Virginia Western Community College
MDL 126
Clinical Immunohematology / Immunology I

Prerequisites

BIO 101 or equivalent

Course Description

Incorporates basic principles of antigen and antibody reactions included in blood grouping and typing, compatibility testing, and serological procedure.

Semester Credits: 4 **Lecture Hours: 2** **Lab/Clinical/Internship Hours: 6**

Required Materials:

Textbook:

Modern Blood Banking & Transfusion Practice, 7th Edition by Denise M. Harmening, Davis Plus, 2012.
ISBN: 9780803668881

Course Outcomes:

At the completion of this course, the student should be able to:

- Identify an atypical antibody or antibodies in an unknown sample
- List and state the significance of the secondary human blood groups
- Distinguish between warm and cold or clinically significant and insignificant antibodies
- Perform quality assurance as related to blood bank reagents and equipment
- Perform routine blood bank tests to include: ABO/Rh, Antibody Detection, Antibody Identification, Direct Antiglobulin Test, Prenatal Antibody Titration
- Identify, prepare, and store blood products using proper product storage requirements, appropriate product selection, means of transfusion and special handling requirements
- Perform calculations relating to blood bank processes to include: RhIg dosage, total blood volume, corrected platelet count increment (CCI)
- Recognize and troubleshoot unusual test results
- Perform advanced testing concepts and techniques utilized in the blood bank or reference laboratory setting
- Recognize how pre-analytical, analytical, and post analytical errors can adversely affect results

Topics and Objectives:

Outline	Objectives
I. Laboratory Safety A. General Safety Principals B. Blood-Borne Pathogen Safety C. Chemical Safety D. Radiation Safety E. Protection from Physical Hazards	(see lab objectives)
II. Fundamental Concepts (Chapter 1) A. Red Blood Cell and Platelet Preservation: Historical Perspectives and Current Trends	• List the major developments in the history of transfusion medicine • Describe several biological properties of red blood cells (RBC) that can affect post-transfusion survival • Identify the metabolic pathways that are essential for normal RBC function and survival. • Define the hemoglobin-oxygen dissociation curve, including how it is related to the delivery of oxygen to tissues by transfused RBCs. • Explain how transfusion of stored blood can cause a shift to the left of the hemoglobin-oxygen dissociation curve. • State the temperature for storage of RBCs in the liquid state. • Define storage lesion and list the associated biochemical changes. • Name the approved anticoagulant preservative solutions and state the maximum storage time for RBCs collected in each. • Explain how additive solutions are used and list their advantages. • Explain rejuvenation of RBCs. • Define the platelet storage lesion. • Describe indications for platelet transfusion and the importance of corrected count increment (CCI). • Explain the storage requirements for platelets. • Explain the swirling phenomenon and its significance. • Discuss various ways to limit, detect or inactivate bacteria in platelet components.
III. Overview of the Routine Blood Bank Laboratory (AABB Technical Manual) A. Organization B. Personnel Requirements C. Standard Operating Procedures	• Describe a modern blood bank laboratory in terms of operations, personnel, facilities, and equipment. • Explain the purpose of the following equipment used in a blood bank laboratory: apheresis machine, flatbed agitator, sterile docking device, and leukoreduction filter.

D. Transfusion Process Oversight

- Summarize blood bank laboratory services in terms of policies, procedures, and tests performed.
- Compare and contrast testing performed on donor units and recipient blood samples.
- Apply knowledge of immunohematology theory and skills developed in classroom practice to actual work situations in a blood bank laboratory

IV. Quality Management and Compliance

A. Quality Management in the Blood Bank (**Chapter 25**)
B. Equipment Preventative Maintenance/Quality Control, qualification/ validation
C. Supply and Reagent receipt, inspection, acceptance testing, QC
D. Nonconformances
E. Laboratory Information Systems in the Blood Bank (**Chapter 28**)

- Explain the differences between compliance and quality management.
- List the three building blocks of quality
- Describe the framework of a quality system for a blood bank and a medical laboratory.
- List 12 quality management systems (QMS) essentials and the blood bank operations to which they are applied.
- Explain the use of validation in introducing a new process.
- Name at least five blood bank process controls.
- Describe the differences between a form and a record.
- Explain the importance of document control for procedures.
- State the difference between remedial and corrective action.
- Describe the role of auditing in a QMS.
- Describe the four types of quality costs.

