CLINICAL LABORATORY SCIENCE TECHNOLOGY 134 / 135 / 136

COLLEGE LABORATORY
CLSC 134 / 135 / 136 COLLEGE LAB POLICIES

The purpose of College Laboratory sessions is to provide students the maximum opportunity to learn and master clinical testing principles and procedures free of the pressures of the actual clinical setting.

Completion of assigned College Laboratory activities is evaluated on a Satisfactory/Unsatisfactory basis. All College Laboratory reports are due no later than one week from the session during which the work was completed. Worksheets and/or assigned objectives must be thoroughly completed to the satisfaction of the instructor for a student to be considered satisfactory in College Laboratory performance. Repeated failure to submit all assigned College Laboratory work on time may result in a Deficiency Notice, followed by an Unsatisfactory College Laboratory grade, and the letter grade for the course will be lowered by one grade (i.e., an ‘A’ becomes a ‘B’, a ‘B’ becomes a ‘C’, etc.).

Students may be required to repeat College Laboratory procedures if the test performance or results obtained are considered unsatisfactory by the instructor. Incorrect or incomplete worksheets will be returned to the student for satisfactory completion of the assigned work.

ALL STUDENT TEST RESULTS ON WORKSHEETS MUST BE INITIALED BY AN INSTRUCTOR BEFORE BEING SUBMITTED FOR GRADING. DO NOT DISCARD ANY SAMPLES, SLIDES, UNOPETTES, BLOOD BANK REACTION TUBES, etc., BEFORE HAVING YOUR RESULTS CHECKED AND INITIALED. ANY RESULTS SUBMITTED FOR GRADING THAT ARE NOT INITIALED BY AN INSTRUCTOR WILL NOT BE ACCEPTED AND THE LAB ASSIGNMENT WILL HAVE TO BE REPEATED.

Failure to complete all assigned CLSC 134 College Laboratory work by the Friday of the last week of the course will result in an Unsatisfactory College Laboratory grade and the letter grade for CLSC 134 will be lowered by one grade. Failure to complete all assigned CLSC 135 College Laboratory work by the second Reading Day (Wednesday May 7th) will result in an Unsatisfactory College Laboratory grade and the letter grade for CLSC 135 will be lowered by one grade. If all College Laboratory work is not completed by the last Final Exam day (Wednesday May 14th) a course grade of Incomplete will be assigned for CLSC 135. A student cannot enter CLSC 123 with a grade of Incomplete in CLSC 135.

Rotation objectives and assignments for the College Laboratory activities throughout the Semester, as well as College Laboratory Procedures are contained in the following section of this Syllabus. Worksheets to be completed for each rotation are distributed in the College Laboratory sessions.
**COLLEGE LABORATORY ATTENDANCE REQUIREMENTS:** College Laboratory sessions involve extensive preparation and setup by the LIA and therefore, an absence creates a great inconvenience for program faculty. In addition, some College Laboratory sessions may be impossible to duplicate. For this reason, a student is only allowed **ONE UNEXCUSED ABSENCE** from College Laboratory throughout CLSC 134 and CLSC 135. The total allowable absences from College Laboratory cannot exceed **TWO DAYS** in a given course or **THREE DAYS** in a given semester. If a student exceeds these limits, their final course grade will be **LOWERED BY ONE LETTER GRADE.**

If a student is absent from College Lab for any reason, they must schedule a makeup session with the LIA or course instructor. A written form will be used for this purpose, so that both the student and the instructor are aware of how and when the missed activities will be rescheduled. **IT IS THE RESPONSIBILITY OF THE STUDENT to see the LIA or course instructor immediately following an absence to schedule the makeup activities.**

**GENERAL COLLEGE LAB OBJECTIVES**

By the end of the college laboratory rotational sequences, the student should be able to:

1. locate all reagents, quality control materials, unknown specimens, supplies, and instruments to be used.
2. correctly perform, log, and interpret all test procedures (within rotational quotas stated) in this syllabus.
3. demonstrate a working knowledge of specimen and reagent use and storage.
4. state the principle behind all college laboratory procedures.
5. determine which steps in performance of a test procedure may be interrupted, and which steps are critically timed.
6. accept the subjectivity inherent in Blood Bank and differential decisions.
7. clean up the laboratory work station they have used.
8. accept differences in terminology and procedures between college laboratory and clinical agency laboratories.
9. recognize the need for assistance (and ask for help) when performing unfamiliar procedures and/or using unfamiliar instruments.
10. locate and read all reagent labels and package inserts when appropriate.
11. make an **independent** attempt to repeat procedures which have resulted in unacceptable results **after a complete review of the procedure and supplies.**
12. seek instructor’s assistance if repeat performance of a procedure fails to yield acceptable results.

13. locate the recorded limits of acceptability for QCs and enter their QC data daily.

14. determine when unknown data is reasonable, based on data obtained from concurrent analysis of quality control materials.

15. submit unknown results to the instructor which satisfy the linearity and reproducibility indicated for selected college laboratory procedures.

16. demonstrate a willingness to overcome procedural errors by practicing procedures until proficient (outside the scheduled lab hours if necessary).
CLSC 134 COLLEGE LABORATORY ROTATIONS

The College Laboratory component of CLSC 134 is composed of four "rotations." Each rotation will be different in its length, complexity, and subject matter content. Some rotations may demand additional practice outside of the scheduled college laboratory sessions, but all are arranged to parallel your clinical rotations as nearly as possible.

In order to perform effectively in the College Laboratory section of CLSC 134, the student must plan for their rotations and come to college lab knowing the theory and main steps of the procedures they will be performing that day. Procedures for laboratory tests performed in each rotation are found in this syllabus, and should be studied prior to a lab session. You will find a list of the seven rotational areas below. The following pages in this syllabus state the objectives and activities for each rotation. Please READ THEM so that you arrive for College Lab sessions informed and prepared about what is to be done during each session.

CLSC 134 College Laboratory Rotations:

Rotation A - Cell Counting Instrumentation (Coulter T540, A5T-5diff, and MAX-M) (3 weeks)

Rotation C – Coagulation (1 week)

Rotation D – “Hematography II” CD-ROM / Abnormal WBC Differential Case Studies (3 weeks)

Rotation F – Body Fluid Cell Counts (1 week)
CLSC 134 COLLEGE LABORATORY

ROTATION A: CELL COUNTING INSTRUMENTATION
(Coulter T540, A^T-5diff, and MAX-M)
CELL COUNTING INSTRUMENTATION ROTATION (ROTATIONS A₁, A₂, and A₃) (3 weeks)

The Cell Counting Instrumentation rotation consists of three separate rotations, A₁, A₂, and A₃. During these rotations, the student will be operating three automated cell counting instruments; the Coulter Model T540, Coulter Model A²T-5diff, and the Coulter Model MAX-M. Quality control data must be within established limits before unknown data is considered acceptable.

COULTER ROTATION ASSIGNMENTS

**ROTATION A₁**

- Use the Instrument Manual to complete the instrument worksheet for the Coulter T540.
- Perform daily startup, shutdown, and preventative maintenance procedures on Coulter T540 and record results on the appropriate logsheets.
- Determine Complete Blood Counts (CBC) on 3 QC samples and record results on the appropriate logsheets.
- Determine Complete Blood Counts (CBC) on five unknown samples using the Coulter T540.
- Calculate the MCV, MCH, and MCHC on those five samples and use those calculated values to predict the morphology of the RBCs.
- Perform "x3" calculations on the T540 data obtained from the five samples to determine the validity of the results.
CLSC 134 / 135 / 136

93

**ROTATION A2**

- Use the Instrument Manual to complete the instrument worksheet for the Coulter A$^{c}$T-5diff.

- **Perform a successful venipuncture** on a classmate to obtain a fresh EDTA sample for analysis on the Coulter A$^{c}$T-5diff.

- **Have a classmate perform a venipuncture on you** to obtain a 2nd fresh EDTA sample for analysis on the Coulter A$^{c}$T-5diff.

- Perform daily startup, shutdown, and preventative maintenance procedures on Coulter A$^{c}$T-5diff and record results on the appropriate logsheets.

- Determine Complete Blood Counts (CBC) on 3 QC and record results on the appropriate logsheets.

- Determine Complete Blood Counts (CBC) on the two freshly-drawn EDTA samples using the Coulter A$^{c}$T-5diff.

- Perform “x3” calculations on the Coulter A$^{c}$T-5diff data obtained from the two samples to determine the validity of the results.

- Examine and evaluate the CBC results produced on the Coulter A$^{c}$T-5diff, including assessment of WBC, RBC, and platelet scatter plots on those two samples.

- Prepare, stain, and examine blood smears on the two samples, performing WBC differentials and RBC/Platelet morphology descriptions on each sample, and compare these results with the Coulter A$^{c}$T-5diff scatter plots of each specimen.
**ROTATION A3**

- Use the Instrument Manual to complete the instrument worksheet for the Coulter MAX-M.

- **Perform a successful venipuncture** on a classmate to obtain a fresh EDTA sample for analysis on the Coulter MAX-M.

- **Have a classmate perform a venipuncture on you** to obtain a 2\textsuperscript{nd} fresh EDTA sample for analysis on the Coulter MAX-M.

- Perform daily startup, shutdown, and preventative maintenance procedures on Coulter MAX-M and record results on the appropriate logsheets.

- Determine Complete Blood Counts (CBC) on 3 QC and record results on the appropriate logsheets.

- Determine Complete Blood Counts (CBC) on the two freshly-drawn EDTA samples using the Coulter MAX-M.

- Perform “x3” calculations on the Coulter MAX-M data obtained from the two samples to determine the validity of the results.

- Examine and evaluate the CBC results produced on the Coulter MAX-M, including assessment of WBC, RBC, and platelet scatter plots on those two samples.

- Prepare, stain, and examine blood smears on the two samples, performing WBC differentials and RBC/Platelet morphology descriptions on each sample, and compare these results with the Coulter MAX-M scatter plots of each specimen.

- Record daily startup, and quality control data obtained on the Coulter MAX-M in appropriate logbooks.

**Note:** QC material must be run each day the student operates the Coulter T540 or A\textsuperscript{c}T-5diff.
CELL COUNTING INSTRUMENTATION ROTATION: OBJECTIVES

ROTATION A1
By the end of this rotation, the student should be able to:

1. explain the "Coulter principle" of cell counting in detail.

2. describe the location and function(s) of each of the following components of the Coulter T-540 instrument:
   a. Uni-T-Pak
   b. Operations keypad
   c. Liquid crystal commands display
   d. Sample aspiration valve (sandwich)
   e. WBC and RBC dilution baths
   f. Hemoglobin cuvette and sourcelamp
   g. Apertures
   h. Aperture displays
   i. Internal and external electrodes
   j. Electronic manometer
   k. Electronic manometer adjusting thumbwheel

3. perform daily startup, shutdown, and preventative maintenance procedures on the Coulter T-540 and determine whether the data generated is valid.

4. correctly aspirate QC and unknown EDTA samples using the Coulter T-540.

5. evaluate all QC and unknown EDTA sample data generated by the T-540 for validity.

6. enter the T-540 startup and QC data into the appropriate logbooks.

7. recall the formulas for calculation of MCV, MCH, and MCHC, and derive these indices on each of the patient samples run on the T-540.

8. Use the indices calculated to predict the expected RBC histogram for each of the patient samples run.

9. recall the proper units and adult normal reference ranges for WBC count, RBC count, hemoglobin, hematocrit, MCV, MCH, MCHC, and platelet count.

10. correlate the hemoglobin, hematocrit, and RBC on all samples using the "times three rule."

11. offer explanations for violations of the "times three rule" when analyzing unknown specimens and suggest additional tests which would prove or disprove your explanation(s).

12. compare QC data to the posted 2SD limits of acceptability for T540 CBC results.
**ROTATION A2**
By the end of this rotation, the student should be able to:

1. describe the location and function(s) of each of the basic components of the Coulter A\textsuperscript{\text{}-T-5diff instrument.

2. perform daily startup, shutdown, and preventative maintenance procedures on the Coulter A\textsuperscript{\text{}-T-5diff and determine whether the data generated is valid.

3. correctly aspirate QC and unknown EDTA samples using the Coulter A\textsuperscript{\text{}-T-5diff.

4. evaluate all QC and unknown EDTA sample data generated by the Coulter A\textsuperscript{\text{}-T-5diff for validity.

5. enter the Coulter A\textsuperscript{\text{}-T-5diff startup and QC data into the appropriate logbooks.

6. evaluate WBC, RBC, and platelet scatter grams for each of the patient samples run, and comment on the probable WBC, RBC, and platelet morphology of patients, predicting abnormalities that might be present.

7. recall the normal ranges of MCV, MCH, and MCHC, and assess these results on patient samples, noting abnormal results and suggesting causes for abnormalities.

8. prepare acceptable quality blood smears on patient samples and stain them using Wright's stain.

9. examine blood smears under the microscope and obtain acceptable WBC differential results, as well as correctly assess RBC and platelet morphology.

10. correlate the hemoglobin, hematocrit, and RBC on all samples using the "times three rule."

11. offer explanations for violations of the "times three rule" when analyzing unknown specimens and suggest additional tests which would prove or disprove your explanation(s).

12. compare QC data to the posted 2SD limits of acceptability for Coulter A\textsuperscript{\text{}-T-5diff CBC results.
**ROTATION A3**
By the end of this rotation, the student should be able to:

2. describe the location and function(s) of each of the basic components of the Coulter MAX-M instrument.

2. perform daily startup, shutdown, and preventative maintenance procedures on the Coulter MAX-M and determine whether the data generated is valid.

3. correctly aspirate QC and unknown EDTA samples using the Coulter MAX-M.

4. evaluate all QC and unknown EDTA sample data generated by the Coulter MAX-M for validity.

5. enter the Coulter MAX-M startup and QC data into the appropriate logbooks.

6. evaluate WBC, RBC, and platelet scatter grams for each of the patient samples run, and comment on the probable WBC, RBC, and platelet morphology of patients, predicting abnormalities that might be present.

7. recall the normal ranges of MCV, MCH, and MCHC, and assess these results on patient samples, noting abnormal results and suggesting causes for abnormalities.

8. prepare acceptable quality blood smears on patient samples and stain them using Wright's stain.

9. examine blood smears under the microscope and obtain acceptable WBC differential results, as well as correctly assess RBC and platelet morphology.

10. correlate the hemoglobin, hematocrit, and RBC on all samples using the "times three rule."

11. offer explanations for violations of the "times three rule" when analyzing unknown specimens and suggest additional tests which would prove or disprove your explanation(s).

