American University of Beirut

Faculty of Medicine
Department of Pathology and Laboratory Medicine

LABM 280

Cytogenetics, Molecular Diagnostics and Histotechniques

2023-2024

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Name:	**Rami Mahfouz MD, MPH**	Course Credits:	2
Office Hours:	7:00am – 6:00pm	Course Meeting:	AUBMC, Pathology Lab
Office Location:	AUBMC, Department of Pathology, Third floor, W-327	Course Time:	M-F 8:00am till 12:00pm
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Course Description:

In this course, the senior Medical Laboratory Sciences students will be exposed to a variety of techniques in Molecular diagnostics, Cytogenetics and Pathology. The practical aspect of each essential technique will be explained and entertained with the student in the context of clinical applications in what serves the patients management and care.

Specific Learning Outcomes:

Distributed as per individual section rotation in Pathology, Cytogenetics, and Molecular Diagnostics.

Molecular Diagnostics:

I. Introduction:

During the rotation in the Molecular Diagnostics Laboratory, the Medical Laboratory Sciences (MLS) student will be exposed to several aspects of the section and learn as much perspectives as possible. It is a mutual responsibility of both the student and the staff of the Molecular Laboratory section to fulfill this goal.

Electronic Resources:
Electronic version of PLM Departmental Service Manual showing detailed instructions for all laboratory tests is accessible at the following link:
https://his.aubmc.org.lb/Lis_emanual/LabTest**.**

II. Exposure to physical aspect of the laboratory:

The MLS student should be aware that the theoretical and best Molecular laboratory setting is composed of the following:

1. Sample Extraction area:
   a. Extraction of genomic material takes place here.
   b. Strict work under sterile conditions (gloves, gowns, goggles).
   c. Adequate labeling and identification of specimens has to be learned very well.
   d. Proper decontamination and cleaning of working area when work is terminated.

2. PCR setup area:
   a. Reagents and PCR components are added (primers, nucleotides, buffers, enzymes)
   b. Also, strict work under sterile conditions (gloves, gowns, goggles)
   c. The student will be introduced to the concepts of ***positive***, ***negative***, and ***no template*** controls.
   d. No spillage to be allowed when working especially with patient samples.

3. Post amplification area:
   a. Loading of amplified DNA onto gels

b. The student will learn the basics of setting up a PCR program and running it
c. This area is usually labeled as "contaminated". Gloves and gowns are to be worn at all times.

III. Exposure to technical aspect of the laboratory:

The MLS student is expected to achieve the following:

1. Identify the various equipment installed in the laboratory. Example:
   a. Hoods (use of UV light, blower, decontamination procedures)
   b. Freezers (-80^0C, -20^0C)
   c. Centrifuges / Vortex mixers / waterbath
   d. Pipettes
   e. Electrophoresis gel units / Digital gel capture
   f. Advanced instrumentation:
      - Thermocyclers (PCR machines)
      - COBAS AmpliPrep
      - Real Time PCR Systems: LightCycler II, RotorGene, CFX Biorad
      - GeneXpert, BioFire, Idylla system
      - Next Generation Sequencing (MiSeq and MiniSeq)

2. Attend the various technical steps to run a specific test.

3. Perform a full procedure himself/herself at the end of the rotation, if interested and where applicable.

IV. Exposure to clinical aspect of the laboratory:

1. The MLS student will be exposed to the various clinical tests offered by the Molecular Diagnostics Laboratory.

   - ❑ HLA typing (Class I & II) at low- and high-resolution levels
   - ❑ Factor V Leiden, Factor II, and MTHFR gene mutations
   - ❑ Advanced molecular thrombophilia testing (Cardiovascular Disease panel, CVD)
   - ❑ Familial Mediterranean Fever gene mutations