Test #1 Sections II – IV

V. Fundamental Concepts

A. Basic Genetics / Blood Group Genetics (**Chapter 2**)

- Explain the Mendel's law of independent segregation and random assortment.
- Correlate the concepts of dominant and recessive traits with examples of the inheritance of blood group antigens.
- Determine the inheritance pattern of a given trait by examining the pedigree analysis,
- Distinguish between X-linked and autosomal traits, and describe how each is inherited.
- Describe the processes of replication, transcription, and translation.
- List the various types of genetic mutations and describe how they can change the function of living cells and organisms.
- Describe the cell's different mechanisms for correcting mutations.
- Identify some of the ways in which genetics can be used in the modern transfusion laboratory.

- Describe in general the modern techniques used in the study of genetics.

B. Fundamentals of Immunology (**Chapter 3**)

- Outline the different components of the immune system and identify their functions.
- Describe the characteristics of the major cells of the immune system and their function.
- Outline the basic steps of hematopoiesis in the immune system.
- Explain the function of the major histocompatibility complex (MHC) class I and II molecules.
- Describe the physical characteristics of immunoglobulins in relation to structure and list the different subtypes.
- Explain the activation Sequences of the three major complement pathways and describe how they come to a common starting point.
- List the methods used in the blood bank to detect antibodies and complement bound to red blood cells.
- Describe the immune response, including antigen-antibody reactions, lymphocyte functions, and host factors that can activate and suppress the immune system.
- List the traditional laboratory techniques used in blood bank testing.
- Identify the various factors that affect agglutination reactions.
- Describe some of the common diseases that can affect blood bank testing.

C. Concepts in Molecular Biology (**Chapter 4**)

- Describe DNA features fundamental in the design of molecular assays and define the applicable terms: complementarity, hydrogen bonding, melting point, denaturation, annealing, and polarity.
- Explain what the central dogma of molecular biology is and how it expanded after the discovery of reverse transcriptase enzyme.
- Explain what the recombinant DNA technology is and describe the tools used in molecular cloning: restriction endonucleases, vectors, and host cells.
- Define gene expression and explain how it may be studied and used in manufacturing of recombinant proteins.
- Summarize the procedures of plasmid DNA isolation and gel electrophoresis.
- Explain the principles of polymerase chain reaction as an in vitro molecular procedure mimicking the

process of semiconserative DNA replication occurring in vivo.
- Discuss the differences between classic PCR, reverse-transcriptase, and real-time PCR.
- Describe the principle of transcription-mediated amplification.
- Describe Sanger's sequencing dideoxy chain termination method.
- Recognize the differences between immunoblotting and hybridization methods: Southern and Northern analysis, microarrays, and fluorescent insitu hybridization.
- Explain major principles of methods used in studying gene polymorphisms: RFLP, VNTR, SSP, and SSOP.
- Explain how nucleic acid testing (NAT) in donor blood improves the process of screening for infectious disease.
- Describe the applications of RBC molecular antigen typing.

Test #2 Section V

VI. Blood Bank Testing Methodologies Overview (AABB Technical Manual and **Chapter 12**)

A. Test tube – reagents, enhancement medias
B. Automated methods – Gel, Solid Phase, other
C. Overview Advanced Methods – adsorption/ elution, inhibition, chemical treatment

- Explain the principles of gel, solid-phase red cell adherence (SPRCA), protein A, and enzyme-linked immunosorbent assay (Elisa) technology.
- Describe the test reactions and how the results are interpreted for each technology.
- List the advantages and disadvantages of each technology.
- Describe the automated equipment that is available for each technology.
- Outline the different components of the immune system and identify their functions.
- Compare the gel and SPRCA technologies in terms of equipment, test reactions, procedures, sensitivity and specificity, and quality control.
- Describe elution methods and give an example of when each would be used
- Explain the principles behind enzyme and neutralization techniques
- Discuss principles of neutralization and inhibition testing.
- Explain titration procedure and how it is used to determine semiquantitative amounts of antibody