12. compare QC data to the posted 2SD limits of acceptability for Coulter MAX-M CBC results.
**CELL COUNTING INSTRUMENTATION ROTATION: PROCEDURES**

**COULTER T540 OPERATION**

**PRINCIPLE OF COULTER T540:**

Coulter Counters operate by the principle of electronic impedance, a method of non-optical, one-by-one counting and sizing of particles. The counter consists of an aperture tube submerged in a sample container. Isoton, an electrolytic solution, is in the sample container and inside the aperture tube. An external electrode is submerged in the sample container, and an internal electrode is suspended inside the aperture tube. When the counter is activated, current begins to flow from the external to the internal electrode. The current flows through a minute tunnel or orifice in the base of the aperture tube. The orifice is approximately 100 microns in diameter and 75 microns in depth and represents the point of maximum resistance to current flow. When the counter is activated, it not only starts an electrical current flow, it also applies vacuum to the inside of the aperture tube. The vacuum draws cells suspended in Isoton from the sample container through the orifice to the inside of the aperture tube. These blood cells being virtually non-conductors of current, produce an additional resistance to the electrical current flow. This resistance at the orifice is directly proportional to the volume of solution the cell displaced and therefore directly proportional to the cell volume. The number of resistance changes produced by the sample is proportional to the number of cells within the suspension. Number and volume of resistance changes counted is displayed on oscilloscopes, and converted by the analyzer computer into cell count values.

**Coulter T-540 Instrument Start-Up:**

1. Turn on instrument and printer. Press **RES1 and RES2 simultaneously**, then press **DIR**. You will see the display “Set Date & Test.”

2. Press **SEL** to select the Set Date & Test option.

3. Press **six** digits for the month, day and year (MMDDYY), then press **ENTER** to store the date in the computer.

4. The next display calls for the test number. Key in a **three** digit test number, i.e., 001) if desired.

5. Press **ENTER**.

6. Open the right front door.

7. Check for proper readings on the Pneumatic Supply gauges on the front pane.

   The gauges should read:  
   
<table>
<thead>
<tr>
<th>Gauge</th>
<th>Reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRESSURE</td>
<td>60 ± 2 psi</td>
</tr>
<tr>
<td>5 PSI</td>
<td>5 ± 0.25 psi</td>
</tr>
<tr>
<td>30 PSI</td>
<td>30 ± 1 psi</td>
</tr>
<tr>
<td>VACUUM</td>
<td>20” Hg minimum</td>
</tr>
</tbody>
</table>

   RECORD GAUGE READINGS ONTO YOUR WORKSHEET.
8. Use the pressure adjust knobs to adjust the 5 psi or the 30 psi gauge, if necessary. Inform instructor or lab assistant if the 60 psi or the vacuum gauge is outside the limits.

9. Check the electronic manometer. The light should appear within the green colored acceptable reference range. If necessary, adjust the manometer within this range using the VACUUM ADJUST wheel. Turn the wheel UP to INCREASE the number of lights; to DECREASE the number of lights, turn the wheel DOWN.

10. Press START UP. When the automatic cycles stop, check the display and printer for the results of the tests and background counts.

   If the tests are within limits, you will see an “Accept” message. If a test did not pass, you will see a “Review” message.

   If “REVIEW” appears, press TEST, 0, 1, ENTER to repeat the tests.

   If “REVIEW” appears again, inform the instructor or lab assistant.

11. Prime the unit with a whole blood sample:
   
a. Place the aspirator tip in the blood.

b. Press the WHOLE BLOOD button.

c. Keep the tip submerged while “Aspirating” is displayed.

12. Watch for “Wipe Tip” on the digital display. When you see it, remove the sample tube and wipe the aspirator tip before “Backwash” is displayed.

   Allow the instrument to cycle the prime sample completely.

13. Analyze three levels of control for your daily quality control. Refer to the package insert for correct mixing and handling.

   Record and check the quality control results in the QC logbook. If the results are unacceptable, inform the instructor or lab assistant.
**Coulter T-540 Sample Analysis:**

1. The display should show the next test number of “Select Function.” If not, press RES1/RES2.

2. Check that the printer is ON.

3. Thoroughly mix the blood sample by gently inverting the tube several times.

   NOTE: Only EDTA-anticoagulated blood should be used.

4. Immerse the aspirator tip in the blood and then press the **WHOLE BLOOD** button.

5. Keep the tip submerged in the blood while the display shows the “Aspirating” message.

6. When the “Wipe Tip” display appears, remove the sample and **wipe** the tip.

   Move your hand away from the tip before the display shows “Backwash” and the backwash arm appears.

7. When the counting cycle has been completed, the CBC results will automatically print on the printer. When removing the printout from the printer, do not use force or try to remove the printout before printing is finished.

    If desired, press PRINT to obtain an extra printout.

8. Repeat Steps 3 through 7 for each sample.

**Coulter T-540 Instrument Shutdown:**

1. Press the **SHUT DOWN** button.

2. Allow the instrument to cycle through an entire **Shut Down** cycle. When the cycle is complete, there will be blue Coulter-Clenz detergent visible in all lines and baths, and the pneumatics of the instrument will power down and go silent.

3. Allow the instrument to stand with Coulter-Clenz in the lines for a minimum of 15-minutes.

4. Press the **START UP** button and allow the instrument to cycle through an entire **Start Up** cycle. When the cycle is complete, Startup and Background Count data will print on the printer.

    If the tests are within limits, you will see an “Accept” message. If a test did not pass, you will see a “Review” message.

    If “REVIEW” appears, press TEST, 0, 1, ENTER to repeat the tests.

    If “REVIEW” appears again, inform the instructor or lab assistant.

5. Turn OFF the main power to the instrument.
COULTER T540 MAINTENANCE PROCEDURES

NOTE: These procedures are only to be performed with the approval and assistance of the instructor or lab assistant.

Sampling Valve Cleaning Procedure:

1. Open the right front door. Remove the clear plastic aspirator panel.
2. Press RES1/RES2 and then press F, 0, 7 ENTER to loosen the BSV.
3. Turn off the main power.
4. Unscrew the black knob assembly, then slide it out.
5. Pull the BSVs three pieces of tubing through the Diluter panel to make it easier to remove the BSV.

IMPORTANT: Leave the tubing attached to the BSV.

6. Grasp the three sections of the BSV. Pull them off the mounting post.
7. Separate the three sections by sliding them apart. Allow them to hang by their tubing.
8. Working with one section at a time, clean each surface with a lint-free tissue that was moistened with detergent reagent.

CAUTION: Do not use gauze to clean the blood sampling valve.

9. Squirt detergent reagent through the holes of each section. Use a container to catch the liquid.

CAUTION: Do not use bleach or a stylus to clean the holes.

10. Rinse the holes and surfaces with distilled water.
11. Dry the center holes with lint-free tissue.
12. Remove the spacer bar and clean the mounting post and guidepost with a tissue moistened with distilled water.

Dry the posts and replace the space bar.

13. Dampen the inside surface of the rear section with a tissue moistened with distilled water.
14. Align the notch with the guidepost and then slide the rear section onto the mounting post.
15. Repeat Steps 13 and 14 for the other two sections, moistening both surfaces of the center section with distilled water.

16. Push the excess tubing through the panel, making sure that the tubing is not pinched. Replace and tighten the black knob.

17. Hold the BSV and pull the black knob. There should be 1/16 inch of space between the knob and the front section.

18. Turn on the main power; wait for the scrolling display to stop. Press RES1/RES2, then press F, 0, 5, ENTER. The BSVs center section should rotate clockwise without hesitation.

19. Press F, 0, 6, ENTER. The center section should rotate smoothly counterclockwise.

   If you observe jerky rotation in Steps 18 and 19, loosen the black knob slightly.

20. Press DRAIN and then RINSE. Verify that the BSV does not leak. If leakage occurs, retighten the knob and repeat Steps 18 and 19.

21. Replace the aspirator panel.

22. Record in your logbook that you performed this procedure.

   NOTE: Allow a 30-minute warmup period and perform “STARTUP” before running patient samples.

Aperture Bleaching Procedure:

1. Press RES1/RES2, then press DRAIN to drain the aperture baths.

2. Locate the check valves below each aperture bath.

3. Remove the RBC bath check valve tubing from its fitting on the Diluter panel.

4. Prepare 50% FRESH bleach solution.

5. Connect the bleach wash bottle to the tubing you disconnected in Step 3.

   Squeeze the bottle until the solution fills the RBC bath up to the top input port. Disconnect the bottle from the tubing.

6. Disconnect the WBC check valve tubing from its fitting on the Diluter panel.

7. Connect the bleach bottle to the disconnected tubing. Fill the WBC bath with bleach up to the input port; then disconnect the bottle.
8. Press F, 0, 9, ENTER to draw the bleach solution into the apertures. 

**WAIT 15 MINUTES.**

9. Press DRAIN.

10. Connect the detergent reagent bottle to the disconnected RBC bath tubing. Fill the RBC bath with reagent up to the input port. Remove the bottle.

11. Repeat Step 10 for the other aperture bath.

12. Reconnect the tubing that you disconnected in Steps 3 and 6 to the Diluter panel fittings.

13. Press DRAIN, then press F, 0, 8, ENTER. Wait for the “Bleach:Clear” function to finish.

14. Press DRAIN; after “Select Function” displays, press RINSE.

15. Press SHUT DN and wait for the cycles to stop.

16. Press START UP and wait for the cycles to stop.

17. When the cycles stop, hold a vial under the tip and press DIL DISP. Discard this liquid.

18. Dispense more liquid into a clean vial by pressing DIL DISP several times. Cycle this through the unit four times:

* Submerge the tip in the liquid.
* Press WHOLE BLOOD.

19. Press TEST, 0, 1, ENTER. Look for an “Accept” display.

20. Before running patient samples or controls, cycle a normal whole blood sample to prime the unit.

21. Record in logbook that you did this procedure.
COULTER AcT-5diff OPERATION

**PRINCIPLE:**

The AcT-5Diff analyzer is a fully automated hematology analyzer providing a complete WBC five-part differential, which is determined simultaneously by the AcV (Absorbance Cytochemistry and Volume) technology and WBC/BASO methodologies.

The AcV technology uses absorbance, cytochemistry, and “focused flow impedance” (hydrodynamic focusing). The WBC/BASO methodology uses differential lysis, impedance technology, and differential thresholds. The following table summarizes the technologies used for the various CBC parameters. More detailed information may be found in the operator's manual.

<table>
<thead>
<tr>
<th>Technology</th>
<th>Measurements</th>
<th>CBC parameters measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coulter Principle (electronic impedance)</td>
<td>Cell volume and count</td>
<td>RBC count, platelet count, and hematocrit</td>
</tr>
<tr>
<td>AcV technology with Dual Focused Flow (hydrodynamic focusing)</td>
<td>Light absorbance of cytochemically-stained cells</td>
<td>Lymphocytes, monocytes, neutrophils, eosinophils, immature cells, and atypical lymphocytes</td>
</tr>
<tr>
<td>Differential lysis using the Coulter principle</td>
<td>Volume and count</td>
<td>WBC count, basophil percentage, and basophil count</td>
</tr>
</tbody>
</table>

**Instrument Startup Procedures:**

1. Before turning the instrument on, check all reagent levels and the waste container. Replenish reagents if necessary, and empty waste container if more than half full. The instrument has the following reagents in these locations:

   - AcT-5diff Rinse: inside the left front door
   - AcT-5diff Fix: inside the left front door
   - AcT-5diff Hgb Lyse: inside the left front door
   - AcT-5diff WBC Lyse: inside the left front door
   - AcT-5diff Diluent: in cabinet under the counter
   - Waste Container: in cabinet under the counter

2. Check the printer for adequate paper supply and replenish if necessary.
3. Turn on the instrument power supply (the metal box that the printer paper sits on) with the switch on the front of the supply box. The printer should turn on at this time. If it does not, turn it on using the power switch on the left side.

4. Turn on the instrument’s main power switch on the left side of the instrument.

5. When turned on, the instrument will automatically go into a Startup cycle, which consists of a rinse cycle followed by a background count (an analysis cycle on the reagent without any blood specimen). As the Rinse Cycle is proceeding, the progress of the cycle can be viewed on the bar display on the instrument screen. This is followed by the Background Count, that can also be observed on the bar display.

6. After the Background Count is completed, the instrument may enter a “warm-up” mode, shown on the screen as a bar display of the Heating Coil temperature. When this warm up is complete, the display will disappear and the screen will display the sample analysis screen.

7. Review the Background Count results. The acceptable ranges for background are:

- **WBC:** $0 - 0.3 \times 10^3/mm^3$
- **RBC:** $0 - 0.03 \times 10^6/mm^3$
- **Hgb:** $0 - 0.3 \, g/dL$
- **Plt:** $0 - 7.0 \times 10^9/mm^3$

8. If the Background Count results are acceptable, proceed to the Quality Control procedure. If they are not acceptable, the instrument will automatically perform Startup up to two more times to obtain an acceptable result. If Startup fails after the third time, a STARTUP FAILED message appears on the screen and will appear on any reports if an attempt is made to run samples. **DO NOT ATTEMPT TO RUN SAMPLES IF YOUR BACKGROUND COUNT IS UNACCEPTABLE. NOTIFY THE INSTRUCTOR.**
QUALITY CONTROL PROCEDURE

1. The quality control samples for the AcT-5diff are called **AcT-5diff Control**. Be sure to use the correct controls on the instrument (not the 5C Controls used for other rotations!).

2. Controls must be warmed to room temperature and well-mixed before running. Be sure to mix GENTLY so that cellular elements in the control are not damaged.

3. Check the display screen and verify that the CBC/DIFF mode is displayed. If the display reads CBC, press the CBC/DIFF button to change the mode to CBC/DIFF.

4. The instrument is set up to reset the specimen ID number to “1” each morning. As samples are run, this number will automatically increase by one with each sample. Be sure to pay attention to what samples are #1, 2, 3, etc. You may write the actual sample numbers/names on the printouts as they come off the printer to avoid sample mixups.

5. A green LED light indicates that the instrument is ready to receive a sample. When the control sample is well-mixed, present the control vial to the sample probe and press the dark gray aspirator plate behind the sample probe. The LED lights flash while the instrument is aspirating. When the red LED lights up, the sample probe retracts into the instrument and the sample can be removed.

6. After the sample cycles, the result will print on the printer. Write the identification of the control on this printout before running your next sample.

7. Proceed to run the other two controls following steps #5 and 6.

8. Verify that the quality control results are acceptable, and log the QC results onto the appropriate worksheets. Have an instructor verify the acceptability of your quality control results. If QC is good, then proceed to Sample Testing.