- ❑ Genetic translocations for Leukemias
- ❑ Genetic translocations for Lymphomas
- ❑ Genetic translocations for solid tumors
- ❑ Molecular testing for Hepatitis B and C, CMV, EBV, VZV, HIV, HPV, and HSV
- ❑ Gene rearrangement studies for B and T cell identification
- ❑ Special molecular tests like: JAK2, FLT3, CALR, and PIK3CA
- ❑ Pharmacogenetic tests: KRAS, BRAF, EGFR, and NRAS

2. The MLS student should meet the following targets in learning about the clinical aspect of the Molecular Diagnostics Laboratory:

    - ❑ Know specific details related to each clinical test
        - Type of anticoagulant needed
        - Amount of sample needed
        - Precautions in handling specimens
        - Expected turnaround time
        - Special tests ordered

    - ❑ Know the technique used for each clinical test:
        - HLA typing (PCR/SSP versus PCR/SSO)
        - Factor V Leiden and FMF (PCR/SSOP)
        - Genetic translocations for leukemias and solid tumors (RT-PCR)
        - Genetic translocations for lymphomas (PCR)
        - Hepatitis B and C, CMV, HIV and EBV (Quantitative PCR)
    - ❑ Get oriented to clinical indications for ordering the various tests. Examples:
        - HLA typing:
            - Bone marrow transplantation
            - Kidney transplantation
            - Specific diseases
                - Ankylosing Spondylitis (B27)
                - Behcet's disease (B5)
        - Genetic translocations:
            - Diagnosis / prognosis
            - Follow-up for minimal residual disease
        - Factor V Leiden, Factor II, and MTHFR:
            - Thrombosis (DVT, PE)
        - FMF gene mutations:
            - Screening and diagnosis of Familial Mediterranean Fever
        - Pharmacogenetic testing for KRAS, BRAF, NRAS, and EGFR:
            - Targeted therapy

        - Importance in oncological classification and prognostication of patient treatment outcome
    - Next Generation Sequencing Tests and Utilization
- ❑ Learn how to interpret the results obtained at the end of a clinical run.

- ❑ Learn how to troubleshoot for major problems that may alter or affect interpretation of the test results.

V. Performance Evaluation:

The MLS student will be evaluated based on the following criteria:

1. Attendance:
   He/she will be requested to fill in and out an attendance form to be kept in the files of the Molecular Diagnostics Laboratory records and a copy in their rotation file care of the Senior MLS Coordinator.

2. Interaction and Interest:
   This will be reflected by their attendance of daily clinical runs performed in the section, their depth of interest in the material in hand, and their relationship with the technologists teaching them the procedures.

3. Special skills:
   The MLS students are highly encouraged to perform, where applicable, a complete run themselves under supervision by a staff member of the Molecular Diagnostics Laboratory.

Cytogenetics:

I. Introduction:

During his/her time spent in the Medical Genetics laboratory, the student should observe all steps of cell culture, harvesting, and slide preparation banding. At the end of his/her rotation the student will be evaluated on the basis of his/her attendance, interaction, professional attitude, performance within the laboratory in addition to the final written examination.

Electronic Resources:
Electronic version of PLM Departmental Service Manual showing detailed instructions for all laboratory tests is accessible at the following link:
https://his.aubmc.org.lb/Lis_emanual/LabTest.

II. Tests encountered:

- Karyotype:
  - Prenatal (Amniotic fluid, CVs)
  - Postnatal (Blood, Bone Marrow, Lymph Nodes)
- Aquired abnormalities (Bone Marrow, lynph node)
- FISH
- DNA analyses: DNA extraction, PCR, Q-FPCR, Sequencing

III. Specimen collection, receiving and registration:

The student is introduced to the various types of samples referred to the laboratory, he/she should know:

1. The appropriate different containers used to collect and transport the sample to the laboratory.
2. The reasons for referral of each sample type.
3. The criteria used in sample rejection.

IV. Aseptic techniques, media, and reagents preparation:

The laboratory technologist will explain the use of proper personal protective equipment (lab coat, gloves, etc..), the proper handling and the use of the biosafety cabinet and practice of sterile techniques. The student will also be instructed on the preparation of all reagents and their proper storage conditions.