VII. Blood Groups and Serologic Testing

A. The Antiglobulin Test (**Chapter 5**)

Chapter 5 Quiz

- State the principle of the antiglobulin test.
- Differentiate monoclonal from polyclonal and monospecific from polyspecific antihuman globulin (AHG) reagents.
- Describe the preparation of monoclonal and polyclonal AHG reagent.
- List the antibody requirements for AHG reagents.
- Explain the use of polyspecific versus monospecific AHG in the indirect antiglobulin test (IAT).
- List the advantages and disadvantages of anticomplement activity in polyspecific AHG.
- Compare and contrast the IAT and the direct antiglobulin test (DAT).
- Explain the principle and applications of red blood cell sensitization.
- Explain the reasons for the procedure steps in the DAT and IAT.
- Interpret the results of a DAT and IAT panel.
- Identify the factors that affect the antiglobulin test.

B. The ABO Blood Group System (**Chapter 6**)

Chapter 6 Quiz

- Describe the reciprocal relationships between ABO antigens and antibodies for blood types O, A, B, and AB.
- Identify the frequencies of the four major blood types in the white, black, Hispanic and Asian populations.
- Explain the effect of age on the production of ABO isoagglutinins.
- Describe the immunoglobulin classes of ABO antibodies in group O, A, and B individuals.
- Predict the ABO phenotypes and genotypes of offspring from various ABO matings.
- Explain the formation of H, A, and B antigens on the red blood cells (RBCs) from precursor substance to immunodominant sugars.
- Describe the formation of H A, and B soluble substances.
- Explain the principle of the hemagglutination inhibition assay for the determination of secretor status.
- Describe the qualitative and quantitative differences between the A1 and A2 phenotypes.
- Escribe the reactivity of *Ulex europaeus* with various ABO groups.
- Describe the characteristics of the weak subgroups of A

- Describe the characteristics of Bombay phenotypes.
- Explain the effects of disease of the expression of ABJH antigens and antibodies.
- Interpret the results from an ABO typing and resolve any discrepancies, if present..
- Explain the use of polyspecific versus monospecific AHG in the indirect antiglobulin test (IAT).
- List the advantages and disadvantages of anticomplement activity in polyspecific AHG.
- Compare and contrast the IAT and the direct antiglobulin test (DAT).
- Explain the principle and applications of red blood cell sensitization.

C. The Rh Blood Group System (**Chapter 7**)

Chapter 7 Quiz

- Differentiate Rh form LW blood group systems.
- Discuss the Fisher-Race and Weiner Rh blood group terminologies
- Translate the five major Rh blood group antigens, haplotypes, and predicted haplotypes, from one nomenclature to another, including Fisher-Race, Weiner, Rosenfield, and ISBT.
- Define the basic biochemical structure of Rh.
- Discuss Rh null type.
- Discuss weakened expression of D antigen on red blood cells.
- List instances when the weak-D status of individuals must be determined.
- List and differentiate some characteristics of Rh typing reagents.
- Define characteristics of Rh antibodies.
- Describe Rh HDN and list some clinical symptoms.
- Discuss Rh antigens other than DCcEe
- Determine the most likely Rh genotype of an individual when given the individual's red cell typing results, haplotype frequencies, and ethnicity.

D. Blood Group terminology and Other Blood Groups (**Chapter 8**)

Chapter 8 Quiz

- Describe how antigens, antibodies, genes and phenotypes are correctly written.
- Define the interaction of Lewis genes with ABO, H and secretor genes
- List substances present in secretions and the Lewis phenotypes based on a given phenotype.
- List the antigen frequencies of the common antigens K, M, S, s, Fya, Fyb, Jka, Jkb, and P1.

- List some low-prevalence and high-prevalence antigens.
- Explain I, P1, and Lutheran antigens as being poorly expressed on cord RBCs.
- List antigens that are denatured by enzymes and antigens whose reactivity are enhanced with enzymes.
- List which antigens are destroyed by treatment with DTT.
- List the characteristics of Lewis antibodies, including clinical significance.
- Define antibodies to M, M, I, and P1 as being typically non-RBC-induces ("naturally occurring"), cold-reacting agglutinins that are usually clinically insignificant.
- Describe antibodies to K, k, S, s, Fya, Fyb, Jka, and Jkb as usually induced by exposure to foreign RBCs (immune), antiglobulin-reactive antibodies that are clinically significant.
- List the antibody specificities that commonly show dosage.
- List and correlate the common 37ºC antihuman globulin-reactive antibodies with hemolytic transfusion reactions (HTR) and hemolytic disease caused by delayed HTR.
- Describe why the Kidd antibodies are common cause of delayed HTR.
- Define the relationship of autoanti-I with Mycoplasma pneumonia infections and with autoanti-I with infectious mononucleosis.
- Describe the association of the Fy(a-b-) phenotype with *Plasmodium vivax* resistance.