SAMPLE TESTING ON THE AcT-5diff

1. Mix the sample well before sampling. Verify that the green LED light is lit indicating the instrument is ready to receive a sample. Remove the cap and present the sample to the probe and press the aspirator plate. When the red LED lights, the sample probe retracts into the instrument and the sample can be removed.

2. When analysis is complete, the sample results will appear on the screen and will print on the printer. Write the sample identification onto the printout before running your next sample.

3. Run your subsequent samples using the procedure in steps #2 and 3.

4. Review your results and verify that all results are valid and no repeats are necessary. Verify the acceptability of your results with an instructor.

5. If all results are acceptable, proceed to Instrument Shutdown.
**INSTRUMENT SHUTDOWN**

1. When all specimens have been run, run a Shutdown procedure on the instrument by pressing the Shutdown button (the **curved** downward arrow). The instrument will cycle Rinse reagent and go into a stand-by mode.

2. Allow the instrument to remain in stand-by for 15 minutes.

3. At the end of the 15 minutes, run a Startup procedure by pressing the Startup button (the **curved** upward arrow).

4. After the Startup cycle is completed, turn off the instrument by pressing the on/off switch on the left side of the instrument, and the on/off switch on the front of the power supply.
CLSC 134 COLLEGE LABORATORY

ROTATION C: COAGULATION
COAGULATION ROTATION (ROTATION C)  
(1 week)

The coagulation rotation provides the student with an opportunity to perform the prothrombin time (PT) and activated partial thromboplastin time (APTT), as well as review the principles and procedure for the bleeding time test.

**COAGULATION ROTATION: QUOTAS**

* The student will perform two PT and two APTT tests on assigned specimens using the MLA 750 and/or KC4-Delta coagulation instruments.

* The student will accurately perform a bleeding time procedure on the instructor.

**COAGULATION ROTATION: OBJECTIVES**

By the end of this rotation, the student should be able to:

1. list the factors in each of the two main branches of the coagulation pathway.

2. describe events that lead to activation of the two coagulation pathways.

3. recall the coagulation processes evaluated by various coagulation tests. (PT, PTT, fibrinogen)

4. identify the major components of the MLA 750 and the KC4-Delta, comparing and contrasting the methods by which these instruments detect clot formation when performing PT and APTT tests.

5. describe the principles of the PT, APTT, and fibrinogen tests, and describe the contents and functions of the various reagents used in the tests.

6. properly reconstitute coagulation controls and reagents.

7. recall stability limits for coagulation reagents following their initial use or reconstitution.

8. complete a minimum of two (2) PT and two (2) APTT tests on patient samples provided, including acceptable quality control results.

9. describe the procedural steps in a Simplate Bleeding time.

10. troubleshoot coagulation testing data when problems occur involving unknowns and QC materials.

11. correctly log QC values for coagulation tests in the college laboratory QC logbook.
COAGULATION is the most complex aspect of hemostasis, which also is dependent on blood vessel integrity, and platelet activity. It involves the interaction of more than 10 different factors contained in normal plasma and tissue, to arrive at the end result, formation of a fibrin clot to stop bleeding.

Coagulation can take one of two different pathways to arrive at the formation of a clot. These are referred to as the intrinsic and extrinsic pathways or cascades.

**The Intrinsic Coagulation Pathway**

The intrinsic (or “contact”) pathway is initiated by either abnormal changes in endothelial cells which release collagen into circulation, or by contact with negatively charged surfaces like glass or plastic. It is this pathway that causes blood to clot when drawn into a syringe or glass tube.

The change in endothelial cell structure or glass contact activate Factor XII to XIIa, which begins a cascade of chemical changes in the Factors. Factor XI is activated to XIa, which in turn activates IX to IXa with the interaction of calcium ions; then IXa, with action from Factor VIII and a phospholipid on the surface of platelets called Platelet Factor 3 (PF3), activates Factor X to Xa, which is the first step of the “final common pathway” of both intrinsic and extrinsic coagulation.

**The Extrinsic Coagulation Pathway**

The extrinsic pathway is the pathway by which bleeding from an open wound or tissue damage is stopped. This pathway begins with Thromboplastin (Factor III), a protein present in all living tissue, which is released into the plasma when tissue is cut or damaged. Thromboplastin, in interaction with calcium and Factor VIIa, is also capable of activating Factor X to Xa, which is the first step of the final common pathway.

**The Final Common Pathway**

The final common pathway begins with the activation of Factor X to Xa through either the intrinsic or extrinsic cascade. Once this has occurred, Xa, with action from calcium, Factor V, and PF3, can convert Factor II (Prothrombin) to IIa (Thrombin). Thrombin acts on Fibrinogen (Factor I) to form soluble fibrin, an unstable clot, and also interacts with calcium to activate Factor XIII and XIIIa. Factor XIIIa then acts on soluble fibrin to form the final, insoluble fibrin clot.

The diagram on the next page is a representation of the Intrinsic, Extrinsic and Final Common Pathways of coagulation.
INNISIC PATHWAY

Kallikrein → Prekallikrein
\[ \text{contact} \]

XII → XIIa

XI → Xla

IX → IXa

XIII

FINAL COMMON PATHWAY

EXTRINSIC PATHWAY

II

(Prothrombin)

IIa

(Thrombin)

Ca^{2+}

XIII

III

(Tissue Thromboplastin)

VII → VIIa

VII

VIII → VIIIa

VIII

IX

IXa

PF3

VIII

X → Xa

Va

PF3

I Ia

fibrinogen

fibrin (soluble)
fibrin (insoluble)

Ia

fibrin (soluble)
fibrin (insoluble)
COLLEGE LABORATORY SUPPLEMENT: COMMON COAGULATION TESTS

Testing the Intrinsic Pathway

The most common laboratory test used to monitor the intrinsic pathway is the APTT. This test utilizes citrate-anticoagulated plasma and combines the specimen with two reagents:

The first is Actin, which has two components. Cephaloplastin (or partial thromboplastin) is a phospholipid which substitutes for Platelet Factor 3 in the activation of Factor XII and XI. It also contains Ellagic Acid, which is a particulate activator that provides a surface for contact activation of Factor XII.

The activation by Actin is followed by the addition of the second reagent, Calcium Chloride, which provides a source of ionic calcium for completion of the intrinsic pathway to fibrin formation. The time is measured in seconds from the addition of the calcium chloride to the detection of a fibrin strand or clot by the detection instrument.

Testing the Extrinsic Pathway

The activity of the extrinsic pathway is measured using the Prothrombin Time, or “Protime.” This test combines citrate plasma with Thromboplastin-C reagent, which has two components.

First is Tissue Thromboplastin (Factor III), which activates Factor VII in the extrinsic pathway.

Second is Calcium, which is needed for the completion of the extrinsic pathway to the formation of fibrin.

The time is measured in seconds from the addition of Thromboplastin-C reagent to the specimen, to the formation of a fibrin strand or clot.
COAGULATION ROTATION: PROCEDURES

PROTHROMBIN TIME
(MLA 750)

PRINCIPLE:

Prothrombin time is the clotting time obtained when excess thromboplastin and optimum calcium are added to citrated plasma under standardized conditions. Under these conditions, the clotting time is essentially a test of the extrinsic pathway of clotting; i.e., tissue extract plus factors VII, V, and X, in the presence of calcium, acting on prothrombin to thrombin, which in turn leads to a fibrin clot.

Reagents: Innovin® (Dade)

Dried recombinant human tissue factor with calcium.

Reconstitute reagent with 20.0 ml of distilled water using a serological pipet.

**Reconstituted Innovin® is stable for 10 days when stored at 2-8° C.**

Quality Control: Lyophilized control material, Levels I and III.

Reconstitute Quality Control material with 1.0 ml distilled water using a volumetric pipet.

**Reconstituted QC material is stable for 8 hours.**

Specimen Collection and Preparation:

1. Collect whole blood in sodium citrate (4.5 ml blood to 0.5 ml 3.8% sodium citrate or one blue-stoppered Vacutainer tube). Mix well.

2. Centrifuge blood specimen for 10 minutes at 3000 rpm. The specimen is stable for 4 hours if the cap has not been removed.

Procedure:

1. Turn on Electra 750. Warm up instrument for 5 minutes or until \textit{AT TEMP} lights, whichever is later.

2. Check that \textit{LAMP LEVEL} switch is in “B” (middle position).

3. Prewarm Innovin in \textit{REAGENT} reservoir for at least 5 minutes. Use magnetic stirring bar for reagents that require agitation.
4. Set Mode Switch to PT.

5. Using a standard 100μL pipette, **not** the instrument pipette, pipet 0.1 ml of control or patient plasma, in duplicate, into the bottom of MLA test cuvettes and place in rack. *Be sure that the entire volume of sample is pipetted into the bottom of the cuvette and no sample droplets cling to the inside walls of the tube.*

6. Place first sample in the test station and start the stopwatch.

7. After 45 seconds, place next sample in incubation station #1.

8. Continue in this manner, adding next tube every 45 seconds. (This interval may be increased for samples with higher PT results, i.e., Control #2).

9. At 2 minutes 45 seconds, place a tip on the 0.2 ml (red top) instrument pipette and aspirate 0.2 ml of warm Innovin. Align pipette over test station. At exactly 3 minutes of incubation, firmly push pipette plunger and hold it down for 1 second to start test.

10. When timer stops, record clot time. Remove and discard cuvette and pipette tip.

11. Place the 2nd sample in the test station, then start the test by delivering 0.2 mL of Innovin at exactly 3 minutes 45 seconds.

12. Use clean pipette tip on instrument pipette for each test to prevent carry-over contamination.

13. Record duplicate results and average the times for the final reported result. *Duplicate measurements must agree within 1.0 second or the test must be repeated.*

15. Check and record QC results in logbook, checking results for acceptability according to the established QC ranges.

*Normals:* 11 - 13 seconds

**NOTES:**

1. All plasma must be run in duplicate and the times must agree within the guidelines indicated in this syllabus. Report the average of the two values obtained.

2. The prothrombin time may be prolonged in patients with liver disease, disseminated intravascular coagulation, or a deficiency of the extrinsic system factors (V, VII, X, II and/or I), as well as in patients receiving oral anticoagulant therapy.

---

**References:**


ACTIVATED PARTIAL THROMBOPLASTIN TIME
(MLA 750)

PRINCIPLE:

The activated partial thromboplastin time consists of recalcifying plasma in the presence of a standardized amount of platelet-like reagent and a plasma activator. The test is a screening procedure used primarily to determine deficiencies in factors necessary for the formation of intrinsic prothrombin activator, namely factors VIII, IX, XI, and XII. Factors II, V, and X deficiencies (normally detected by the prothrombin time) can also be detected by the APTT.

Reagents:

1. Actin FS (Dade)
2. 0.025 M CaCl₂ (Dade)

Quality Control: Lyophilized control material, Levels I and III.

Reconstitute Quality Control material with 1.0 ml distilled water using a volumetric pipet.

Reconstituted QC material is stable for 8 hours.

Specimen Collection and Preparation:

1. Collect whole blood in sodium citrate (4.5 ml blood to 0.5 ml 3.8% sodium citrate or one blue-stoppered Vacutainer tube). Venipuncture must be atraumatic.

2. Centrifuge blood specimen for 10 minutes at 3000 rpm. The specimen is stable for 4 hours if the cap has not been removed. Heparin patient specimens should be run within 1/2 hour.

Procedure:

1. Turn on Electra 750. Warm up instrument for 5 minutes or until AT TEMP lights, whichever is later.

2. Check that LAMP LEVEL switch is in “B” (middle position).

3. Prewarm calcium chloride in calcium reservoir for at least 5 minutes.

4. For ellagic acid type reagent, set mode switch to APTT. For particulate type activator materials, set mode switch to SPEC 2.
NOTE: At time of this printing, common ellagic acid activators are Dade Cephaloplastin or Actin, or Ortho Thrombofax. A common type of particulate activator is General Diagnostic's Platelin Plus, or Automated APTT Reagent.

5. Using a standard 100μL pipette, (not the instrument pipette), pipet 0.1 ml of control or patient plasma, in duplicate, into the bottom of MLA test cuvettes and place in rack. Be sure that the entire volume of sample is pipetted into the bottom of the cuvette and no sample droplets cling to the inside walls of the tube.

6. Add 0.1 ml of Actin Reagent to the first test tube, shake gently, and place in the test station, then start the stopwatch.

7. After 60 seconds, add 0.1 ml of Actin to the next cuvette with plasma, shake gently and place in incubation station #1.

8. At 2 minutes 45 seconds, place a tip on the 0.1 ml (blue top) instrument pipette and aspirate 0.1 ml of warm Calcium Chloride reagent. Align pipette over test station. At exactly 3 minutes of incubation, firmly push pipette plunger and hold it down for 1 second to start test.

9. When timer stops, record clot time. Remove and discard cuvette and pipette tip.

10. Place the 2nd sample in the test station, then start the test by delivering 0.2 mL of Calcium Chloride reagent at exactly 4 minutes.

11. Use clean pipette tip on instrument pipette for each test to prevent carryover contamination.

12. Record duplicate results and average the times for the final reported result. Duplicate measurements must agree within 2.0 seconds or the test must be repeated.

13. Check and record QC results in the logbook, checking results for acceptability according to the established QC ranges.

Normals: 24 - 38 seconds

NOTES:
1. All plasmas must be run in duplicate and the times must agree within the guidelines indicated in this syllabus. Report the average of the two values obtained.

2. In patients with hematocrit values greater than 55%, the citrate concentration must be adjusted so that a proper plasma/anticoagulation volume is maintained.

References:
SIMPLATE BLEEDING TIME

PRINCIPLE:

Bleeding time is defined as the time taken for a standardized skin wound to stop bleeding. Upon vessel injury, platelets adhere and form a hemostatic plug. Bleeding time measures the ability of these platelets to arrest bleeding and therefore measures platelet number and function. Bleeding time is measured as a screening procedure used to detect both congenital and acquired disorders of platelet function.

Equipment:

1. Simplate bleeding time device. (This is a spring-loaded blade in a disposable holder that makes a standardized incision 5 mm x 1 mm deep.)
2. Sphygmomanometer
3. Stopwatch
4. Filter paper

Procedure:

1. Place a sphygmomanometer cuff on the upper arm, cleanse the muscular area of the lateral forearm approximately 5 cm below the antecubital crease with alcohol, and allow to air dry.
2. Remove the Simplate device from its package and twist off the white tear-away safety tab. Inflate the cuff to 40 mmHg. This pressure should be maintained throughout the entire test.
3. Place the Simplate firmly on the forearm and depress the trigger, starting the timer at the same time.
4. At 30 seconds, blot the flow of blood with filter paper. Bring the filter paper close to the incision without touching the edge of the wound and allow it to absorb the blood. Blot in a similar manner every 30 seconds until blood no longer stains the filter paper. Stop the timer at this point.
5. Remove the cuff, cleanse the arm with water, and apply a bandage.