V. Tissue culture techniques, harvesting and slide preparation:

During his/her rotation, the student will be able to:

1. Differentiate between the two main culture techniques: the suspension (the short-term culture), and the anchored (long-term tissue culture).
2. Observe and learn some trouble shooting techniques used in culture set-up, maintenance, harvesting and slide preparation for all sample types.

VI. Chromosome banding and staining technique:

Observation of G-banding technique using trypsin and staining with Leishman is a requirement for every student.

VII. Chromosome analysis:

The student is introduced to the classification and identification of chromosomes on a printed image of a metaphase spread and is required to practice at least one additional printed case and return it for correction and discussion.

VIII. Fluorescence In-Situ Hybridization (FISH) technique

The student will be acquainted with the principle of the FISH technique and its application in the laboratory.

IX. DNA analyses:

The student will be introduced to Molecular genetics techniques including:

1. DNA extraction:
    a. Sample processing
    b. Assessment of DNA concentration using spectrophotometry (Nanodrop)

2. PCR set-up:
    a. Addition of reagents and PCR components
    b. Amplification of a DNA sequence

3. Electrophoresis:
    a. Loading of agarose gel
    b. Bands pattern evaluation

4. Q-F PCR and Sequencing:
    a. Addition of reagents and components
    b. Loading on the ABI3500 genetic analyzer
    c. Result interpretation

Pathology:

I. Introduction:

By definition, Pathology is the study of disease. It is devoted to the investigation of the structural and functional changes in cells, tissues, and organs that underlie disease. The study of pathology is divided into groups, general pathology and special or systemic pathology. The former is concerned with the basic reactions of cells and tissues to abnormal stimuli that underlie all diseases. The latter examines the specific responses of specialized organs and tissues to more or less well-defined stimuli.

The purpose of this rotation is to introduce the students to the different aspects of pathology from a technical standpoint. Upon completing the rotation, the student will be able to understand better the importance of each step, from receiving specimens to the release of the final report.

The rotation is divided into 6 steps as follows:

1. Specimen reception and logging in the laboratory information system (LIS).
2. Grossing, cassetting, and processing of specimens.
3. Embedding and sectioning of tissue blocks.
4. H&E Staining and mounting of slides.
5. Special (histochemical) stains.
6. Immunohistochemistry.

II. Specimen Reception and Logging in the LIS:

All specimens taken out in the operating rooms and private clinics are sent to the pathology lab for examination. This includes a wide variety of tissue samples. Examples include lymph nodes, gastric biopsies, lung biopsies, liver biopsies, renal biopsies, bone marrow biopsies, bladder biopsies, brain tumors, prostate biopsies, skin biopsies, breast biopsies, etc…

Once the specimens are received in the lab, the first step is to submerge the entire tissue sample in 10% neutral buffered formalin to prevent autolysis (self-destruction of the tissue), unless special work-up (Frozen Section, Flow Cytometry, Karyotyping, etc…) is required. The specimen is then logged in the LIS and given a pathology number. All cases are logged manually on a daily log sheet as well.

III. Grossing, Cassetting, and Processing of specimens:

Once specimens are logged in the LIS, cassettes are made in order to place the parts of the tissue that require examination in them. Grossing refers to the visual examination that is usually done by the resident / pathologist on duty in order to determine which part of the tissue is most relevant to the diagnosis of the patient's medical condition. At the end of the day, all cassettes are placed on the processing machine overnight. Tissue processing includes several steps such as formalin fixation, alcohol dehydration, xylene clearing, and finally paraffin wax infiltration in order to harden the tissue and make it suitable for sectioning.

