Immunophenotyping and Flow Cytometry at University of Minnesota Medical Center, East Bank

The goal of flow cytometry rotation is to provide a basic understanding of the principles underlying the application of flow cytometry for immunophenotyping and DNA ploidy-cell cycle analysis.

The immunophenotyping component of this rotation includes principles of flow cytometry instrumentation, of the monoclonal antibodies and staining procedures used, of the lineage and differentiation antigens studied, and of the use of these techniques as an adjunct to the diagnosis of hematologic malignancies. It will also include application of marrow and solid tissue transplants. Exposure to processing and interpretation of peripheral blood, lymph nodes, bone marrow and body fluids will be done.

The DNA ploidy-cell cycle analysis component includes a basic understanding of the principles underlying flow cytometry instrumentation for DNA ploidy analysis, of the DNA dyes and staining procedure used, of the analysis methods used for cell cycle determinations, and of the prognostic/diagnostic information obtained. Applications will include hematopoietic malignancies, and a wide variety of solid tumors analyzed by single and dual color assays.

Institutional Site Director
Anthony Killeen, MD,PhD

Training Site:
University of Minnesota Medical Center

Duration of Rotation:
One month

Post Graduate Level of Residents Involved
PGY levels 2-5

Guidelines for Patient Care and Specimen Handling

Specimen handling in the Immunophenotyping Laboratory is the direct responsibility of the laboratory technologists. Resident decision making in the laboratory is under the direct supervision of the teaching faculty. The on service teaching faculty members are physically present during standard operating hours; faculty members not physically present are rapidly available by phone or pager. No diagnosis is communicated to the clinicians before a faculty member has evaluated the case.

Overview of Daily Duties and Responsibilities
The residents experience during this rotation will include "hands-on" time involving the instrumentation and staining procedure, in addition to theoretical background and independent analysis of archival cases. It will include the following:

1. Attend daily teaching sessions conducted by the Director.
2. Read appropriate articles from residents’ folder and additional readings as assigned by the Director.
3. Process, stain, analyze, and interpret all cases assigned by the Director.
4. Obtain clinical-pathologic correlation information. Determine appropriate testing methods and panel of monoclonals to be used.
5. Meet with Director for sign out of flow cytometry results, correlate with morphology or other biologic markers, and prepare interpretative flow cytometry / immunophenotyping report when assigned.
6. Perform computer analysis and interpret all historical cases provided by the Director.
7. Prepare and present cases at appropriate clinical pathology conference.

First Week

1. Introduction to IFC Lab.
2. Sample preparation overview:
   a. Ficoll/Hypaque cell separation.
   b. Lysing.
   c. Viability tests.
   d. Introduction to direct and indirect immunofluorescence staining.
3. Read assigned literature.
4. Introduction to FACScan-assist in flow cytometric acquisition and on screen analysis of cases.

Middle Weeks

1. Continue FACScan.
2. Assist in preparation, staining and flow analysis of selected daily cases.
3. Q & A- discussion session with Lab Director- immunophenotyping portion.
4. Read assigned literature.
5. DNA ploidy and cell cycle analysis.

Last Week

1. Introduction to DNA ploidy and cell cycle analysis:
   a. preparation of fresh tissue.
   b. preparation of paraffin embedded tissue.
   c. multiparameter flow cytometric analysis.
2. Assist in preparation, staining and flow analysis of daily cases.
3. Read assigned literature.
4. Q & A- discussion session with Lab Director.
5. Complete the evaluation form.

**Goals and Objectives**

1. **Instrumentation**
   a. Basic physics of flow cytometry instrumentation
   b. Understanding of effects of instrument set-up on data results
   c. Perform necessary instrument quality control

2. **Immunophenotyping**
   A. **T-cell subsets analysis:**
      a. Select an appropriate panel of monoclonal antibodies for single color, two-color and eventually three color procedure, and explain why each is required.
      b. Stain, analyze peripheral blood samples, and interpret results.
      c. Understand principles and be able to perform two-color evaluation using both manual settings and analysis in CELLQuest software and automatic gating and analysis using SimulSET/MultiSET software.
      d. Understand and demonstrate sources of error in each test. Understand advantages/disadvantages of each.
      e. Describe patient variables (i.e. drugs, infections, etc.) which can affect results of T cell subsets and analysis.
      f. Determine when it is appropriate to add additional interpretative comments and/or clinical consults.
      g. Describe the NCCLS and CDC Guidelines.
   B. **Leukemia/Lymphoma Immunophenotyping:**
      1. Understand direct versus indirect immunofluorescence assays and their application for flow cytometry and for immunofluorescence microscopic examination of frozen sections and cytospin smears
      2. Perform each and describe the advantages/disadvantages of whole blood and bone marrow lysis versus ficol-hypaque methods.
      3. Describe the patterns of expression of lineage-associated/differentiation antigens recognized by monoclonal antibodies on normal hematopoietic cells.
      4. Describe the patterns of expression of these antigens in various hematologic malignancies.
      5. Determine appropriate preparative method, select an appropriate panel of monoclonal antibodies and explain why each is required.
6. Understand and perform alternative gating and analysis using various combinations of flow cytometric parameters and various computer assisted procedures to identify abnormal populations.
7. Determine when it is appropriate and what immunohistochemical or immunofluorescence tests should be performed.
8. Analyze each of the specimen type on the flow cytometer, and/or by the microscope.
9. Determine whether straining is adequate and whether additional antibodies are needed.
10. Correlate flow cytometric/immunofluorescence straining results with the morphology, histology, and clinical information. Write an interpretive report.