Normal Range: 2.5 - 9.5 minutes

Reference:
CLSC 134 COLLEGE LABORATORY

ROTATION D: CD-ROM “HEMATOGRAPHY II” / ABNORMAL WBC DIFFERENTIAL CASE STUDIES
ROTATION D: CD-ROM “HEMATOGRAPHY II’” AND ABNORMAL WBC DIFFERENTIAL CASE STUDIES

(3 weeks)

During Rotation D, the student will spend three weeks studying WBC morphology and performing abnormal WBC differentials. The following are the assigned activities for this rotation:

**PART I: CD-ROM “HEMATOGRAPHY II” PROGRAM**

During this part of the college laboratory, the student will view the CD-ROM instructional program “Hematography II”, which covers maturation and morphology of WBCs, focusing on WBCs not normally found in differentials, but often present in disease states. The student will complete the accompanying worksheet from information presented on the CD.

**PART II: ABNORMAL WBC DIFFERENTIAL CASE STUDIES**

At the beginning of their hematology college laboratory and clinical rotations, CLSC 122 students will meet in the college laboratory for one clinical session for a review of abnormal WBC morphology and an introduction to the reading of abnormal WBC differentials.

In addition to completion of the “Hematography II” CD-ROM during their three-week college lab abnormal differential rotation, students will complete a minimum of 6 abnormal WBC differentials. These will be presented to the student in the form of case studies with complete case history and CBC information on each patient. The student will complete the WBC differential with RBC morphology report, and submit their results along with the accompanying worksheet. The instructor may assign additional abnormal WBC differentials to be completed if it is felt that the student’s performance on the 6 case studies is less than satisfactory. Student performance of abnormal WBC differentials will be assessed through the Abnormal WBC Differential Practicum described on the next page of this Syllabus.
WBC DIFFERENTIAL PRACTICUM EXAM

- Competency in the reading of abnormal WBC differentials is assessed after completion of this rotation through an Abnormal WBC Differential Practicum Exam.

**THIS EXAM IS GIVEN DURING THE LAST SESSION OF THE STUDENT’S COLLEGE LABORATORY HEMATOLOGY ROTATIONS.**

This Exam consists of two parts:

**Part 1:** The students will be shown two 35mm Kodachrome slides each of ten normal and abnormal WBCs to identify by name.

For successful completion of this practicum exam, the student must identify **8 out of the 10 WBCs correctly** on Part 1 of the WBC Differential Practicum Exam.

**Part 2:** The students will be given 6 Wright-stained abnormal human blood smears to perform a complete differential exam on each, including a WBC differential, and evaluation of the RBC and platelet morphology of the specimen. All differentials will be accompanied with the complete CBC data.

Each of the six unknown differentials will be worth ten points for a combined total possible score of 60 points. Each diff will be scored as follows:

- WBC diff percentages: 5 points
- WBC inclusions / abnormalities: 1 point
- RBC size / shape / color: 2 points
- RBC inclusions and/or NRBCs: 1 point
- Platelet morphology / estimate: 1 point

For successful completion of this practicum exam, the student must achieve a **minimum score of 77% (46 points)** on Part 2 of the WBC Differential Practicum Exam.

Failure to achieve the above minimum criteria (8 out of 10 cells correct in Part 1 and 77% - 46 points in Part 2) on this Exam will result in an clinical grade of Unsatisfactory, and a course grade of “F”.
Rotation D (CD-ROM / Abnormal WBC Differentials): Objectives

By the end of this rotation, the student should be able to:

1. locate normal erythrocytes, leukocytes, and thrombocytes on Wright’s stained blood smears using a microscope.

2. demonstrate basic competency in the use of microscopes and differential counting devices.

3. review the expected differential percentages of a normal adult leukocyte population.

4. correctly categorize immature cells of the granulocytic, lymphocytic, and erythrocytic cell lines.

5. express differential results in a percentage format.

6. recall the expected peripheral blood findings in:
   a. anemias
   b. leukemias
   c. thrombocytopenias

7. explain what is meant by a "left-shift."

8. perform acceptable differentials representing the following disorders:
   a. left-shifts
   b. granulocytic leukemias
   c. lymphocytic leukemias
   d. other WBC disorders

9. perform acceptable differentials representing the following disorders:
   a. hypo/micro anemias
   b. macro/normo anemias
   c. normo/normo anemias
   b. random disorders of RBCs or platelets

10. correctly identify 8 out of 10 selected normal and abnormal WBC types on 35mm Kodachrome slides, and accurately perform six abnormal WBC differentials, achieving a minimum score of 77% according to the scoring criteria described in this syllabus.
The grading of hematologic abnormalities is based on the number of cells affected per oil field. The average number of cells per oil field that display the abnormality should be determined by viewing no less than 10 oil fields. In addition to performing the 10 field average, the smear must be scanned to ensure the abnormality is not an isolated artifact of smear preparation or staining. The amount of each abnormality is reported using words or numerical ratings. Descriptive words and their numerical equivalencies are listed in the table below.

**Grading Remarks and Numeric Equivalents:**

<table>
<thead>
<tr>
<th>Remark</th>
<th>Numeric Equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Rare, Trace</td>
<td>0 to 1+</td>
</tr>
<tr>
<td>Occasional, Few, Slight, Mild</td>
<td>1+</td>
</tr>
<tr>
<td>Mild to Moderate</td>
<td>1+ to 2+</td>
</tr>
<tr>
<td>Moderate</td>
<td>2+</td>
</tr>
<tr>
<td>Moderate to Marked</td>
<td>3+</td>
</tr>
<tr>
<td>Marked</td>
<td>4+</td>
</tr>
</tbody>
</table>

**RBC Abnormalities**

**Anisocytosis** *(variation in size)*

<table>
<thead>
<tr>
<th># Cells/Oil Fields</th>
<th>Report As:</th>
<th>RDW Should Be:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 5</td>
<td>Normal</td>
<td>10 - 15 %</td>
</tr>
<tr>
<td>6 - 15</td>
<td>Slight</td>
<td>15 - 17 %</td>
</tr>
<tr>
<td>16 - 30</td>
<td>Moderate</td>
<td>17 - 19 %</td>
</tr>
<tr>
<td>&gt; 30</td>
<td>Marked</td>
<td>&gt; 19 %</td>
</tr>
</tbody>
</table>

**Macrocytosis** *(largeness of size)*

<table>
<thead>
<tr>
<th># Cells/Oil Fields</th>
<th>Report As:</th>
<th>MCV Should Be:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 5</td>
<td>Normal</td>
<td>80 - 95 u³</td>
</tr>
<tr>
<td>6 - 15</td>
<td>Slight</td>
<td>98 - 108 u³</td>
</tr>
<tr>
<td>16 - 30</td>
<td>moderate</td>
<td>109 - 120 u³</td>
</tr>
<tr>
<td>&gt; 30</td>
<td>Marked</td>
<td>&gt; 120 u³</td>
</tr>
</tbody>
</table>

**Microcytosis** *(smallness of size)*

<table>
<thead>
<tr>
<th># Cells/Oil Fields</th>
<th>Report As:</th>
<th>MCV Should Be:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 5</td>
<td>Normal</td>
<td>80 - 85 u³</td>
</tr>
<tr>
<td>6 - 15</td>
<td>Slight</td>
<td>76 - 80 u³</td>
</tr>
<tr>
<td>16 - 30</td>
<td>Moderate</td>
<td>66 - 75 u³</td>
</tr>
<tr>
<td>&gt; 30</td>
<td>Marked</td>
<td>&lt; 65 u³</td>
</tr>
</tbody>
</table>
Hypochromasia (paleness of the RBC beyond center 1/3 of cell)

<table>
<thead>
<tr>
<th># Cells/Oil Fields</th>
<th>Report As:</th>
<th>MCHC Should Be:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 5</td>
<td>Normal</td>
<td>32 - 36 %</td>
</tr>
<tr>
<td>6 - 15</td>
<td>Slight</td>
<td>30 - 32 %</td>
</tr>
<tr>
<td>16 - 30</td>
<td>moderate</td>
<td>29 - 30 %</td>
</tr>
<tr>
<td>&gt; 30</td>
<td>Marked</td>
<td>&gt; 29 %</td>
</tr>
</tbody>
</table>

Poikilocytosis (variation in shape)

<table>
<thead>
<tr>
<th># Cells/Oil Fields</th>
<th>Report As:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 1</td>
<td>Normal</td>
</tr>
<tr>
<td>2 - 8</td>
<td>Slight</td>
</tr>
<tr>
<td>9 - 20</td>
<td>moderate</td>
</tr>
<tr>
<td>&gt; 20</td>
<td>Marked</td>
</tr>
</tbody>
</table>

RBC Inclusion Howell Jolly Bodies, Basophilic Stippling, Cabot’s Rings, Pappenheimer Bodies, Siderotic Granules

<table>
<thead>
<tr>
<th># Cells/Oil Fields</th>
<th>Report As:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>1 - 2</td>
<td>Slight</td>
</tr>
<tr>
<td>3 - 6</td>
<td>moderate</td>
</tr>
<tr>
<td>&gt; 6</td>
<td>Marked</td>
</tr>
</tbody>
</table>

Polychromasia (variation in color within the RBC population present)

<table>
<thead>
<tr>
<th># Cells/Oil Fields</th>
<th>Report As:</th>
<th>Expect Count:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 2</td>
<td>Normal</td>
<td>0 - 2 %</td>
</tr>
<tr>
<td>2 - 3</td>
<td>Slight</td>
<td>2 - 4 %</td>
</tr>
<tr>
<td>4 - 5</td>
<td>moderate</td>
<td>4 - 6 %</td>
</tr>
<tr>
<td>&gt; 6</td>
<td>Marked</td>
<td>&gt; 6 %</td>
</tr>
</tbody>
</table>

Nucleated RBCs

- Count separately from 100 WBCs while doing the differential.
- Must correct the WBC count if > 5 nRBCs/100 Cell Diff.
WBC Abnormalities

Toxic Changes in Neutrophils (Toxic granulation, Dohle Bodies, and Hypervacuolization)

1+ (slight)  Dark granulation in neutrophils that is excessive but not heavy

2-3+ (moderate)  Heavy granulation or Dohle Bodies or both

4+ (marked)  Presence of vacuoles. Dohle Bodies or heavy granulation may be present. Each toxic manifestation may be reported.

Hypersegmentation (≥ 6 lobes/nucleus)

20 - 25 % of neutrophils  =  slight

26 - 35 % of neutrophils  =  moderate

> 35 % of neutrophils  =  marked

Atypical Lymphocytes

- Record separately from typical lymphocytes and report as separate cell.
  (30 lymphs, 10 atypical lymphs)  *(This is done at LCCC.)*

OR other protocols:

- Record as a percentage of total lymphocytes seen on the diff:
  (25% of lymphocytes are atypical)

  OR

- Record as a fraction of the total lymphs seen on the diff:
  (10/40 lymphocytes atypical)

Other WBC/Platelet Abnormalities

- Smudge cells (if > 10/diff estimate few/mod/many and report

- Giant platelets (report if supported by MPV)
Estimating the Total WBC Count from the Smear

1. Count number of WBCs/4 high dry fields.
2. Divide by 4 to get #WBCs/1 high dry field.
3. Multiply by 100.
4. Should APPROXIMATE the automated count within 10%.

Estimating the Platelet Count from the Smear

1. Count the number of PLTS in 10 oil immersion fields.
2. Divide by 10 to get the average PLTS/1 oil field.
3. Multiply by 20,000.
4. Should APPROXIMATE the automated count within 10%.

Reporting Platelet Estimates

<table>
<thead>
<tr>
<th>AVG #/Oil Field</th>
<th>Report As:</th>
<th>Automated PLT Should Be:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 2</td>
<td>marked decrease</td>
<td>0 - 40,000/mm³</td>
</tr>
<tr>
<td>2 – 4</td>
<td>moderate decrease</td>
<td>40,000 - 80,000/mm³</td>
</tr>
<tr>
<td>4 – 7</td>
<td>slight decrease</td>
<td>80,000 - 140,000/mm³</td>
</tr>
<tr>
<td>7 – 20</td>
<td>normal or adequate</td>
<td>140,000 - 400,000/mm³</td>
</tr>
<tr>
<td>20 – 30</td>
<td>slight increase</td>
<td>400,000 - 600,000/mm³</td>
</tr>
<tr>
<td>30 – 40</td>
<td>Moderate increase</td>
<td>600,000 - 800,000/mm³</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>Marked increase</td>
<td>&gt; 800,000/mm³</td>
</tr>
</tbody>
</table>
CLSC 134 COLLEGE LABORATORY

ROTATION F: BODY FLUID CELL COUNTS
BODY FLUID CELL COUNTS ROTATION (ROTATION F) (1 week)

During this rotation, students will perform WBC and RBC cell counts on non-blood body fluids provided, including a WBC differential if necessary based on the total WBC count.

**BODY FLUID CELL COUNT ROTATION: ASSIGNMENTS**

- Perform one (1) WBC and RBC cell count using a hemocytometer, with manual WBC differential if necessary, on a cerebrospinal fluid specimen.

- Perform one (1) WBC and RBC cell count using a hemocytometer, with manual WBC differential if necessary, on a serous body fluid (i.e., synovial fluid, peritoneal fluid, etc.).

- Perform the necessary manual calculations to correctly report RBC and WBC counts on the body fluids counted, using whatever dilution techniques and counting techniques necessary as determined by the nature of the fluid.

**BODY FLUID CELL COUNT ROTATION: OBJECTIVES**

By the end of this rotation, the student should be able to:

1. explain the principle of and accurately perform the procedure for WBC and RBC cell counts on various body fluids.

2. explain when it is necessary to perform a WBC differential on body fluid counts, and accurately perform this procedure on the sample fluids provided.

3. identify the most important indication for collection of cerebrospinal fluid, briefly describe the procedure for collection, and indicate the correct tube specimen for each department in the laboratory.

4. identify the cellular elements normally found in CSF, and identify their normal reference ranges.

5. suggest laboratory findings that would distinguish bacterial meningitis from viral meningitis.

6. define synovial fluid, describing its normal appearance and cell count results.

7. describe synovial fluid findings in cases of infectious arthritis.

8. define peritoneal, pericardial, and pleural fluid.