IV. Embedding and Sectioning of tissue blocks:

Embedding or casting refers to the process by which already hardened tissue due to paraffin wax infiltration is turned into blocks suitable for sectioning on the microtome. This is done by placing the tissue in a metal mold then dispensing enough

paraffin wax all around the tissue. The metal mold is then placed on a cold plate which in turn will cause the entire specimen block to come off the metal block. Once all blocks have been completed, sectioning begins. Trimming is first done to shave off all paraffin wax covering the surface of the tissue, after which the blocks are placed on ice to cool down, then sectioning is done.

V. H&E Staining and Mounting of slides:

Hematoxylin and **E**osin (H&E) staining is the first step in the testing process. This can be done manually or using automation. The staining machine has several pre-set programs, but they all share several basic steps such as deparaffinization (dewaxing), hydration, staining with hematoxylin and eosin, dehydration, and then clearing. Mounting refers to the process by which a thin glass cover is placed on top of the stained sections to make the slides permanent and permits microscopic examination. H&E staining is very important because it demonstrates the overall morphology of the tissue section as well as whether there are any abnormal cells present. Some diagnoses are made using only H&E-stained, while others require the use of special (chemical) stains, immunohistochemistry, or both.

VI. Special (histochemical) Stains:

In this rotation, special dyes or stains will be used to demonstrate the presence or absence of certain groups of cells, or at times non-cellular material such as fat, collagen, iron, etc… The basis of this technique is affinity which is considered as a measure of the tendency of, say, a dyestuff to transfer from a dyebath onto a section. Various contributions to dye-tissue and reagent tissue affinities will be considered. Examples of these contributions include solvent-solvent interactions, reagent-reagent interactions, and reagent-tissue interactions.

VII. Immunohistochemistry:

Immunohistochemistry is a staining technique used for identifying cellular or tissue constituents (antigens) by using specific antibodies. The resulting antigen-antibody reaction is visualized by labeling the antibody. The most widely used enzyme is horseradish peroxidase, hence the term immunoperoxidase. Another type of label used in our laboratory is fluorescein, hence the term immunofluorescence.

Once the rotation in Immunohistochemistry is completed, the students will have a clear idea regarding the precision of the technique, as well as the importance of each step, from choosing the right control tissue, to visualizing the end-result under the microscope.

Course Content:

As per above description of individual rotations.

Dr Mahfouz will be presenting one session (50 minutes duration) as part of the rotation in the Molecular Diagnostics Laboratory.

Evaluation:

	Percentage of Grade
Attendance & Practical Training	10%
Written Exam includes: Molecular (50%), Cytogenetics (25%), and Pathology (25%)	90%

Recommended References / Books:

Henry. Clinical Diagnosis and Management by Laboratory Methods. Saunders, Edition 2012.

Course Policy:

1. Attendance: you are expected to attend all classes and participate in class activities. If you miss a class, it is your responsibility to make up for the material missed and inquire about any announcements made. If you miss more than one fifth of class sessions, you are subject to withdrawing from the course with a-w-grade. Please refer to the AUB catalogue.

2. Exams: Examinations must be taken as scheduled. **Make up exams will not be given unless a valid excuse is given**. Only authorized medical reports will be accepted.

3. Withdrawal date: Please observe withdrawal dates set by the Registrar's Office.

4. Cheating and Plagiarism: **Cheating and plagiarism will not be tolerated**. Kindly review the Student Code of Conduct in your handbook and familiarize yourself with definitions and penalties. If you are in doubt about what constitutes plagiarism, please ask your instructor because it is your responsibility to know. **The American University of Beirut has a strict anti-cheating policy**. Penalties include *failing marks* on the assignment in question, *suspension* or *expulsion* from University and a *permanent mention of the disciplinary action in the student's records.*

5. AUB strives to make learning experiences as accessible as possible. If you anticipate or experience academic barriers due to a disability (including mental health, chronic or temporary medical conditions), please inform me immediately so that we can privately discuss options. In order to help establish reasonable accommodations and facilitate a smooth accommodations process, you are encouraged to contact the Accessible Education Office: accessibility@aub.edu.lb; +961-1-350000, x3246; West Hall, 314

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