3. DNA Ploidy-Cell Cycle Analysis
   a) Perform processing and staining of paraffin-embedded tissue, fresh solid tissue tumor and/or peripheral blood, lymph node and body fluids specimens.
   b) Determine the appropriate quality control, and internal/external controls to be utilized with each of the above specimen type.
   c) Analyze each of the above specimen types on the flow cytometer.
   d) Perform cell cycle analysis on each of the above specimen types.
   e) Describe the source of error involved with each specimen type, and with numerical analysis routine.
   f) Collect appropriate clinical/laboratory results for correlation and prepare an interpretative report.

Structured Formal Education in the Management of the Cytogenetics Laboratory

The resident is invited to attend the monthly Laboratory Staff Meeting. The resident also becomes involved in management issues, as they arise during the course of the workday. These issues are further discussed in the daily focused tutorial sessions with the Laboratory Director.

On-call Duties

The resident is expected to be available to laboratory personnel, either in person or by pager, throughout the working day. In an urgent situation, the resident should contact either the supervisor or a faculty member. No on-call duties outside of regular laboratory working hours are assigned to the resident.

Communication with On-Duty Faculty

Teaching faculty members on service are physically present during standard operating hours (8:00 AM - 5:00PM); specific faculty members when not physically present in the laboratory are available by phone or pager. At all times, a supervising faculty member is on call for evening and week-end questions. No diagnosis is communicated to clinicians before a faculty member has evaluated the case.
Resident Opportunities to Function as a Consultant to Other Physicians

The resident is expected to answer queries with regard to appropriate testing and patient results. These interactions may include physicians from Medicine Clinic, Hematology/Oncology, HIV Clinic, Bone Marrow and Solid Organ Transplant Services, and others. The resident is also expected to be able to discuss results with residents, fellows and staff from the other Special Diagnostics Laboratories.

Required Conferences

- Hematologic Malignancy Conference: Staff and residents from Special Hematology, Cytogenetics, Molecular Diagnostics and Flow Cytometry attend and present applicable results and discuss their significance. This clinical conference provides trainees with the opportunity to correlate and discuss pathologic findings.

- Clinical Pathology Conference, weekly, Residents and faculty present and discuss interesting clinical pathology cases, recent advancements in clinical pathology, and the interpretation of laboratory values in terms of a clinical setting. This conference provides a weekly forum for trainee and faculty discussion of difficult and unusual cases. The conference is composed of both a 30 minute resident and 30 minute faculty presentation. Residents develop their presentation under the guidance of a faculty member with whom they are currently rotating. This conference provides trainees with the opportunity to correlate and discuss pathologic findings and a regular avenue for trainee peer teaching.

- Laboratory Medicine Grand Rounds, weekly, residents attend conferences on a variety of basic science and clinical topics. Conference is held on the University of Minnesota Medical School Campus.

- Rosai/Sinard Conference, weekly, residents present a variety of real cases on a theme related to a recent or upcoming faculty Resident's Conference. This conference provides a regular avenue for trainee peer teaching with feedback given by the Chief Resident's Subcommittee.

- Resident's Conference, weekly, residents attend conferences on a variety of scheduled pathology topics given by the faculty.

Optional Conferences

- Surgical Pathology Unknown Conference, weekly, Residents present their interpretation of unknown cases. Conference is held in the Division of Surgical Pathology at University of Minnesota Medical Center. Residents are responsible for reviewing the cases prior to the conference. Slides are put out for review one week in advance. This conference provides a weekly forum for trainee and faculty discussion of difficult and unusual cases.

- City Wide Surgical Pathology Conference, weekly, Pathologists from the Twin Cities bring interesting cases to share and discuss. This conference provides a weekly forum for trainee and faculty discussion of difficult and unusual cases.
• Cytology Conference, weekly, Residents review cytology cases mixed with didactic resident, technologist, and faculty teaching. This conference provides a weekly forum for trainee and faculty discussion of difficult and unusual cases and a regular avenue for trainee peer teaching.

Scholarly Activities and Research During Rotation
Residents are provided with continuous access to literature searching programs. The expectation is that residents will utilize the medical literature to find up-to-date information on their cases. It is further expected that residents will utilize the medical literature to help provide our clinical colleagues with up-to-date knowledge related to the cases they complete. During sign-out of cases, the residents and teaching faculty discuss each case, from a scholarly perspective. It is hoped that these discussions will foster an interest in research and the development of new knowledge.

Basis and Method of Resident Evaluation
Residents will be evaluated based on their mastery of the instrument and staining procedures, ability to analyze, interpret and determine adequacy of results. The residents are provided with continuous feedback on their performance during the rotation. In general, only deficiencies are noted in writing. Residents are evaluated on their demonstrated ability to provide informative consultation to the clinical service teams, their medical knowledge, their application of this knowledge to efficient/quality patient care, and their microscopic diagnostic, technical and observational skills. Residents are also evaluated on their interpersonal skills, professional attitudes, reliability, and ethics with members of the teaching faculty, peers, laboratory staff, and clinicians. They are further evaluated on their initiative in fostering quality patient care and use of the medical literature, as it relates to their assigned cases. Their timely completion of assigned interpretive reports is another component of the evaluation. Residents on probation receive a written mid-rotation evaluation.

Educational Resources Available
In addition to the service library and reference book collections of the teaching faculty, residents have access to the University of Minnesota Medical School Library (Diehl Hall) on the same campus.

Computer Information Systems for Resident Education and Service Duties
Residents have continuous access to the laboratory and hospital clinical information systems, which relate to patient care on the University of Minnesota Medical Center. The residents also have access to on-line literature searching.