9. explain the clinical significance of cell count results for the fluids listed in objective #8.
BODY FLUID CELL COUNT ROTATION: PROCEDURES

WBC AND RBC CELL COUNTS ON BODY FLUIDS

PRINCIPLE:

The manual enumeration of blood cells in a body fluid is accomplished by use of a hemocytometer. The numbers and types of RBCs, WBCs, and other cells seen in a body fluid provides significant information for the diagnosis and treatment of various illnesses, including bacterial and viral meningitis, various types of arthritis, cardiac disorders, and pneumonia and other pulmonary conditions. Depending on the type and character of the fluid, cells can be counted in an undiluted specimen, or if necessary, diluted with diluting fluid to allow easier differentiation and counting. WBC differentials on smears prepared from concentrated fluids are normally performed on body fluid specimens if more than 10 WBCs per cubic millimeter are observed during the cell count procedure.

Procedure for Clear Body Fluids:

1. Body fluids that are clear usually do not require a dilution for counting. Mix the fluid well before proceeding.

2. Using a glass Pasteur pipette or microhematocrit capillary tube, carefully plate the undiluted fluid onto one side of a properly cleaned Neubauer hemocytometer. Allow to stand undisturbed for 10 minutes to allow the cells to settle in the chamber.

3. Examine the fluid under low (10x) power. If the cells are not overlapping at all, the cell count can be performed on the undiluted specimen. Proceed to load the fluid into the 2nd side of the hemocytometer and allow to settle.

4. If 10 or more cells of a given type (RBCs or WBCs) are seen in each large “W” square in the chamber, a valid count can be obtained by counting 5 “W” squares on each side of the hemocytometer. If less than 10 cells are seen in each “W” square, all 9 “W” squares on each side of the hemocytometer should be counted.

5. Count the cells seen in the appropriate number of “W” squares, differentiating between WBCs and RBCs. RBCs should also be tabulated into two groups as they are counted, those with crenated membranes, and those with normal membranes (“fresh” RBCs). The diagrams below attempt to illustrate the different appearances RBCs and WBCs will have in an unstained fluid.

Diagram here! P. 220 Ross

6. After counting the WBCs and RBCs in the appropriate squares, refer to the Calculations portion of this procedure to obtain final cell count results.
**Procedure for Cloudy Body Fluids:**

1. Body fluids that are visibly cloudy, or where the cells overlap upon initial examination in the previous procedure, require dilution with a diluting fluid before counting cells in a hemocytometer.

2. For *slightly cloudy fluids*, a 1:10 dilution of the fluid with isotonic saline is usually sufficient. This may be obtained by pipetting 1.0ml of saline using a volumetric pipette into a 12x75 glass tube. Then use a 100µl semiautomated pipette to withdraw 0.1ml of saline and discard. Using a clean tip, pipette 0.1ml of fluid into the remaining saline and mix thoroughly.

3. For *moderately cloudy fluids*, a 1:20 dilution with isotonic saline is usually sufficient. This may be obtained by pipetting 1.0ml of saline using a volumetric pipette into a 12x75 glass tube. Then use a 50µl semiautomated pipette to withdraw 0.05ml of saline and discard. Using a clean tip, pipette 0.05ml of fluid into the remaining saline and mix thoroughly.

4. For *extremely cloudy*, a 1:100 dilution with isotonic saline may be required. This may be obtained by pipetting 1.0ml of saline using a volumetric pipette into a 12x75 glass tube. Then use a 10µl semiautomated pipette to withdraw 0.01ml of saline and discard. Using a clean tip, pipette 0.01ml of fluid into the remaining saline and mix thoroughly.

5. Once the fluid has been diluted, the cell count is performed following the previous procedures for counting clear fluids.

6. Refer to the *Calculations* portion of this procedure to obtain final cell count results.

**Procedure for Very Bloody Body Fluids:**

**RBC Count on Bloody Fluids:**

1. For performing an RBC count on an *extremely bloody fluid*, a 1:100 dilution with isotonic saline will be required. This may be obtained by pipetting 1.0ml of saline using a volumetric pipette into a 12x75 glass tube. Then use a 10µl semiautomated pipette to withdraw 0.01ml of saline and discard. Using a clean tip, pipette 0.01ml of fluid into the remaining saline and mix thoroughly.

2. Once the fluid has been diluted, the cell count is performed following the previous procedures for counting clear fluids.

3. Refer to the *Calculations* portion of this procedure to obtain final cell count results.
Procedure for Very Bloody Body Fluids: (continued)

WBC Count on Blood Fluids:

(Glacial Acetic Acid Procedure for removal of RBCs from a body fluid)

1. If a fluid is extremely bloody, it is difficult or impossible to clearly observe the WBCs among the numerous RBCs in the fluid. To resolve this problem, the RBCs are lysed with acid to allow counting of just WBCs according to the following steps:

2. Add four drops of well-mixed fluid to a labeled 12x75mm test tube.

3. Take a plastic transfer pipette and aspirate concentrated glacial acetic acid into the pipette and drain it carefully, wipe the outside completely dry with a Kimwipe, and touch the tip of the pipette to the Kimwipe to remove any excess acid.

   (This is done to coat the inside of the transfer pipette with glacial acetic acid before aspirating your sample in the next step. This step will lyse the RBCs, so that the WBCs will be the only cells left to count.)

4. Place the rinsed glacial acetic acid transfer pipette into the 12x75mm tube and aspirate the 4 drops of specimen into the pipette. Let the specimen sit in the pipette for at least 1 minute.

5. Discharge the acidified specimen back into the 12x75mm tube and discard the plastic transfer pipette into a biohazard container. Carefully load the acidified fluid with a plain (blue ringed) microhematocrit capillary pipette into the hemocytometer. Allow cells to settle for 10 minutes then perform the WBC cell count, counting the appropriate number of “W” squared on each side of the chamber. Refer to the Calculations portion of this procedure to obtain final cell count results.

6. This method is generally useful because the amount of acetic acid present in the tube is not enough to significantly dilute the specimen, but is capable of lysing the RBCs and delineating the nuclear material in the WBCs to aid in the distinction between mononuclear and polynuclear cells. If this distinction is visibly obvious, a WBC differential can be performed as WBCs are counted by tabulating WBCs in these two groups. This may eliminate the need for a stained differential.
**Calculations:**

**Calculation of the Dilution Correction Factor:**

1. Body fluids that have not been diluted do not require a Dilution Correction Factor calculation. Any fluid that has been diluted requires this calculation.

2. Determine the body fluid sample volume and total volume (sample volume + saline volume) used in your dilution procedure.

3. Use these two volumes in the following calculation to determine the Dilution Correction Factor \( x \):

\[
\frac{\text{sample volume}}{\text{total volume}} = \frac{1}{x}
\]

**Example:** If 1.0ml of saline are pipetted into a tube, then 0.1ml (100µL) is removed and discarded, the remaining saline volume is 0.9ml. If 100µL of sample is then added to the saline,

- the sample volume = 0.1ml
- the saline volume = 0.9ml
- the total volume = 1.0ml

Therefore the calculation would be:

\[
\frac{0.1}{1.0} = \frac{1}{x}
\]

\[x = 10\]

The **Dilution Correction Factor** for this example is: **10**
**Final Calculation:**

The final calculation of the WBC and RBC counts is performed using the following formula:

\[
\text{Total # of cells counted} \times \text{Dilution Factor} \times 10 = \# \text{cells per mm}^3 (\mu\text{L})
\]

\[
\# \text{ of large (W) squares counted (mm}^2\text{)}
\]

The percentage (%) of RBCs that are fresh or crenated should also be reported with the RBC count:

If 339 total RBCs are counted on two sides of the chamber, and 120 are fresh and 219 are crenated, the percentages would be calculated as follows:

\[
\frac{120}{339} \times 100 = 35\% \text{ fresh RBCs}
\]

\[
\frac{219}{339} \times 100 = 65\% \text{ crenated RBCs}
\]

**REMEMBER:** Body Fluid cell counts are reported out as whole numbers per mm³, regardless of how big the number is. Scientific notation format (x 10⁶ / mm³, x 10⁵ / mm³, etc.) is used only for cell counts performed on whole blood samples.
**Procedure for WBC differential of Body Fluids**

If there are **more than 10 WBCs seen in a body fluid cell count**, most hospital laboratories perform a modified WBC differential procedure on the specimen. Optimally, a slide is prepared of the body fluid using a cytocentrifuge, yielding a very concentrated WBC smear. Many laboratories do not have access to a cytocentrifuge, so a centrifuge sedimentation method is substituted, and this method yields acceptable smears for Wright's staining. WBCs are usually differentiated only into mononuclear and polynuclear categories and reported out as percentages. The following are the procedural steps.

1. If the volume of fluid exceeds 2ml, pour about 1-2ml of well-mixed fluid into a separate tube. If there is less than 2ml present, use the entire specimen.

2. Centrifuge the specimen at 2500-3000rpm for 10 minutes to concentrate the cellular elements at the bottom of the tube.

3. Remove the tube from the centrifuge and carefully aspirate the fluid into another tube, being careful not to touch the tip of your pipette to the bottom of the tube. Remove the majority of the fluid, leaving about ¼ inch of fluid in the bottom of the tube. In clear fluids with low numbers of cells, there may not be a visible layer of cells at the bottom of the tube, but they are still present, and care must be taken not to touch the bottom of the tube with your pipette while removing the supernatant.

4. Vortex the centrifuged tube for 10-15 seconds to dislodge the cell button and mix the cells into the remaining fluid.

5. With a large drop of the fluid remaining in the tube, prepare two smears with the fluid:

   - Prepare one of the smears using the “wedge” technique employed when preparing whole blood differential smears, holding the spreader slide at a higher angle to make the smear relatively thick.

   - Prepare the second smear by placing a drop of the fluid in the center of the slide and spreading it around in a small circle with the dropper tip, making the film fairly thick.

6. Allow these smears to **air dry very thoroughly**, and then stain the smears by the standard Wright's stain procedure used for whole blood differential smears.

7. Once the smear is stained and thoroughly dry, examine under oil immersion (1000x) and count 100 WBCs, separating into mononuclear and polynuclear categories. Report the differential as % mononuclear and % polynuclear WBCs.
NORMAL REFERENCE RANGES FOR BODY FLUID CELL COUNTS:

<table>
<thead>
<tr>
<th>Type of Fluid</th>
<th>WBCs</th>
<th>RBCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrospinal Fluid</td>
<td>0-5/mm³</td>
<td>0/mm³</td>
</tr>
<tr>
<td>Synovial Fluid</td>
<td>0-150/mm³</td>
<td>0/mm³</td>
</tr>
<tr>
<td>Other serous fluids (exudates &amp; transudates)</td>
<td>0-1000/mm³</td>
<td>0/mm³</td>
</tr>
</tbody>
</table>
CLSC 135 COLLEGE LABORATORY

ROTATION B:  BLOOD BANKING
BLOOD BANK ROTATION (ROTATION B)  
(7 weeks)

During this rotation, the student will practice the basic blood bank procedures, including grouping, typing, antibody screens and identification, crossmatching, and direct antiglobulin testing. The blood bank rotation will last for seven weeks, with specific assigned activities for each week. Students should come to college laboratory prepared (knowing the theory) of the procedures which they will be performing on unknown specimens. Students cannot afford to be absent for any of these sessions, and as the work must be performed sequentially, and the skill development in these procedures is cumulative. In addition, the student specimens require a large amount of the Laboratory Assistant’s time to prepare, and out of respect for her, students are requested to make all attempts to be present and on time for all laboratory sessions.

Each College Laboratory session during the Blood Bank rotation will begin with a group session in which the previous week’s procedures, objectives, and written assignments are reviewed. Then, the current session’s procedures will be explained before the students begin their individual work.

BLOOD BANK ROTATION SESSION ASSIGNED ACTIVITIES AND OBJECTIVES

**BLOOD BANK SESSION #1: ASSIGNED ACTIVITIES**

During this session, the student will be performing ABO/Rh type and Indirect Antibody Screen procedures on two (2) unknown blood samples, including performance of a Weak D on all D-negative samples.

**BLOOD BANK SESSION #1: OBJECTIVES**

Following this laboratory session, the student should be able to:

1. properly prepare a 2-5% red cell suspension in isotonic saline.
2. properly perform and accurately interpret the following tests:
   a. ABO forward grouping
   b. ABO reverse grouping
   c. D typing.
   d. Weak D typing.
3. properly enter serologic data on college laboratory blood bank logsheets, using accepted notations, and writing legibly.
4. discuss the guidelines for estimating the strength of agglutination reactions in vitro and indicate the expected strength of ABO reactions.
5. identify the locations of antigens and antibodies found in test tubes during ABO forward and reverse grouping and D typing procedures.

6. explain the principle of the weak D test.

7. explain what is happening within the test tube during the incubation phase of weak D testing.

8. describe the function of AHG (coombs serum) reagent in the weak D test.

9. explain the purpose of the washing step performed in an AHG-phase procedure, and indicate the effects of inadequate washing.

10. explain why check cells (coombs control cells) are added to negative tests following the AHG phase in weak D testing, listing three specific conclusions supported by obtaining the correct results in this step of testing.

11. properly perform and accurately interpret all steps of the weak D test.

12. complete ABO forward and reverse grouping, and D typing, including the weak D test when indicated, and indirect antibody screen procedures on two (2) specimens with 100% accuracy.

13. state the ABO/Rh type called the "Universal Donor" and explain why.

14. state the ABO/Rh type called the "Universal Recipient" and explain why.

15. demonstrate an understanding of the need for QC in blood banking, and describe the general procedures for QC of blood bank antiserum and reagent cells.

16. given a patient ABO/Rh type, list the expected antigens and antibodies found in that patient's blood specimen.
BLOOD BANK SESSION #1: PROCEDURES

PREPARATION OF A 3-5 % RBC SUSPENSION (CLOT TUBES)

1. Label one 13 x 100 and one 12 x 75 tube with patient information.

2. Using a disposable pipette, carefully transfer the serum into the 13 x 100 pre-labeled tube.
   * Hold the clot tube in a steady manner so that RBCs will not become re-suspended into the serum.
   * Stop transferring serum when no more can be removed from the clot tube without aspirating RBCs.
   * Step #2 (removal of serum from clot) may have already been performed on specimens used in college lab.

3. Using the same transfer pipette or a wooden applicator stick, gently dislodge many RBCs from the clot into the residual serum.
   * A gentle circular stirring motion is preferred.
   * Stop stirring when the residual serum is a deep red color (contains many re-suspended RBCs).

