and processing</p> <p>Qualitative assays</p>	<p><b>Review:</b> DNA structure, melting, T<sub>m</sub>, absorbance Specimen handling and DNA extraction and quantification</p> <p><b>Observe:</b> Specimen receipt and DNA extraction procedure for DNA testing</p> <p><b>Review:</b> Hybridization standard PCR assay Restriction enzyme digestion Gel electrophoresis Allele specific PCR Detection methods Real time PCR theory Limitations, sources of error</p>
	10:30-11:30	Inherited Risk Factors for Thrombosis (I)	Thrombosis risk testing	<p><b>Review:</b> Inherited defects in coagulant proteins resulting in increased risk for DVT functional testing: APC resistance whole blood PCR method theory</p> <p><b>Observe:</b> WB PCR method procedure and equipment (FV and FII assays)</p>
	13:30-14:00	Inherited Risk Factors for Thrombosis (II)	Case study FV Leiden and FII polymorphism	<p><b>Case study:</b> Discuss data interpretation and QC/QA issues</p>



Day	Time	Topic	Objectives	Details
	14:00-15:00	Gene Inversion in Severe Hemophilia A	Inverse PCR testing for FVIII gene inversion	<b>Review:</b> Gene inversions and severe hemophilia A Original southern blotting method vs. new inverse PCR method Reagents and equipment <b>Case study:</b> Limitations and sources of error QC/QA issues
	15:00-16:40	Prognostic indicators in AML	DNA-based testing for mutations in <i>FLT3</i> , <i>NPM1</i> genes	<b>Review:</b> Role of <i>FLT3</i> and <i>NPM1</i> proteins in cell signalling PCR methodology AML in children and adults: Karyotypically normal population <b>Case Study:</b> Screening for mutations in acute leukemia QC/QA issues and test sensitivity
Wednesday,	8:30- 11:00	RNA- based Assays	Review of RNA-based Assays  Types and biochemical properties of RNA  Specimen handling and processing  RNA extraction and quantification  Qualitative PCR assays  Quantitative PCR Assays	<b>Review:</b> RNA species, stability, primary and secondary structure RNA liability, RNase inhibitors Manual and automated methods cDNA reactions Primary and nested PCR detection methods Quantitative Real-time PCR Reagents and equipment Assay set up, standards etc Computer data analysis Limitations and sources of error
	11:00-12:00	Molecular Oncology	Translocations in leukemia CML/ALL, APL, and other AML	<b>Review:</b> Translocations and gene fusions Cellular consequences
	13:00-15:30	Minimal Residual Disease Detection (I)	Molecular assays for fusion genes  Qualitative assays	<b>Review:</b> BCR-ABL1 and PML-RARA fusion gene assays by nested PCR <b>Observe:</b> Specimen receipt, processing, and RNA extraction and storage Equipment and assay reagents
	15:30-16:30	MRD (II)	RT directed, nested PCR	<b>Case study:</b> M-bcr and m-bcr translocation identification Data interpretation and reporting Appropriate use of testing

Day	Time	Topic	Objectives	Details
Thursday,	8:30-10:00	MRD assay (III)	Quantitative, real time PCR	<b>Review:</b> Q-PCR theory Assay reagents, controls and standards Data interpretation and sources of error
	10:00-11:30	Monitoring Cancer Therapy	RT-directed Q-PCR for BCR-ABL1 fusion gene transcripts	<b>Review:</b> Assay reagents and set-up reverse transcription reaction PCR controls <b>Observe:</b> M-bcr PCR assay set-up <b>Case study:</b> Data interpretation and report generation

\* Residents are expected to attend Hematol Rounds 12:00-1:00, TBCC Auditorium (CC104)

Day	Time	Topic	Objectives	Details
Thursday	13:30-14:00	Hematopoietic Cell Chimerism	Review of blood cell chimerism in BM/PBSC transplantation recipients	<b>Review:</b> Definition and types Importance to assessment of engraftment, residual disease, GvH disease development, disease relapse Frequency of testing
	14:00-15:00	Molecular Assays for Chimerism Determination	Review of STR PCR and SNP (indel) PCR techniques	<b>Review:</b> Theory and assay set-up Reagents and controls Specimen requirements, DNA banking Whole blood vs. Flow separated cells
	15:00-17:00	Chimerism Assessment	STR PCR Assay	<b>Observe:</b> DNA banking Specimen manipulation Capillary analyzer overview Electropherogram interpretation <b>Case study:</b> Complete, mixed and no chimerism examples Flow sorted cells and chimerism determination

Day	Time	Topic	Objectives	Details
Friday	8:30-9:30	Laboratory Report Sign-out Meeting	Result review, and data interpretation. Appropriate use of molecular testing and importance of relationship to other laboratory data/patient clinical history QA/QC and TAT issues.	Discussions with hematopathologists and laboratory section head Question and answer session
	9:30-10:00	Chronic Myeloproliferative Disorders	Review of PV, ET, IMF	<b>Review:</b> Diagnostic challenges Laboratory testing Mutations
	10:00-12:00	JAK2 V617F Assay	Real time PCR allelic discrimination assay protocol  Assay procedure and data analysis	<b>Review:</b> Theory of allelic discrimination PCR Fluorescent probes and assay set up Data interpretation and reporting Sources of error and QA/QC <b>Observe:</b> Sample manipulation Assay set-up and run Equipment operation QC/QA and sources of error <b>Case study:</b> Raw data analysis Report Generation and interpretation
	13:00-14:00	QA and QC	QA/QC issues in the Molecular Laboratory	<b>Review:</b> Pre-analytic, analytic and post- analytic errors Incident reporting
	14:00-15:00	Short Quiz	Assessment of trainee understanding of molecular techniques and their role in diagnosis and monitoring of hematologic diseases	25 Short answer/multiple choice question quiz
	15:00-16:30	Quiz Answers	Review of quiz answers with trainee and feedback session	<b>Quiz review:</b> Question and answer session Review of trainee feedback

**NOTE:**

Although this chart outlines the anticipated schedule for rotation through the Molecular Laboratory, the actual testing schedule is determined by specimen volume and batching. Therefore, the timing and amount of laboratory observation must remain flexible during the rotation week. Ample time will be provided for case study review with the Laboratory Director, for those procedures not currently carried out in week of rotation.