E. Uncommon Blood Groups (**Chapter 9**)

- List some uncommon blood group systems
- For each of the blood group systems in the chapter:
  - List some low- and high- prevalence antigens
  - Identify some antigens which have varying prevalence in different ethnic groups.
  - List some antigens which are destroyed by enzyme or DTT
- List some characteristics of antibodies to these antigens
- Describe clinical significance and disease association for some uncommon antigens.

Test #3 Section VI - VII

VIII. Blood Collection (**Chapters 13 & 18**)

A. Donor selection and qualification – health history questions, physical exam
B. Collection type-
   i. Whole blood venipuncture
   ii. Apheresis – blood, platelet, plasma
   iii. Special Collections: Autologous, Homologous, and Directed
C. Collection Processes and testing

- Identify the organizations that regulate or accredit the immunohematology laboratory.
- List and differentiate instances when donors would be acceptable or unacceptable to donate.
- Differentiate among the different types of donations.
- Define leukapheresis, plateletpheresis, plasmapheresis, and erythropheresis.
- Describe the procedure for whole blood donation.
- State the acceptable interval of different types of donation.
- Differentiate among mild, moderate, and severe reactions and state recommended treatments for each.
- List the components which can be collected using apheresis technology.
- List the tests required for allogeneic, autologous, and apheresis donation.
- Discuss record keeping procedures for donors.
- Define prion and these acronyms: HIV, HCV, HBV, HTLV, CJD, vCJD, WNV.
- Discuss donor infectious disease testing, deferral, donor reentry protocol.
- List some endemic countries for malaria.
- State deferral criteria for Babesia, Zika, and Ebola.

IX. Blood Components (**Chapter 15**)

A. Component Production
B. Blood Component Labeling
C. Product Requirements and QC
D. Product Storage and Distribution

- Discuss CFR and AABB Circular of Information in regards to component production, labeling, storage, indications and contraindications
- Identify the storage conditions and shelf life of blood products routinely encountered in the hospital blood bank.
- Discuss blood derivative products.

Final Exam – Cumulative

Notes:

- **Study guides for tests are provided at the discretion of the instructor.**
- **MDL 227 continues from the point that MDL 126 ends. These topics are usually covered in MDL126; if not completed any remaining topics will be covered in MDL 227.**

MDL 126 Laboratory Schedule

Week	Topic:	Objectives:
1 - 2	**Laboratory Safety**	• Identify the components of the chain of infection and give examples of each, describe infection-control procedures used to break the chain, and identify four functions of infection-control programs. • Describe proper procedures for hand hygiene, putting on and removing protective clothing, and entering the nursery or neonatal ICU. • Describe standard and transmission-based precautions and identify the organizations that developed them. • State safety rules to follow when working in the laboratory and in patient areas. • List examples of blood-borne pathogens and describe their means of transmission in a healthcare setting. • Discuss the major points of the blood-borne pathogens (BBP) standard, including required needlestick and sharps safety, and identify key elements of a BBP exposure control plan. • Describe hazards, identify warning symbols, list actions to take if incidents occur, and specify rules to follow for proper biological, electrical, fire, radiation, and chemical safety.
	Lab Quiz – Safety	
	Equipment and Reagent Overview • Manufacturer's Directions • Timer • Temperature • Incubators • Water bath • Centrifuge • Routine Blood Bank Reagents Receipt, MSDS, Package Inserts	• Review and interpret Manufacturer's Directions and MSDS (Material Safety Data Sheets) • Discuss reagent manufacturers' directions to include: reagent storage, preparation (if required), procedures for use, quality control, limitations, reagent source, and acceptable samples • Explain importance of lot number and expiration dating of supplies and reagents • List basic equipment utilized in the blood bank laboratory (timer, thermometer, incubators, heat blocks, water bath, centrifuge, cell washer, etc.)