4. Transfer 2 drops of the RBC-laden serum to the 12 x 75 tube.

5. Using a squirt bottle, add isotonic saline to the RBC/serum mixture until the tube is 3/4 full.
   * The saline stream must be added with enough force to evenly suspend the RBCs into the saline. If the suspension is uneven, it will be necessary to repeat this washing step after centrifugation.
   * Never allow the top of the squirt bottle to enter the test tube. This may allow RBCs to contaminate the saline affecting all subsequent tubes.
   * If small pieces of the clot are noted at the bottom of the tube after saline addition, remove them with a wooden applicator stick.

6. Centrifuge the RBC suspension at high speed for 1 minute.
   * If excessive vibration is noted, shut off the centrifuge immediately and recheck both balancing and rotor engagement with the shaft.
   * The centrifuge must be correctly balanced and lid latched.
   * The centrifuge rotor must be fully engaged with the shaft. (Green line shows on Dade Immufuge shaft.)
7. Remove the tube from the centrifuge after the rotor has decelerated to a complete stop.
   * Never open the lid until the rotor has stopped turning.
   * Never attempt to use objects or fingers as a brake to stop the centrifuge more quickly.

8. Discard the supernatant saline by completely inverting the tube over a waste beaker or sink. You do not need to use a pipette to remove supernatant saline. Inversion is effective if performed deliberately and quickly.
   * The RBCs will remain packed onto the bottom of the tube.
   * Do not shake the inverted tube.
   * RBCs will be lost if the tube is inverted more than once.

9. After decanting, return the tube to its normal upright position and gently shake the tube until all cells are re-suspended in the small amount of residual saline.

10. Using the squirt bottle, forcefully add saline to the cells until the tube is approximately 1/4 to 1/3 full.
    * Saline addition must be forceful enough to mix the cells into the saline to result in a uniform suspension. If unsuccessful, it is necessary to re-centrifuge, decant and add saline again.

11. Adjust the strength of the suspension to equal that of a known 3-5% suspension of RBCs, such as a well-mixed bottle of reagent cells.
    * Add additional saline if too red, or re-centrifuge to remove saline if too pale.
PREPARATION OF A 3-5 % RBC SUSPENSION (EDTA TUBES)

1. Label one 13 x 100 and one 12 x 75 tube with patient information.

2. Using a disposable pipette, carefully transfer the plasma into the 13 x 100 pre-labeled tube.
   * Hold the tube in a steady manner to prevent re-mixing of the cells into the plasma layer.
   * Stop transferring plasma when no more can be removed from the clot tube without aspirating RBCs.
   * Step #2 (removal of plasma layer) may have already been performed on specimens used in college lab.

3. Using the same transfer pipette, deliver 1 drop of RBCs from the specimen into the pre-labeled 12 x 75 tube.
   * Mixed EDTA blood that has not been separated is also acceptable for this procedure; 1-2 drops will provide sufficient cells for the suspension.

4. Using a squirt bottle, add isotonic saline to the RBC/serum mixture until the tube is 3/4 full.
   * The saline stream must be added with enough force to evenly suspend the RBCs into the saline. If the suspension is uneven, it will be necessary to repeat this washing step after centrifugation.
   * Never allow the top of the squirt bottle to enter the test tube. This may allow RBCs to contaminate the saline affecting all subsequent tubes.

5. Centrifuge the RBC suspension at high speed for 1 minute.
   * If excessive vibration is noted, shut off the centrifuge immediately and recheck both balancing and rotor engagement with the shaft.
   * The centrifuge must be correctly balanced and lid latched.
   * The centrifuge rotor must be fully engaged with the shaft. (Green line shows on Dade Immufuge shaft.)

6. Remove the tube from the centrifuge after the rotor has decelerated to a complete stop.
   * Never open the lid until the rotor has stopped turning.
   * Never attempt to use objects or fingers as a brake to stop the centrifuge more quickly.
7. Discard the supernatant saline by completely inverting the tube over a waste beaker or sink. **You do not need to use a pipette to remove supernatant saline. Inversion is effective if performed deliberately and quickly.**

   * The RBCs will remain packed onto the bottom of the tube.
   * Do not shake the inverted tube.
   * RBCs will be lost if the tube is inverted more than once.

8. After decanting, return the tube to its normal upright position and gently shake the tube until all cells are re-suspended in the small amount of residual saline.

9. Using the squirt bottle, forcefully add saline to the cells until the tube is approximately 1/4 to 1/3 full.

   * Saline addition must be forceful enough to mix the cells into the saline to result in a uniform suspension. If unsuccessful, it is necessary to re-centrifuge, decant and add saline again.

10. Adjust the strength of the suspension to equal that of a known 3-5% suspension of RBCs, such as a well-mixed bottle of reagent cells.

    * Add additional saline if too red, or re-centrifuge to remove saline if too pale.
ABO GROUPING PROCEDURE

PRINCIPLE:

*Forward:* Red blood cells have antigens in their cell membranes. These antigens react with commercially obtained antiserum (antibodies) to produce visible agglutination. Agglutination, or lack of such, with Anti-A or Anti-B antisera will determine the ABO antigenic group.

*Reverse:* Naturally occurring antibodies Anti-A and Anti-B are present in the serum of those lacking the A and B antigens on their red blood cell membranes. Serum, which may or may not contain either or both of these antibodies, is reacted with reagent A cells and reagent B cells. Agglutination patterns should confirm the results of the forward grouping.

*Forward and Reverse Grouping Procedure:*

1. Prepare a 3-5% saline suspension of the red blood cells to be tested, according to the procedures on pp. 108-111.

2. For the Forward Grouping test, label two 12 x 75 mm tubes as A and B.

3. For the Reverse Grouping test, label two tubes as $A_r$ and $B_r$.

4. Add 1 drop of Anti-A (blue) to the A tube.

5. Add 1 drop of Anti-B (yellow) to the B tube.

6. Add 1 drop of re-suspended reagent A1 cells to the $A_r$ tube.

7. Add 1 drop of re-suspended reagent B cells to the $B_r$ tube.

8. Add 1 drop of the red cell suspension to each of the Forward Grouping tubes.

9. Add 2 drops of patient serum or plasma to each of the Reverse Grouping tubes.

10. Gently shake to mix and centrifuge on High speed for 15 seconds.

11. Gently tilt the tubes, observing for agglutination or hemolysis.

12. Record the strength of agglutination in each tube.

Guidelines:

- $4+$ solid aggregate, clear background
- $3+$ large clumps, clear background
- $2+$ very small clumps, pink background
- $1+$ very tiny clumps, pink background
- $\pm$ granular appearance, pink background
- $0$ no agglutination
13. Determine ABO Group of the patient according to the following guidelines:

<table>
<thead>
<tr>
<th>ABO Group</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>A1 Cells</th>
<th>B Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4+</td>
<td>0</td>
<td>0</td>
<td>4+</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
<td>0</td>
</tr>
<tr>
<td>AB</td>
<td>4+</td>
<td>4+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O</td>
<td>0</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
</tr>
</tbody>
</table>

**Expected Results:**

<table>
<thead>
<tr>
<th>Expected Results:</th>
<th>Forward Grouping</th>
<th>Reverse Grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient cells plus</td>
<td>Anti-A</td>
<td>A1 Cells</td>
</tr>
<tr>
<td>Patient serum plus</td>
<td>Anti-B</td>
<td>B Cells</td>
</tr>
</tbody>
</table>

**Technical Errors**

**Forward Groupings**

1. False positives
   a. over-centrifugation
   b. wrong antiserum used
   c. wrong specimen used

2. False negatives
   a. weak or strong cell suspensions used
   b. antiserum not added or wrong ones were used
   c. under-centrifugation
   d. shaking too hard when tilting tube to view agglutination
   e. wrong specimen used
   f. failure to record hemolysis

**Reverse Groupings**

1. False positives
   a. over-centrifugation
   b. wrong serum used
   c. wrong reagent cells used

2. False Negatives
   a. failure to remove patient’s RBCs from serum or plasma
   b. failure to add serum or plasma
   c. hemolyzed serum used
   d. under-centrifugation
   e. improper reagent cells added
   f. improper re-suspension of reagent cells
   g. excessive shaking when reading results
   h. failure to record hemolysis

References:

RH TYPING PROCEDURE

PRINCIPLE:

Rh (D) antigens present on the red blood cell membrane are reacted with commercially available antisera, containing antibodies to the D antigen. Agglutination results if the RBC membrane has the D antigen and should not occur if this antigen is lacking. (Presence of the D antigen is called Rh+.)

Older formulas of Anti-D antiserum were composed of Anti-D antibodies suspended in an albumin-protein diluent. When using such protein based antisera, one should run an Rh control (containing only protein diluent) to be sure agglutination, if present, is due to the interaction of Anti-D and D antigens on the red blood cell, rather than caused by the protein diluent.

More recent formulas for Anti-D are saline based or blended formulas and an Rh control tube is only necessary for patient samples exhibiting ABO/Rh forward typing results of AB Positive. In these cases, a control tube is still necessary to rule out possible non-specific agglutination of patient cells.

Consult with the instructor to determine which type of Rh typing serum is in use in the College Laboratory.

Procedure:

1. Label one tube for each Rh typing to be performed as D.
2. Add 1 drop of Anti-D antisera to the “D” tube.
3. Add 1 drop of patient cells (3-5% suspension) to the tube.
4. Mix gently, centrifuge, and observe for agglutination and/or hemolysis.
5. Record strength of reactions (if any).
6. If the patient’s ABO/Rh type is AB pos, a reagent control must be run to rule out non-specific agglutination between the patient RBCs and reagent diluent:

   Label a tube “C” for Control. Add 1 drop of reagent control and 1 drop of patient RBC suspension. Centrifuge and examine for agglutination or hemolysis.
Interpretation:

Agglutination with anti-D indicates the patient to be positive for the D antigen (Rh positive).

If a reagent control is run, it must be negative. Agglutination in the control tube indicates the possibility of non-specific agglutination between the patient RBCs and reagent diluent. The ABO and Rh results are considered invalid until further testing is performed.

NOTE:  Rh negative typings can be further tested for the presence of weak D antigens using the “Weak D Procedure” found on the next page, if indicated by the procedures of the institution. In College Lab, ALL Rh negatives will be tested for weak D.

References:

WEAK D TEST PROCEDURE

PRINCIPLE:

Red blood cells having D antigens on their membranes occasionally fail to demonstrate visible agglutination when combined with Anti-D antisera. This is due to presence of weak variants of the D antigen, referred to as weak D (formerly “Du”), on the red cell membrane. In the weak D test, Anti-D is allowed to attach to weak D antigenic sites during a warm incubation, after which visible agglutination may result. Additional enhancement may be provided by the indirect antiglobulin test.

Procedure:

1. Retain the tube used in the Rh typing procedure, which was labeled “D.” In addition, set up a reagent control tube labeled “DC,” containing 1 drop of reagent control and 1 drop of patient RBCs. A control tube must always be run when testing for weak D.

2. Place these tubes in a 37°C heat block or water bath for 15-30 minutes.

3. Centrifuge on high speed 15 seconds and observe for agglutination and/or hemolysis.

4. Wash cells 4 times with large volumes of saline. Saline must be added forcefully enough to re-suspend the cell button at the bottom of the tube. Spin all washes at least 1 minute (high speed) or excessive cell losses during decantation will result.

5. After removing the fourth wash from the centrifuge, decant by inversion and blot the rim of each test tube with a tissue to remove residual saline. Shake the tube to re-suspend the cell button.

6. Add 1 drop of Anti-Human Globulin (AHG) serum to each tube.

7. Mix gently, centrifuge for 15 seconds (high speed), and observe for agglutination and/or hemolysis. (Microscopic exam is encouraged for all negatives.) Positive reactions indicate weak D antigens are present on the membranes of the cells.

8. Add 1 drop of Antiglobulin Control Cells (“Check Cells”) to all negative tubes and mix gently. **(Do NOT add to any positive tubes!)**

9. Re-spin 15 seconds (high speed) and examine for agglutination.

10. ALL tubes should show agglutination. This indicates the following:
    a. Anti-Human Globulin was added.
    b. Anti-Human Globulin was functional.
    c. The washing technique was adequate.
    d. The patient is truly weak D negative and therefore Rh negative.

11. If no agglutination is observed following the addition of Check Cells, re-check the reagents used for expiration and repeat the procedure. The most probable reason is inactivation of Anti-Human Globulin by inadequate washing, which does not completely remove reagent Anti-D from the tube.
Expected Results:

All weak D positive bloods are considered as “Rh positive.”
All weak D negative bloods are considered as “Rh negative.”

Unexpected Results:

A positive reaction in the reagent control tube indicates non-specific reactivity between the patient’s RBCs and the reagent diluent, probably caused by the presence of a positive DAT. Further investigation is necessary before the ABO and Rh of this patient can be reported out.

References:

INDIRECT ANTIGLOBULIN TEST PROCEDURE (IDAT)
(Used in Antibody Screen, Antibody Panel, Extended Crossmatch, and Elution Testing)

PRINCIPLE:

Antibodies present in the serum, plasma, or eluate are added to reagent or test cells of known (screens and panels) or unknown (crossmatches) antigenic makeup. This mixture is allowed to incubate in conditions which favor the attachment of antibody to RBC antigens (thermal or coating phase). Excess or unbound antibodies are removed during the washing phase, allowing for agglutination of the antibody coated RBCs by addition of anti-human globulin reagent.

Antiglobulin check cells are antibody-coated RBCs which are added to all negative reactions. They should cause negative reactions to become positive and validate the results accordingly.

Procedure:

1. Label 12 x 75 tubes for screening cells, panel cells, or donor cells (usually I, II, and III for screens, or 1, 2, 3, ...for panels). When performing an Antibody Screen, include a tube for auto control, labeled as AC, to detect the presence of autoantibodies.

2. To each tube, add 2 drops of the serum to be tested for antibodies.

3. If performing an Antibody Screen or Panel, re-suspend each vial of reagent red blood cells by gentle inversion and add 1 drop to each appropriately labeled tube.

   If performing a Major Crossmatch, add 1 drop of 3-5% suspension of donor RBCs to the appropriately labeled tube.

4. Gently mix the tubes, centrifuge for 15 seconds (high speed), observe for agglutination or hemolysis. (Room temp. phase: optional)

5. Add 2 drops of GAMMA N-Hance Low Ionic Strength Saline (LISS) or PEG to each tube. Incubate at 37°C for 10 minutes. 22% Bovine Albumin may be used in place of N-Hance, but should be incubated 30 minutes. All of these reagents reduce the zeta potential of the mixture and are referred to as “potentiators.”

6. Following incubation at 37°C (thermal phase), spin and read again, recording the strength of all positive reactions, unless using PEG. Tests using PEG as a potentiator are not spun and read at this phase.

7. Fill all tubes with isotonic saline. A squirt bottle should be used forcefully enough to evenly re-suspend all cells in the saline, filling the tube at least 3/4 full.

