I. **Definition of Manual/Special Tests Unit**

A. **Specimen handling:**
   1. Evaluate sample acceptability by checking labeling, quantity, and quality.
   2. Prepare acceptable peripheral blood smears.
   3. Demonstrate correct handling of specimens and contaminated equipment.

B. Perform the following:
   1. WBC/PLT estimates from the blood smear that agree with the automated result ± 20%.
   2. Manual Reticulocyte counts which agree within 0.5% of correct value on retics <3.0%, agree within 1.0% of correct value on retics ≥3.0%, and must agree within 2.0% on retics ≥6.0%.
   3. Kleihauer-Betke stain Case, count and interpretation^^
   4. LAP stain Case, count and interpretation^^
   5. Acute leukemia stain panel Case, interpretation^^

^^Read procedure provided and answer the stain questions located on Blackboard, Special Tests Unit before doing each case.

C. Answer the Osmotic fragility, Ham’s, Sugar water and Donath-Landsteiner questions located on Blackboard, Special Tests Unit.

D. For all testing designated to this unit, refer to department or provided procedures, Student Lab material and/or McKenzie text. You are responsible for tests not performed, as stated in objectives.

E. After completion of questions and cases, take the ‘Closed-Book’ Special Tests Exam over KB, LAP, AL Stains, OF, Ham’s, Sugar and DL tests when ready but by the end of Hematology Rotation II. The “Open-Book” Manual/Special Tests Exam is also due by the end of Rotation II.

F. **Practicals** will include manual Reticulocyte counts (relative & absolute) and WBC/PLT estimates.

II. **Objectives regarding Acceptable Specimens and Specimen Handling**

A. **Acceptable Specimens**
   1. Explain the action of anticoagulants used to prevent blood coagulation.
   2. Describe specimen requirements for each test performed, including expiration times.
   3. Assess sources of collection error that may invalidate laboratory determinations, including corrective action for pre-analytic errors.

B. **Specimen Handling**
   1. Describe safety requirements of the laboratory.
   2. Explain the proper procedure to follow in the event of an accident (e.g., spill), including the location of the MSDS manual.
III. Objectives regarding Blood Smears, Reticulocyte Counts, Hemoglobin S Prep, and Test for Infectious Mononucleosis.

A. Blood Smears

1. Discuss the following components in the preparation and staining of peripheral blood smears using Wright’s stain:
   a. Principle of Romanowsky-type stains
   b. Action of the reagents/dyes
   c. Characteristics of a properly made smear, including adjustments for poor smears (e.g., changing the angle of pusher slide or size of the blood drop)

2. Describe methods to correct improperly stained Wright’s smears.

3. Discuss the use of albumin for preparing blood smears when excessive smudge or broken cells are present.

B. Manual Reticulocyte Counts

1. Discuss manual reticulocyte counts including each of the following:
   a. Principle of supravital stains
   b. Procedure
   c. Acceptable specimens
   d. Sources of error
   e. Normal reference values expressed in both relative and absolute numbers

2. Calculate relative and absolute reticulocyte counts.

3. Discuss the significance of increased or decreased reticulocytes (absolute number) in the blood.

4. Correlate retic findings with the presence or absence of polychromasia on the Wright’s stained smear.

C. Prep for Hemoglobin S

1. Describe the principle, clinical significance, controls, reagents and acceptable specimens for the hemoglobin S tube turbidity test.

2. Discuss causes of false positive and false negative sickle cell prep results.

3. Explain why a hemoglobin electrophoresis is done to confirm a positive sickle cell prep.

D. Test for Infectious Mononucleosis

1. Explain the principle, specimen, procedure, interpretation, and diagnostic value of the test for infectious mononucleosis.

2. Correlate results of the IM test with clinical symptoms and hematologic blood picture.

3. Differentiate the nonspecific heterophile antibodies of IM with specific EBV antibodies.
   a. Discuss limitations of the IM test.
   b. Discuss when testing for EBV specific antibodies might be performed.
IV. Objectives regarding Special Hematological Tests – For the most part, these objectives are new for Hematology Rotation II

A. Quality control on special tests

1. Describe the specimen used for control(s) for the following procedures:
   a. Peroxidase, Sudan Black B, PAS, and Esterase stains
   b. Leukocyte Alkaline Phosphatase stain
   c. Kleihauer-Betke stain
   d. Osmotic Fragility test

2. Analyze deviations in control results (e.g., negative or not within acceptable score range as described by the procedure), describing corrective action(s).

B. Discuss the following special tests:

1. Describe the principle, importance of controls, and acceptable specimens for each of the following cytochemical stains: SL pgs 190-191
   a. Peroxidase
   b. Sudan Black B (SBB)
   c. Periodic Acid Schiff (PAS)
   d. Specific (Chloracetate) Esterase
   e. Non-Specific Esterase stains

2. Correlate the cytochemical staining pattern with clinical information, features of cell morphology, surface marker analysis and cytogenetics for the diagnosis of hematologic disorders.

3. Leukocyte alkaline phosphatase stain (LAP) – SL pg 191
   a. Describe the principle, reagents, specimen used, scoring and acceptable control score for the LAP stain.
   b. Identify normal and abnormal results.
   c. Discuss disorders for which the procedure has diagnostic value.

4. Kleihauer-Betke stain – SL pg 129
   a. Describe the principle, controls, reagents, and acceptable specimens used for testing.
   b. Calculate the percentage/mls of fetal cells present.
   c. Identify normal results and instances of increased percentage/mls of fetal cells.
   d. Correlate results with administration of Rh Immune Globulin.

5. Osmotic fragility test – SL pgs 110-111
   a. Describe the principle, importance of the control and acceptable specimens used for the osmotic fragility test (immediate/unincubated test).
   b. Identify normal and abnormal values for the osmotic fragility test (immediate).
   c. Discuss RBC disorders for which the procedure has diagnostic value.

6. Sucrose hemolysis/Sugar water test, Ham’s (acid) test and Donath-Landsteiner test
   a. Describe the principle and diagnostic value of each test.
   b. Discuss the etiology and significant lab findings in Paroxysmal Nocturnal Hemoglobinuria (PNH) and Paroxysmal Cold Hemoglobinuria (PCH).
   c. Contrast PNH and PCH (see SL pages 126 & 133)

^^Take ‘Closed-Book’ Special Tests Exam when ready but by the end of Hemo Rotation II.