		• Discuss for equipment used in the BB the importance of: quality control/ preventative maintenance and maintenance schedules • Practice completing reagent log for blood bank reagents: Order requisition, purchase requisition, packing sheets, inspection upon receipt, MSDS, package inserts, etc.

3 -4	**Equipment Checks / Calibration /Qualification / Validation**
	• Timer qualification
	• Thermometer qualification
	• Pipette calibration
	• Incubators /Water bath maintenance
	• Serofuge qualification and serologic calibration
		• Define and differentiate between quality systems essentials, quality assurance, quality control, qualification, validation, calibration, preventative maintenance and corrective maintenance
		• State daily preventative maintenance, quality control, start-up and shutdown of the following equipment routinely found in the blood bank: Timers, pipettes, thermometers, heat block/ incubators/ water bath, serofuge, cell washers, etc.
		• Perform and discuss processes / procedures for the following:
		  • Timer qualification
		  • Thermometer qualification
		  • Pipette calibration using gravimetric method
		  • Incubators /Water bath maintenance
		  • Serofuge qualification and serologic calibration
		  • Serofuge operation and balancing load
		  • Operates maintains calibrates Incubators /Water bath / Heat Blocks
		  • Serofuge qualification and serologic calibration

5	**Lab Quiz – Equipment** **Basic Skills**	• Centrifuge samples • Performs volume transfers using various types of pipettes using good technique • Execute preparation of 3-5% RBC suspensions • Remove plasma from rbcs and saline from rbcs without re-mixing specimen • Demonstrate labeling required for test tubes when performing blood bank testing • Perform centrifugation using serofuge with balanced load

		• Discuss importance of concurrent documentation of lab results • Perform and calculate dilutions
6 - 7	**Grading Test Tube Reactions / Titration**	• Perform accurate grading of test tube reactions after viewing re-suspension / reaction grading demonstrations, • Calculates dilutions accurately • Performs titration procedure accurately • Calculates dilution and prepares solutions
	Lab Practical – Reaction Grading and Titration	
8	**Genetics and Inheritance of Blood Group Antigens (Worksheets and Case Studies)**	• Explain red blood cell antigen inheritance patterns, sex linked inheritance patterns, calculate frequency • Solve inheritance pattern given a pedigree
9 - 11	**ABO Rh Testing** (includes case studies) • Reagents Used / QC • Forward (red Cell) Typing • Reverse (plasma)Testing • ABO discrepancy resolution • Rh phenotyping	• Discuss sample suitability • Discuss reagents used for forward ABO group testing • Perform slide ABO typing • After observing demonstration, performs reagent QC for forward group testing • Accurately labels test tubes • Prepares suspensions labels tubes • Perform forward (red Cell) typing by test tube method • Discuss reagents used for reverse ABO group testing • After observing demonstration, performs reagent QC for reverse group testing • Performs reverse (plasma) testing by test tube method • Analyze samples and recognize discrepant results • Propose possible causes for discrepancies • Perform Rh phenotype testing with available reagents • Discuss Rh reagent qc requirements. • List Rh genotypes in different nomenclature • Discuss equipment requirements. • Understands the requirement for documentation of reagent information to include: manufacturer, lot number, expiration date, open dating and reagent inspection. • Understands the need for concurrent documentation of results.

	Lab Practical – ABO Rh	

12 - 13	**DAT Testing**
	• Reagents Used /QC
		• Discuss and list QC requirements for reagents used when performing a DAT
		• Perform:
		  • Preparation of cell suspensions (washing)
		  • DAT testing using poly, IgG
		  • Verification of negative results using Coombs Control Cells
		• Explain valid versus invalid testing for DAT

14 - 15	**IAT – Weak D Testing and Antigen Typing**	• Perform IAT test (weak D test, antigen typing at IAT) • Correctly explain difference between DAT and IAT • Understand use of anti-human globulin reagents and Coombs Control • Explain valid versus invalid testing for weak D test • Discuss use of IAT in other testing for the blood bank (antibody screen, antibody identification, antigen typing)
	Final Lab Practical – Cumulative	