8. Centrifuge 1 minute on high speed, decant by inversion, gently re-suspend the “cell button” in residual saline, and repeat step #7 for a total of 4 washes.

9. Following the last washing, blot the rims of the test tubes being decanted to remove the majority of residual saline. (This will dilute the AHG reagent.)
10. Add 1 drop AHG reagent to each tube.

11. Mix gently, spin 15 seconds (high speed), and read for agglutination and/or hemolysis. All negative tubes should be examined microscopically.

12. To all negative tubes, add 1 drop of check cells, mix, spin 15 seconds (high speed) and read. They should become positive for agglutination.

This indicates:
   a. AHG was added correctly to all tubes.
   b. the AHG reagent added is functional.
   c. the washing procedure was adequate.

13. Repeat all tests in which the check cells fail to cause the negatives to agglutinate, taking special care to use appropriate reagents and wash correctly.

**Expected Results:**
Most serums tested do not contain atypical antibodies and therefore will not cause agglutination in the IDAT. Positive reactions by IDAT with screening cells I, II, and III indicates the presence of an atypical antibody, which can then be identified using panel cells. Positive Auto Control tubes indicate the presence of an autoantibody.

**False Negative IDATs:**

1. Improper storage of serum, cells, and AHG.
2. Failure to add patient's serum.
3. Improper incubation temperature or time.
4. Less than optimal ratios of serum to cells.
5. Inadequate washing.
6. Elution of antibodies due to washing phase delays.
8. Failure to add AHG reagents.
10. Failure to check negatives microscopically.

**False Positive IDATs:**

1. Agglutination of RBCs prior to washing (cold agglutinins).
2. Improper storage of saline (glass and metal).
3. Dirty glassware.
4. Over-centrifugation.
5. Direct coombs positive cells present in AC tube.

**References:**
**BLOOD BANK SESSION #2: ACTIVITIES**

During this session, the student will perform two (2) ABO and Rh typings and 2 Indirect Antibody Screens, using the method in use at their clinical site (LISS N-Hance Low Ionic Strength Saline additive or PEG Polyethylene Glycol additive), resolving any serological discrepancies encountered in their ABO testing.

When their sample testing is complete, students will receive **group instruction on the identification of antibodies** using antibody identification panels. Sample antibody panel case studies will be used to demonstrate the interpretation of antibody ID panels.

**BLOOD BANK SESSIONS # 3, 4, & 5: ACTIVITIES**

During these sessions, the student will perform two (2) ABO and Rh typings, 2 Indirect Antibody Screens, and Crossmatches on each specimen for 2 units of compatible donor blood, including antibody identification panels when necessary. This testing will be performed using one of the following three procedures:

a. Indirect Antiglobulin procedure using N-Hance Low Ionic Strength Saline additive

b. Indirect Antiglobulin procedure using PEG (Polyethylene Glycol) additive

c. Indirect Antiglobulin procedure using the Ortho ID MicroTyping System Gel Test (Ortho ID-MTS).

Students will rotate through these three methods over the three week period so that experience is gained performing testing with all methods. This rotational approach will be continued through the assigned activities during Weeks #6-7, so that ultimately, a student will have at least two opportunities to use the Ortho ID-MTS system. While on a method, they will use that method to identify any antibodies identified in the screening procedure.
BLOOD BANK SESSIONS #2, 3, 4, & 5:  OBJECTIVES

Gamma N-Hance LISS / PolyEthyleneGlycol Objectives:

Following this session, the student should be able to:

1. describe the general principle of an IDAT test.

2. explain the basic test principle employed when performing IDAT testing using LISS and PEG, including the differences between the two procedures.

3. describe each step of Anti-Human Globulin testing listed below, and explain the significance of the steps:
   a. immediate spin phase
   c. incubation phase
   d. washing step
   d. antihuman globulin phase
   e. addition of check cells

4. describe the actual contents of Trio Screening Cells, Panel Cells, and Check Cells.

5. explain what information is provided by comparing Indirect Antiglobulin Screening (IAS) serologic results with screening cell package insert "antigrams."

6. determine when it is appropriate to perform "panels."

7. perform and interpret procedures on unknown samples provided, including Indirect Antibody Screens, and Antibody Identification Panels using LISS and PEG procedures.

8. explain the most common causes of ABO discrepancies in ABO forward and reverse grouping procedures, and describe how these are resolved using further serological testing.

9. explain the meaning of the phrase a “subgroup of A”.

10. describe the contents of anti-A\textsubscript{1} lectin and A\textsubscript{2} cell reagents.

11. explain the principle of ABO discrepancy resolution using anti-A\textsubscript{1} lectin and A\textsubscript{2} cell reagents, including examples of when these tests are appropriate, and ABO discrepancies which are not appropriately resolved using these reagents.

12. resolve ABO grouping discrepancies found in patient samples assigned by proper performance of serologic testing.

13. explain how the serologic data obtained in various samples demonstrating ABO discrepancies resolves the discrepancy seen.

14. explain the principle of each phase of the major crossmatch procedure and describe instances when each of the phases is appropriate to perform.
15. perform major crossmatches using specimens provided, through the appropriate phases.

16. resolve any discrepancies / incompatibilities detected in testing, and summarize and explain the serological findings in an acceptable written format.

17. demonstrate an increased proficiency in washing, grading reactions, and other steps in AHG methods.

18. complete ABO, Rh, Indirect Antibody Screen, and crossmatch testing for two donor units on a minimum of two (2) samples each session, including Antibody Identification Panels if indicated, using the procedure assigned, with 100% accuracy.

**Ortho ID-MTS Gel Test System Objectives:**

Following this session, the student should be able to:

1. describe the principle of the Ortho ID-MTS Gel Test system.

2. briefly describe each step of the Type and Screen using the ID-MTS system.

3. note the difference between the RBC reagents used for traditional tube methods versus those used for the ID-MTS system.

4. describe the appearances of a 4+, 3+, 2+, 1+, and negative reactions when interpreting results from the ID-MTS gel test system.

5. identify several advantages the ID-MTS gel system has over traditional tube methods for blood bank procedures.

6. perform and interpret procedures on unknown samples provided, including Antibody Screens, Antibody Identification Panels, and donor crossmatching procedures, using the Ortho ID-MTS Gel Test system.

7. resolve any discrepancies / incompatibilities detected in testing, and summarize and explain the serological findings in an acceptable written format.

8. demonstrate an increased proficiency in washing, grading reactions, and other steps in AHG methods.

9. complete ABO, Rh, Indirect Antibody Screen, and crossmatch testing for two donor units on a minimum of two (2) samples each session, including Antibody Identification Panels if indicated, using the procedure assigned, with 100% accuracy.
RESOLVING DISCREPANCIES IN ABO TYPING PROCEDURES

FIRST: Determine ABO Group of the patient according to the following guidelines:

<table>
<thead>
<tr>
<th>ABO Group</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>A1 Cells</th>
<th>B Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4+</td>
<td>0</td>
<td>0</td>
<td>4+</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
<td>0</td>
</tr>
<tr>
<td>AB</td>
<td>4+</td>
<td>4+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O</td>
<td>0</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
</tr>
</tbody>
</table>

Many conditions occur which cause discrepancy between the forward and reverse ABO grouping results. These results are unexpected, and must be resolved by additional testing. Among the causes for such grouping discrepancies are previous transfusion, pregnancy, patient conditions that result in missing or weak antigens or antibodies, and subgroups of antigens.

COMMON SEROLOGICAL PROCEDURES FOR RESOLUTION OF DISCREPANCIES OBSERVED IN ABO FORWARD / REVERSE GROUPING RESULTS

1. Examine the patient’s forward and reverse grouping results for correlation, as see in the table above.

2. If there is a discrepancy in the results, circle the mismatched results on your worksheet.

3. Begin to consider possible causes of the discrepancy, recalling the fact that most discrepancies arise from situations involving lack of or unexpected serum antibodies in the reverse results.
A. Lack of serum antibody activity in reverse grouping results:

1) This will be observed as a negative or only weakly positive reverse grouping result where a strong positive is expected to correspond with a negative forward grouping result.

(For example, a forward group O with negative results seen in either reverse grouping tube, or a forward group A with a negative reverse result with B cells.)

2) The missing antibody can often be strengthened and demonstrated by enhancing the reaction mixture. This can be accomplished by either adding 2 – 3 more drops of serum to the tube, and/or incubating the tube at room temperature for 15-30 minutes.

B. Unexpected Anti-A1 antibody activity in reverse grouping results:

1) This will be observed as a strongly positive reverse grouping result in the A1 cells where a negative positive is expected to correspond with a positive forward grouping result.

2) The most likely explanation for this discrepancy is a patient belonging to a non-A1 subgroup forming an anti-A1 antibody.

3) This can be demonstrated by testing 1 drop of patient's RBC suspension with 1 drop of anti-A1 lectin. A negative result verifies that the patient belongs to a non-A1 subgroup (most likely A2), thus confirming the explanation of the discrepancy.

4) The result can further be supported by testing 2 drops of patient serum with 1 drop of reagent A2 RBCs. A negative result in this tube demonstrates that the antibody being detected is a specific anti-A1 antibody and not a non-specific, possibly cold-reacting, antibody.
MAJOR CROSSMATCH PROCEDURE  
(COMPATIBILITY TESTING)

PRINCIPLE:

Donor Red Blood Cell antigens are combined with recipient serum, which may contain antibodies. If the recipient’s serum contains an antibody which is specific for the donor’s RBC antigens, coating of the RBCs will occur. The steps in this procedure essentially follow the IDAT procedure (previous pages), using donor RBCs with patient serum.

More recently, blood banks are performing only the immediate spin (room temp.) phase of this test, provided the antibody screening of the recipient’s serum is negative for unexpected (atypical) antibodies. This protocol will also be acceptable for college lab testing.

Procedure:

1. Perform an IDAT test on the recipient’s serum concurrently with this procedure. (See previous procedure)

2. Label one tube with the donor segment number for each unit to be crossmatched.

3. Prepare a 3-5% suspension of donor’s RBCs form a blood bag segment, which have been washed at least once to remove anticoagulants and plasma.

4. Place 2 drops of recipient's serum into each crossmatch tube.

5. Add 1 drop of the 3-5% donor’s RBCs to the appropriately labeled tube which contains recipient serum.

6. Mix gently, spin 15 seconds (high speed), read for agglutination and/or hemolysis.

7. If positive reactions occur, verify group and type of donor and recipient immediately. (See proper donor blood selection guide.)

8. If no reactions are observed, the blood is considered compatible with the recipient, provided the IDAT (Antibody Screen) is negative and the group and type of both recipient and donor is verified. Do not discard your crossmatch tubes until the IDAT results are known.

9. Recipients having positive antibody screens (IDATs) must have the crossmatch carried through all phases of IDAT testing. (See previous procedures.)
NOTE: This procedure has many limitations. It will only detect incompatibility when the recipient’s serum contains antibodies, in sufficient strength, and specific for donor RBC antigens which are present. It will not detect all errors in blood typing, blood grouping, or donor blood selection. It does not ensure the prevention of a transfusion reaction in the recipient, nor does it ensure survival of donor red blood cells in the recipient’s circulation.

Expected Results:

No agglutination or hemolysis in any phase.

Unexpected Results:

Agglutination or hemolysis in any phase of testing indicates that recipient antibodies are reacting with the intended donor cells. The unit should not be given to this recipient until the incompatibility is resolved.

False Negatives:

1. Failure to add recipient’s serum to tubes.
2. Insufficient quantity of recipient’s serum added.
3. Improper donor cells added to tubes.
5. Failure to record hemolysis as incompatible result.
6. Others listed in IDAT procedure (if performed).

False Positives:

1. Presence of Rouleaux.
2. Contamination of bloods or reagents.
3. Presence of fibrin strands (incomplete clotting).
4. Cold agglutinins.
5. DAT positive donor cells.
6. Dirty glassware.
7. Others listed in IDAT procedure (if performed).

References:

TYPE AND SCREEN: ORTHO ID-MTS GEL TEST SYSTEM

PRINCIPLE: The gel test procedure is based on the principle of hemagglutination in which a red cell antigen will react with a corresponding antibody resulting in red cell agglutination. In the gel test, the specific antibody is incorporated into the gel and the gel has been pre-filled into the microtubes of the plastic card. During centrifugation the red cells pass through the gel and come in contact with the antibody. Agglutination occurs if these red cells have the specific antigen that corresponds to the antibody in the gel. Agglutinated red cells become trapped in the gel. Strong antigen-antibody reactions result in large agglutinates, which form a red line of cells layered at the top of the gel. Weaker positive reactions will have visible red cell agglutinates suspended throughout the gel. Non-agglutinated cells are not trapped by the gel and will form a button of red cells at the bottom of the microtube.

REAGENTS AND SUPPLIES:
- ABD Monoclonal and Reverse and anti-IgG gel cards
- Diluent 2-plus
- 0.8% Affirmagen (A & B Cells)
- 0.8% Selectogen screening cells
- Manual pipettor(s) to dispense 50, 25, 12.5 microliters and tips
- 12 x 75mm tubes
- ID-MTS incubator and centrifuge
- ID-MTS worktable
- Patient’s anticoagulated sample (preferably EDTA)

PROCEDURE:
1. Before starting any procedures, be sure to turn the Ortho ID-MTS incubator on. The switch is on the back panel of the instrument.

2. Spin all plasma samples to be tested for 2 minutes in a Blood Bank Serofuge to remove debris or fibrin. Bring all cards and reagents to room temperature.

3. Bring all cards and reagents to room temperature. Check the appearance of the cards before using, making sure they contain the appropriate amounts of antisera, and the color of the gel is correct.

4. Label one ABD card and two microtubes of an Anti-IgG card with the appropriate patient information. Label the two Buffer microtubes of the ABD card “A,” and “B,” for your Reverse Grouping test. Label the two Anti-IgG microtubes “I” and “II” for your Antibody Screen test. Remove the foil only from the portion of the cards that you will use for testing. Be sure to label the cards BEFORE removing the foil. Set the labeled cards upright in your workstation rack.
5. Label one 12x75mm tube with patient name or number for the 4% patient cell suspension. Dispense 0.5ml of Diluent 2-Plus into the labeled tube.

6. Set the pipettor at 25µL and prepare a 4% patient RBC suspension by pipetting 25µL of patient packed RBCs from the bottom of the specimen tube into this tube containing the Diluent. Put the tip of the pipettor all the way down into the Diluent when dispensing the RBCs to assure complete delivery.

7. Change the pipettor setting to 12.5µL and dispense 12.5µL of the patient 4% RBC suspension into the microtubes labeled anti-A, anti-B, anti-D, and Control on the ABD card. Check the volume levels of all microtubes before incubation to make sure they are all equal and that all cells and plasma have been properly pipetted.

8. Set the pipettor to 50µL and dispense 50µL of Affirmagen A1 cells and B reverse grouping reagent cells into the appropriate labeled buffered gel microtubes of the ABD card.

9. Keeping the pipettor at the same setting, dispense 50µL of Selectogen screening cells I and II into the appropriately labeled microtubes of the Anti-IgG card.

10. Set the pipettor to 25µL and dispense 2 shots (50µL) if patient plasma into each of the A1 and B cell microtubes.

11. Dispense 25µL of patient plasma into each of the Selectogen screening cell microtubes.

12. Place the Anti-IgG antibody screen card in the incubator at 37°C for 15 minutes. (The card may be incubated up to 40 minutes).

13. At the end of the 15 minute incubation, place both cards in the ID-MTS centrifuge and spin for 10 minutes.
After the centrifugation, the strips are removed from the centrifuge and read for agglutinates trapped in the gel column:

a. **negative reaction**: unagglutinated red cells forming a well-delineated pellet at the bottom of the microtube.

b. **1+ reaction**: red cell agglutinates predominantly observed in the lower half of the gel column. Unagglutinated cells form a pellet in the bottom of the microtube.

c. **2+ reaction**: red cell agglutinates dispersed throughout the length of the gel column. Few agglutinates may be observed in the bottom of the microtube.

d. **3+ reaction**: the majority of red cell agglutinates trapped in the upper half of the gel column.

e. **4+ reaction**: a solid band of red cell agglutinates on top of the gel. A few agglutinates may filter into the gel but remain near the predominant band.

f. **mixed field reaction**: a band of red cell agglutinates on top of the gel, accompanied by a pellet of unagglutinated cells at the bottom of the microtube.

**NOTE**: Mixed Field reactions are a rare occurrence and could be an indication of contamination of the plasma (serum) with fibrin or other debris. Plasma (serum) tests yielding a MF reaction should be repeated on a sample that has been clarified by centrifugation.

See below for a diagram representing these reaction strengths.

<table>
<thead>
<tr>
<th>Negative</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>4+</th>
<th>Mixed Field</th>
</tr>
</thead>
</table>

13. ABO/Rh type of the patient is interpreted according to standard practice. Any positive result in the Selectogen columns of the Anti-IgG card indicates the presence of an antibody requiring identification with a panel.
Crossmatch Procedure if Antibody Screen is NEGATIVE:

14. If the antibody screen is negative, the crossmatch may be completed by selecting donor units of a compatible ABO/Rh type and performing an immediate spin crossmatch according to these steps:

   a. prepare a 0.8% RBC suspension of the donor unit by pipettting 10 – 12.5 µL of donor packed cells into 1mL (2 shots) of Diluent-2-Plus.

   b. Label one microtube of a Buffer Card for each donor unit to be tested with the patient and donor information. Set the pipettor to 50µL and pipette 50µL of 0.8% donor RBCs into the appropriate microtube.

   c. Set the pipettor to 25µL and pipette 25µL of patient plasma into the microtube.

   d. Place the card into the centrifuge and spin for 10 minutes. Remove the card and read for agglutination in the microtubes.

Crossmatch Procedure if Antibody Screen is POSITIVE:

15. A positive antibody screen will require that an antibody identification panel be performed using 50µL of 0.8% Panel A panel cells and 25µL of patient plasma in appropriately-labeled anti-IgG cards, according to the Selectogen procedure outlined in steps #6-10 above.

   In addition to the Panel A cells, an auto control should also be run with the panel:

   - Convert the 4% patient RBC suspension to 0.8% by adding 2mL (4 shots) of Diluent-2-Plus to the tube.

   - Add 50µL of this RBC suspension and 25µL of patient plasma to a Anti-IgG microtube on the Panel A card and proceed with the procedure.

16. Panel results are recorded on a Panel A Worksheet and interpreted in the usual way to identify the antibody(ies) present.
17. Once the antibody has been identified, **crossmatches on a patient with a positive antibody screen** must be run through the entire Anti-IgG procedure:

   a. Prepare a 0.8% RBC suspension of each donor unit to be tested by pipetting 10 – 12.5 µL of donor packed cells into 1mL (2 shots) of Diluent-2-Plus.

   b. Label **one microtube of an Anti-IgG card for each donor unit** to be tested with the patient and donor information.

   c. Add 50µL of 0.8% donor RBC suspension and 25µL of patient plasma to the appropriate microtubes.

   d. Incubate for 15 minutes, spin for 10 minutes, and read for agglutination in the microtubes.

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References:
**BLOOD BANK SESSION #6: ACTIVITIES**

During this session, the student will submit the completed "Dry-Panel" homework assignment and will perform:

1. **one** (1) ABO, Rh, and Direct Antiglobulin Tests (DAT) on the newborn cord blood sample provided, with an elution and antibody identification panel, if indicated.

2. **one** (1) Type and Screen on the maternal sample provided, with antibody identification panel, if indicated. (One or more students may be assigned to the Ortho ID-MTS Gel System for this specimen.)

3. an acceptably written summary explaining the results of the above lab work.

**BLOOD BANK SESSION #6: OBJECTIVES**

Following this session, the student should be able to:

1. explain the principle of Direct Antiglobulin Testing, indicating situations when this test would be appropriate.

2. differentiate between polyspecific, anti-IgG, and anti-C3d Antihuman Globulin reagent, indicating the RBC coatings detected by each.

3. state the basic principle of an "elution," and describe when this test is indicated.

4. list several methods by which elutions may be done.

5. explain the relationship between a positive DAT with different monospecific and polyspecific reagents, and the presence or absence of antibodies in the eluate.

6. perform an antibody identification panel, by IAT methodology, on eluates or serums found to be IAS positive.

7. correctly identify all antibodies detected in panels using antigrams.

8. successfully interpret antigram patterns provided in a "dry-panel" homework assignment.

9. complete ABO, Rh, and DAT testing on a minimum of one (1) newborn cord blood specimen, including an elution procedure if indicated, and interpret the results of all serological testing performed with 100% accuracy.

10. complete ABO, Rh, and Indirect Antibody Screen testing on a minimum of one (1) maternal specimen, including Antibody Identification Panel if indicated, and interpret the results of all serological testing performed with 100% accuracy.
DIRECT ANTIGLOBULIN TEST PROCEDURE (DAT)

PRINCIPLE: Red blood cells may have antibodies, drugs, or other mechanisms coating the antigens on their cell membranes. Anti-human globulin reagent is added to such antibody-coated cells, causing them to agglutinate, a positive DAT result. All DAT negatives must be verified by the addition of check cells and observation of agglutination.

Procedure:
1. Prepare a 3-5% saline suspension of red cells from an EDTA specimen.
2. Place 1-2 drops of 3-5% suspension into a properly labeled test tube. Wash 4 times.
3. Add 1 drop of AHG to the tube, mix, spin 15 seconds (high speed), and read for agglutination. All negatives should be confirmed microscopically.
4. Add 1 drop of check cells to each negative, mix gently, centrifuge, and re-examine.
5. All negatives should now become positive. (See explanation in IDAT principle.)
6. Positive reactions indicate that coated RBCs were present in the sample.

NOTES: Binding of antibody onto RBCs may occur in the body or in the test tube if a red-top specimen is used. Therefore, an EDTA specimen should always be used for DAT testing. The anticoagulant binds calcium, which is necessary for antibody coating. If the DAT result is positive on the EDTA RBCs, antibody coating of the RBCs must have occurred in the body, as the lack of calcium in the tube prevented such coating within the specimen tube.

Elution is the process whereby the antibody which is coating the RBC antigens may be removed. These antibodies are contained in the “eluate,” which is substituted for the serum in the IDAT procedure to identify the coating antibody. (See IDAT, elution procedures.)

Expected Results: Negative.

False Positives:
1. Refrigerated red-top tubes containing cold auto antibodies (cold agglutinins).
2. Septicemia.
3. Bacterial contamination of stored blood.
4. Many drugs.
5. Others listed in false positive IDAT procedure.

False Negatives:
See the IDAT procedure.

GAMMA ELU-KIT II FOR RAPID ACID ELUTION

PRINCIPLE:

Red blood cells coated with antibody are first thoroughly washed to remove all traces of unbound protein, using a special wash solution to maintain the association of bound antibody. The washed cells are then suspended in a solution at low pH to dissociate the bound antibody. After centrifugation, the supernate containing any dissociated antibody is separated from the red cells and neutralized by adding a buffering solution. The eluate is then ready to be tested for antibody detection and/or identification.

Reagent Preparation:

Working Wash Solution must be prepared by diluting the Concentrated Wash Solution 1 in 10 with distilled water.

Specimen:

A specimen drawn in EDTA anticoagulant is the specimen of choice for this procedure.

Procedure:

Preparation of the Eluate:

1. Fill a 12x75mm tube with specimen to within ½” of the rim, then centrifuge the specimen for 2 minutes. Using a plastic transfer pipette, remove and discard as much plasma as possible. Wash the specimen one time with physiologic saline. Remove the supernatant saline with a transfer pipette. **Do not invert the tube to decant the supernatant!**

2. Wash the specimen an additional 4 times using the Working Wash Solution.

3. Transfer 20 drops of the washed RBCs to a clean, labeled tube, then add 20 drops of reagent Eluting Solution and mix gently by inverting the tube 4 times.

4. Centrifuge immediately for 45-60 seconds. Use a transfer pipette to transfer the supernatant eluate to a clean, labeled test tube. The sedimented RBCs should be discarded.

5. To the separated acid eluate, add 20 drops (equal volume) of reagent Buffering Solution and invert to mix thoroughly. Be sure the color of the eluate turns a pale blue color and remains blue upon mixing. If the eluate is not blue, add additional Buffering Solution, drop by drop, mixing each time, until the eluate remains blue.

6. Centrifuge to clear the specimen, and transfer the supernatant to a clean tube. The eluate is now ready for testing.
Testing the Eluate:

1. The eluate is now tested for the presence/identification of antibodies. In most cases, the eluate will be tested against Panel Cells.

2. Place 1 drop of each of the reagent RBCs to be used in appropriately labeled tubes. Add a small volume of physiologic saline (5-10 drops) and centrifuge for 30 seconds; decant the saline and blot the tubes dry.

3. Add 2 drops of Eluate to the dry RBCs in each tube.

4. Mix the contents of all tubes thoroughly and incubate for 15 minutes at 37°C. No potentiator is added to the tubes!

5. After appropriate incubation, add 5-10 drops of Working Wash to each tube, centrifuge for 30 seconds, decant the tubes, and blot dry.

6. Without further washing, add 1 drop of Anti-Human Globulin to each tube, mix, and centrifuge for 15 seconds.

7. Re-suspend the cells in each tube and read for agglutination or hemolysis.

Interpretation:

Eluate Specimen: Any positive reactions in these tubes indicate the presence of an antibody in the eluate, which originated on the membrane of the specimen RBCs being tested. This antibody can be identified using the usual panel identification procedures ("crossing off").

**BLOOD BANK SESSION #7: ACTIVITIES**

During this session, the student will perform:

1. **one** (1) ABO/Rh, IAS, and antibody identification panel. (One or more students may be assigned to the Ortho ID-MTS Gel System for this specimen.)

2. **one** (1) antibody titer procedure for the antibody identified.

3. **any number** of other tests required to resolve unexpected reactions or confirm serologic evidence.

4. an acceptably written summary of the serologic evidence obtained, thoroughly explaining any discrepancies or incompatibilities encountered.

**BLOOD BANK SESSION #7: OBJECTIVES**

Following this session, the student should be able to:

1. describe the principle and procedure for the quantitative titer of an antibody identified in a patient serum.

2. explain several clinical situations where the titering of an antibody would be appropriate.

3. complete ABO, Rh, Indirect Antibody Screen, Antibody Identification panels, and antibody titer procedures on a minimum of one (1) specimens, resolving all ABO discrepancies and antibody identifications, and interpreting the titer results with 100% accuracy.
BLOOD BANK SESSION #7: PROCEDURES

TITRATION OF UNEXPECTED ALLOANTIBODIES

PRINCIPLE:

Alloantibodies detected in the serum of patients can be titered by serial dilution of the serum, to determine the relative concentration of the antibody detected. This procedure is useful in several circumstances:

1. Prenatal Studies: When the antibody is of specificity known to cause HDN, the results of titration studies are used to assess the need for amniocentesis.

2. Antibody Identification: Some antibodies cause agglutination of virtually all reagent RBC samples, but specificity is indicated by differences in the strength of reactivity with each sample in titration studies.

3. High-Titer-Low-Avidity (HTLA) Antibodies: These are antibodies that are weakly reactive in the antibodies that are weakly reactive in the undiluted state but continue to react at extremely high dilution, unlike most weakly reactive antibodies which quickly dilute out to negative reactivity.

Procedure:

1. Prior to performing an antibody titer, an alloantibody must have been detected in the Indirect Antibody Screen. A panel must then be performed to identify the antibody.

2. Label ten 12x75mm test tubes #1 through 10. Refer to the table below to complete the following steps.

3. Pipet 0.1 ml of physiologic saline into all of the tubes.

4. Add 0.1 ml of patient serum to tube #1 and mix thoroughly. This creates a 1:2 dilution of the serum.

5. Transfer 0.1 ml of tube #1 into tube #2, mix and transfer 0.1 ml of tube #2 into tube #3, mix, and so on through tube #10. Discard 0.1 ml of the dilution in tube #10 after thoroughly mixing.
SUMMARY TABLE FOR ANTIBODY TITER PROCEDURE

<table>
<thead>
<tr>
<th>Tube #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>mL saline</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>mL serum</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>mL of previous tube</td>
<td>-</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>dilution made of previous tube</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>final dilution</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

6. From either the Trio Screening Cells or the Panel-Two cells, an appropriate reagent RBC must now be selected to correspond to the antibody identified in the serum. The RBC selected must have the antigen on its membrane, and if the antibody is one known to demonstrate dosage, *the cell must be homozygous for the antigen.*

7. Add one drop of the appropriate reagent RBCs to all tubes. The titer is then run through the optimal testing phases for the antibody identified in the panel. *In most cases,* this will involve addition of 2 drops of N-HANCE potentiator to each tube, incubation at 37°C for 5-15 minutes, washing four times with saline, addition of Anti-Human Globulin reagent, centrifuging and interpreting each tube for agglutination.

**Interpretation:**

The titer of the alloantibody is interpreted as the reciprocal of the highest dilution of serum exhibiting agglutination of 1+ or stronger.

References: