CLINICAL LABORATORY SCIENCE 131/132

COLLEGE LABORATORY
GENERAL COLLEGE LABORATORY OBJECTIVES

During the college laboratory sessions, each student will:

1. rotate through a schedule of related tests according to a rotation schedule.

2. **read each test procedure before starting a test procedure.**

3. locate all reagents, quality control materials, unknown specimens, supplies, and instruments to be used.

4. perform each test in the rotation schedule with **acceptable performance** identified in each test procedure.

5. demonstrate an interest in the college laboratory by following directions, asking questions when perplexed, listening to directions and completing the test procedures on the rotation schedule.

6. **practice laboratory safety** while in the college laboratory. Examples: pipetting, wearing laboratory coats, wearing gloves when handling all body fluids, disinfecting work areas before and after performing a test procedure.

7. answer, in writing, the specific objectives associated with each laboratory procedure, place them in the laboratory notebook, and hand them into the instructor no later than one week after the completion of the rotation.

8. correctly perform, log, and interpret all test procedures (within rotational quotas stated) in this syllabus.

9. obtain an **instructor/LIA signature** on all completed laboratory work **before** leaving the lab for the day.

10. demonstrate a working knowledge of specimen and reagent use and storage.

11. state the principle behind all college laboratory procedures.

12. determine which steps in performance of a test procedure may be interrupted, and which steps are critically timed.

13. clean up the laboratory workstation they have used.

14. accept differences in terminology and procedures between college laboratory and clinical agency laboratories.

15. recognize the need for assistance (and ask for help) when performing unfamiliar procedures and/or using unfamiliar instruments.

16. locate and read all reagent labels and package inserts when appropriate.

17. make an **independent attempt** to repeat procedures, which have resulted in unacceptable results **after a complete review of the procedure and supplies.**

18. seek instructor's assistance if repeat performance of a procedure fails to yield acceptable results.
19. locate the recorded limits of acceptability for QCs and enter their QC data daily.

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

21. submit unknown results to the instructor in the laboratory notebook, which satisfy the linearity and reproducibility, indicated for selected college laboratory procedures.

22. demonstrate a willingness to overcome procedural errors by practicing procedures until proficient (outside the scheduled lab hours if necessary).
GENEAL COLLEGE LABORATORY INFORMATION

The CLSC program maintains a college laboratory in HS 109. The college laboratory will be an essential part of your CLSC education, allowing you access to reagents, instruments, and supplies for perfecting your skills in medical laboratory techniques. Should you experience difficulties in your clinical rotations; the college laboratory can also provide you with additional opportunity for practice and individual instruction. This extra practice and instruction will occur only if you arrange it around open lab hours and instructor schedules.

College Laboratory Policy: 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 after the completion of the laboratory rotation (A, B, C, D, etc). 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. Each student will keep a laboratory notebook which will house worksheets, laboratory objective sheets, and graded assignments.

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. Failure to submit all assigned College Laboratory work before the final exam, completed to the satisfaction of the instructor, will result in 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”, etc.) Failure to submit laboratory work to the satisfaction of the instructor by the Final Exam will result in an “incomplete” grade for the course.

At the beginning of fall semester, all students will enter a two-week CLINICAL ORIENTATION. These orientation sessions will be held during the students’ clinical days and times in the CLSC/Phlebotomy College Laboratory. The normal College Laboratory sessions during those two weeks will be an extension of this Clinical Orientation. Students will begin their actual CLINICAL rotations and their various COLLEGE LAB rotations the third (3) week of the fall semester at their assigned clinical site. At the end of CLSC 132 (Weeks #6 and #7), all students will enter a two-week CLINICAL ORIENTATION for Immunohematology (Blood Bank) in preparation for Spring Semester. These orientation sessions will be held during the students’ clinical days and times in the CLSC/Phlebotomy College Laboratory. Again, the normal College Laboratory sessions during those two weeks will be an extension of this Clinical Orientation.

In CLSC 131/132, college lab you will learn how to perform many laboratory tests in three subjects: urinalysis, hematology, and miscellaneous. In order to do this effectively, 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.

The following pages in this syllabus state the minimum quotas, objectives, questions, procedures, and worksheets for each rotation. Please READ THEM prior to your assigned College Lab session so that you don’t enter college lab rotations wondering what is to be done during each session.
**COLLEGE LABORATORY CLSC 131 SCHEDULE**

Section 01: Tuesdays 2:00 – 4:50 P.M.

Section 02: Thursdays 2:00 – 4:50 P.M.

<table>
<thead>
<tr>
<th>Week #1</th>
<th>[Continuation of Clinical Orientation]</th>
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<tr>
<td>Week #2</td>
<td>[Continuation of Clinical Orientation]</td>
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</table>

**Week #3** Begin College Laboratory Rotations

<table>
<thead>
<tr>
<th>Rotation A</th>
<th>Video: Preventing Pre-analytical Errors</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Macro and Micro Pipetting</td>
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<td></td>
<td>Receive 24 hour urine container</td>
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<table>
<thead>
<tr>
<th>Rotation B</th>
<th>Erythrocyte Sedimentation Rate</th>
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<tbody>
<tr>
<td></td>
<td>Manual Microhematocrit</td>
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</table>

<table>
<thead>
<tr>
<th>Rotation C</th>
<th>Hematology:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Manual WBC Counts</td>
</tr>
<tr>
<td></td>
<td>Manual Platelet Counts</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rotation D</th>
<th>Hematology:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Making Differential Smears/Staining</td>
</tr>
<tr>
<td></td>
<td>Reticulocyte Counts</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rotation E (2 weeks)</th>
<th>Hematology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal Differential Counts</td>
</tr>
</tbody>
</table>
# COLLEGE LABORATORY CLSC 132 SCHEDULE

Section 01: Tuesdays 2:00 – 4:50 P.M.  
Section 02: Thursdays 2:00 – 4:50 P.M.

<table>
<thead>
<tr>
<th>Rotation</th>
<th>Weekly Focus</th>
</tr>
</thead>
</table>
| Rotation F | Routine Urinalysis Week 1  
Quality Control  
Physical Properties  
Chemical Tests  
Microscopic Tests  
Confirmation Tests |
| Rotation G | Routine Urinalysis Week 2  
Quality Control  
Physical Properties  
Chemical Tests  
Microscopic Tests  
Confirmation Tests |
| Rotation H | Routine Urinalysis Week 3  
Quality Control  
Physical Properties  
Chemical Tests  
Microscopic Tests  
Confirmation Tests |
| Rotation I | Quantitative Urine Total Protein |
| Rotation J | Body Fluids  
Body Fluid counts |

Weeks #7 & #8  
Immunohematology Orientation (Continuation of Clinicals)
SAFETY RULES
(COLLEGE LABORATORY)

1. Remember where you are and why you’re here. Horseplay is not permitted.

2. Locate the following safety equipment in the laboratory and know how to use them:
   a. Eye wash stations
   b. Fire extinguishers
   c. Fire blankets
   d. Showers

3. Eyesight must be protected when dealing with strong reagents. Many states require the use of safety goggles in all laboratories for all purposes.

4. Laboratory coats must be worn at all times while working in the laboratory.

5. Long hair should be tied back TIGHTLY. Many accidents have occurred due to flowing hair being caught in the flame of a Bunsen burner, moving mechanisms of instruments, etc.

6. All chemical spills should be cleaned up immediately, USING THE CORRECT CLEANUP PROCEDURE FOR THE TYPE OF CHEMICAL. Some chemicals can cause severe skin irritation. THE INSTRUCTOR MUST BE NOTIFIED OF ALL CHEMICAL/REAGENT SPILLS.

7. In any physical contact with chemicals, the area should be immediately flushed with copious amounts of water.

8. The student should know which chemicals need special care in handling.

9. In the laboratory area the student should become familiar with fire safety procedures.

10. Caution should be taken when pipetting serum, plasma, or whole blood. There should be no pipetting by mouth. Any body fluid spills must be cleaned up with 10% household bleach (Clorox) or other disinfectant spray.

11. NO eating or drinking is allowed in the College laboratory work areas.

12. Disposable gloves must be worn when handling all body fluid samples.

13. Know the location of the Material Safety Data Sheets (MSDS) for laboratory reagents, and know the correct usage of these sheets.

14. Proper Laboratory attire should be worn during any laboratory exercise (including venipunctures) including laboratory coats, gloves, closed toe shoes, no clogs, no shorts, no dangling jewelry, etc.

15. Laboratory rules (including attire) will be enforced anytime a student is working in the college laboratory (even during unscheduled laboratory time).

16. Laboratory rules will be enforced during venipuncture drawing/practicing sessions.

17. An instructor must be present during blood draws from volunteers.
NOTE: Any accidents (broken glassware, serum spills, whole blood spills, puncture wounds, reagent spills, etc.) must be reported to a full-time faculty member immediately.
### HAZARDS INVOLVED IN HANDLING COMMON CHEMICAL SUBSTANCES

<table>
<thead>
<tr>
<th>TYPE</th>
<th>HEALTH HAZARD</th>
<th>FLAMMABILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrated Acids</td>
<td>high: very corrosive, some have noxious fumes (inhalation risk)</td>
<td>negligible</td>
</tr>
<tr>
<td>Alcohols</td>
<td>some: varies with type of alcohol</td>
<td>high</td>
</tr>
<tr>
<td>Alkali (NaOH, KOH, etc.)</td>
<td>high: eye contact is very dangerous</td>
<td>negligible</td>
</tr>
<tr>
<td>Ether</td>
<td>high: inhalation, depression of CNS</td>
<td>extremely high</td>
</tr>
<tr>
<td>Hydrocarbons (gasoline)</td>
<td>high: ingestion may cause pneumonia</td>
<td>high</td>
</tr>
<tr>
<td>Acetic Acid, glacial</td>
<td>moderate: very irritating to eyes</td>
<td>moderate</td>
</tr>
<tr>
<td>Acetone</td>
<td>some</td>
<td>high</td>
</tr>
<tr>
<td>Ammonia</td>
<td>high: toxic and irritating fumes</td>
<td>negligible</td>
</tr>
<tr>
<td>Chloroform</td>
<td>high: possible liver damage from inhalation; may be absorbed through skin</td>
<td>negligible</td>
</tr>
</tbody>
</table>
## Reproducibility Limits in the College Lab

<table>
<thead>
<tr>
<th>Test</th>
<th>Method</th>
<th>Limits (±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macro and Micro Pipetting</td>
<td>Spectrophotometer</td>
<td>0.010 Absorbance Units from the Mean Absorbance Unit Value</td>
</tr>
<tr>
<td>Quantitative Urine Total Protein</td>
<td>Pyrogallol Red-Molybdenum Complex</td>
<td>± 2 S.D. of the Quality Control Samples</td>
</tr>
<tr>
<td>Routine Urinalysis</td>
<td>Macroscopic Test Results</td>
<td>95% Accurate from results obtained by Instructors</td>
</tr>
<tr>
<td>Routine Urinalysis</td>
<td>Microscopic Test Results</td>
<td>95% Accurate from results obtained by Instructors</td>
</tr>
<tr>
<td>Manual WBC Count</td>
<td>Unopette/Counting Chamber</td>
<td>15%</td>
</tr>
<tr>
<td>Manual Platelet Count</td>
<td>Unopette/Counting Chamber</td>
<td>15%</td>
</tr>
<tr>
<td>Reticulocyte Count</td>
<td>Manually Counted %</td>
<td>10%</td>
</tr>
<tr>
<td>Erythrocyte Sedimentation Rates</td>
<td>Dispette Method and Westergren Method</td>
<td>10%</td>
</tr>
<tr>
<td>Manual Eosinophil Counts</td>
<td>Unopette/Counting Chamber</td>
<td>10%</td>
</tr>
<tr>
<td>Body Fluid Counts</td>
<td>Counting Chamber</td>
<td>15%</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>Manual Microhematocrit</td>
<td>3%</td>
</tr>
<tr>
<td>Normal Differentials</td>
<td>Manual Counting</td>
<td>95% Accurate from results obtained by Instructors.</td>
</tr>
</tbody>
</table>
* Videotape:  Applied Phlebotomy Video Series: Preventing Preanalytical Errors:

**OBJECTIVES:**

After watching the video: Preventing Preanalytical Errors, the student will be able to:

1. List four possible serious consequences of specimen collection errors.
2. Describe the six job tasks where Preanalytical Error can occur.
3. Describe the cost of Preanalytical Error to the Medical Facility.
4. Identify the most potentially life-threatening preanalytical error.
5. Explain the appropriate procedure for Outpatient Identification as required by NCCLS (CLSI)
6. Explain the appropriate procedure for Inpatient Identification as required by NCCLS (CLSI)
7. List several unacceptable means of patient identification and describe why they are inappropriate.
8. Explain why it is important to have a two-step identification process.
9. Describe the importance of dietary considerations when collecting blood specimens.
10. Explain the importance of chronobiology in the collection of blood specimens.
11. List the five tests that are prone to diurnal variation.
12. Describe the importance of timed blood collection for Therapeutic Drugs.
13. Describe the accepted procedure when drawing a blood specimen in an arm that has an I.V.
14. Explain why it is inappropriate to draw above an I.V.
15. Describe the six adverse effects that can occur when drawing from a Vascular Access Device.
16. Identify the amount of blood that should be discarded when drawing from a Vascular Access Device.
Preventing Preanalytical Error Notes

Note: The organization NCCLS referenced in this videotape has changed its name to Clinical Laboratory Standards Institute (CLSI)

The effect of specimen collection errors has on individuals is often irreversible and sometimes fatal.

Serious consequences of specimen collection error can be:
- over medication
- under medication
- misdiagnosis
- even death

The laboratory provides 70% of all objective information on health status that a physician receives—Medical Laboratory Observer

Because the accuracy of every laboratory result is heavily dependent upon how the specimen was drawn, processed, and transported, phlebotomist are critical to the quality of care that the patient receives.

When you consider all of the errors that occur during specimen analysis, most occur during the preanalytical phase.

Preanalytical: 56% of Errors
Analytical: 13% of Errors
Postanalytical: 28% of Errors
Undetermined: 3% of Errors

Preanalytical Error is the error that occurs between the time the blood is ordered up to the time the blood is tested.

Preanalytical Error can occur during:
- ordering
- accessioning
- specimen collection
- processing
- transportation
- storage

Because this process cannot be fully automated, it remains the most complex and difficult to control.

It is estimated that specimen collection errors cost the average 400-bed hospital over $200,000 per year in recollection cost and medication errors.
Preanalytical Errors that Occur Before the Puncture

The most potentially life-threatening preanalytical error is misidentification of the patient.

NCCLS (CLSI) Recommends:

Outpatient Identification
Ask for their full name, address, identification number, and/or birth date

Compare the information given with the information on the request form.

Inpatient Identification
Ask for their full name, address, identification number, and/or birth date.

Compare the information given with the information on the request form and the patient’s identification bracelet.

Emergency Room patients should be identified even if it is a temporary identification bracelet.

The following should not be considered for reliable identification of patients:
- Name on Chart
- Name on Water Pitcher
- Identification bracelets attached to anything other than the patient (bed rail, I.V. pole, or lying on the bedside table).

When we rely on unreliable identification methods, we take a chance with the patient’s life.

When checking identification bracelets, it is not sufficient to rely on this alone to be accurate or on the right patient. CAP study found up to 5% of identification bracelets had erroneous information. A repeat study found up to 16% in other institutions had erroneous information. That is why we have the patient state their name and information to verify that the identification bracelet is correct.

It is not acceptable to address the patient: “Are you John Smith”. Hard of hearing patients may misunderstand the pronunciation of the name and nod in the affirmative.

Time of Collection Errors

Time- of –Collection considerations include:

- dietary considerations
- chronobiology (body clock)
- medication metabolism
- coordinating collections with the patients condition
Dietary Considerations:

Most reference ranges (normal values) are established on fasting ranges, therefore, fasting specimens are ideal for testing purposes. Non fasting specimens may not compare well to the fasting ranges.

Glucose and Triglyceride testing are critical for fasting. If fasting is required, and the patient has not fasted, and the testing cannot be done at another time, it should be noted on the requisition so the physician can interpret the results accordingly.

Chronobiology:

Some blood levels vary naturally according to body clock changes. For example, serum Iron results are 30% lower in the evening than in the morning.

Test that are prone to diurnal variation are:

- Cortisol
- Progesterone
- Catecholamine
- Prolactin
- Testosterone

There may be restriction in draw times. Review your facilities procedures for the collection of these tests.

Medication Metabolism:

Therapeutic Drug Tests are drawn to ensure patient dosing is correct. Some drug testing are to be timed according to when the nursing staff is to administer medication. Without a specified time of collection, the rule of thumb is for some levels to be collected just before the nurse gives the next dose. This is not always possible, so the Time of Collection along with the Time of the patient’s last dose should accompany the specimen results so that the physician can monitor dosing effectively.

Patients are subjected to a side range of procedures, therapies, and tests. Many can have an effect on laboratory results.

I. V. Fluids:

NCCLS recommends not drawing from an arm with I.V. Fluid.

If drawing from that arm is unavoidable, then:
- shut off the IV for 2 minutes
- tighten the tourniquet below the IV
- draw the specimen from below the infusion site
- discard the first 5 cc of blood
- document that the patient was drawn from the same arm as infusion fluids

NCCLS warns against drawing above an IV even if it has been temporarily turned off.
Patients have suffered serious complications even death as a result of specimens being drawn above a temporarily turned off IV.

Facilities should establish and enforce their own policy regarding drawing from an arm with an I.V.

Vascular Access Devices

Draws from a Vascular Access Device are associated with:

- blood culture contamination
- contamination with I.V. fluid
- high rate of hemolysis
- potential for air embolism in blood stream
- risk of bacteria in blood stream
- risk of line occlusion

Drawing from a vascular device can save a patient a stick, however, samples are often unacceptable or incapable of producing accurate results.

Vascular Access devices are designed for fluids to flow into the vein and not for blood to be withdrawn.

Whenever possible, avoid drawing from a vascular access device to prevent errors inherent in line draws.

If a Vascular Access Device is going to used for Blood Sample:

Non-Coagulation Testing
- discard twice the dead space volume

Coagulation Testing
- discard 6 times the dead space volume

5 cc is usually adequate.

Blood Cultures

Timing is critical for Blood Cultures. Blood Cultures are most accurate if collected at the onset of chills or during the rise in fever.

Exercise

Exercise has a profound effect on many tests. It is best to avoid collecting the blood sample after the patient has had a strenuous workout.

Many outpatients combine trips to the hospital with physical therapy or cardio rehab programs.
Exercise temporarily increases:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>LD</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>ACTH</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>HDL</td>
</tr>
<tr>
<td>CK</td>
<td>WBC Count</td>
</tr>
<tr>
<td>Cortisol</td>
<td>% Neutrophils in a CBC</td>
</tr>
</tbody>
</table>

Should note on the requisition that patient has exercised prior to blood specimen collection.

When collecting specimens, patient should not pump fist to make the vein more visible. Pumping the fist temporarily increases levels of Potassium and Ionized Calcium.

*Should educate the patient that pumping their fist may alter some of their laboratory results so that they do not repeat the behavior in the future.*

When infants or children cry during or in anticipation of the blood drawing procedure, it raises the WBC Count. Take time to pre-warm micro skin puncture sites and calm the fears of pediatric patients to minimize this effect.

**Patient Posture**

Reference ranges (normal values) are based upon ambulatory results.

Most inpatients are lying down. For most tests this difference is clinically insignificant. But for some tests, it can be significant.

When patients go from lying down to standing up, the body knows it will take extra effort to supply the brain with blood now that it is elevated above the heart. It responds by sending a signal to release hormones that raise blood pressure. With the increased blood pressure, small analytes efflux to surrounding tissue, leaving larger molecules in the blood in greater concentrations. This is referred to as “Hemoconcentration”.

Posture Sensitive Analytes include:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Analyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>Catecholamines</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>Alk Phos</td>
<td>ALT</td>
</tr>
<tr>
<td>ADH</td>
<td>Drugs</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>Renin</td>
</tr>
<tr>
<td>Calcium</td>
<td>Total Protein</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
</tr>
</tbody>
</table>

Even though all of these tests are sensitive to posture, not all of these tests have posture restrictions for blood collection. Check your facilities procedures for posture requirements. Consistently adhering to your facilities test requirements is imperative.

Hemoconcentration also occurs when the tourniquet is left on too long or greater than one minute.
Leaving the tourniquet on too long can affect:

- Potassium
- Lactate
- Ionized Calcium
- Magnesium

Some patients require extensive survey before finding an acceptable vein. Take as much time as needed to find a suitable vein. Must wait 2 minutes before reapplying the tourniquet to complete the puncture. Waiting 2 minutes allows the hemoconcentration to disperse.

**Release of the Tourniquet**

NCCLS (CLSI) recommends that the tourniquet be released as soon as the vein is accessed, however, not all circumstances allow for a successful venipuncture if the tourniquet is released before the venipuncture is complete.

Be aware of the effects of excessive tourniquet time.

Release upon venous access whenever possible and does not jeopardize the collection.

**Cleansing the Draw Site**

Alcohol should be allowed to dry before puncturing.

If not allowed to dry, alcohol residue is not only painful to the patient but can hemolyze the red cells. Hemolysis leads to erroneous test results, especially Potassium Tests.

**Betadine Cleansing**

If iodine gets into the sample it can elevate the following tests:

- Potassium
- Phosphorous
- Uric Acid

Even when allowed to dry before the puncture.

Not usually a problem because during routine laboratory procedures, the above tests are drawn after the blood cultures unless the blood has been drawn in a syringe.

When blood is drawn in a syringe, iodine can contaminate chemistry specimens.

It is important to remove iodine with an alcohol pad prior to puncture.

**False Positive Blood Cultures**

Contamination of the puncture site after cleansing can cause false positives leading a physician to treat a patient unnecessarily.
Financial Cost of Contaminated Blood Cultures;
- increased stay by 4.5 days
- increased cost by $5,000 due to hospital, pharmacy, and laboratory procedures
- increased microbiology laboratory overtime by 30%
- 26% of pediatric patients are unnecessarily hospitalized.

It is not a good practice to re-palpate the site after preparing for venipuncture.

Gloves are not sterile.

Increases the chance of blood culture contamination with skin organisms.

Bottle tops must be disinfected or become a source of contamination.

Minimize Contamination by:
- Cleanse site with a friction scrub for 30 – 60 seconds
- Allow the antiseptic to dry for at least 30 seconds prior to puncture
- Do not re-palpate cleansed site
- Clean top of culture vials with antiseptic if recommended

Preanalytical Errors that Occur During Puncture

Hemolysis: RBC’s rupture releasing Hemoglobin into serum or plasma.

Plasma or Serum becomes red/pink colored.

**Causes of Hemolysis:**
- drawing through Vascular Access Device
- excessive probing
- improper needle placement
- excessive pulling pressure on the plunger of a syringe
- vigorous specimen mixing
- small needle size
- inappropriate blood to anticoagulant ratio
- “milking” the site of a capillary puncture
- premature or excessive centrifugation

**Effects of Hemolysis:**

| Increased Potassium | Increased Magnesium |
| Increased LDH       | Increased Ammonia   |
| Increased AST       | Decreased RBC       |
| Increased ALT       | Decreased Hgb/ Hct  |
| Increased Phosphorous |

Dilutional Effect on all Analytes
Preventing Hemolysis

- avoid draws through IV cannulas
- avoid slow draws that come from improperly positioned needles
- avoid pulling too hard on plunger of the syringe
- do not under fill tubes containing additives
- gently invert tubes
- avoid using a 25 gauge or smaller needle
- pre-warm infant heels or the fingers of older children or adults
- do not centrifuge specimen at an excessive speed or before they are allowed to clot completely

Order of Draw

Important to follow the established order of draw to prevent carry over of additives between tubes.

NCCLS (CLSI) recommended Order of Draw

1. Sterile blood culture tubes or vials
2. Blue-stopper Sodium Citrate tubes
3. Serum tubes
4. Heparin or green-stopper tubes
5. Lavender-stopper EDTA tubes
6. Gray-stopper or glycolytic inhibitor tubes

Same Order for Syringe Draws

Capillary Order of Draw

1. EDTA tube
2. Other additive tubes
3. Non-additive tubes

Discard Tube for Citrate Tubes

If Citrate is the only tube being drawn or the 1st tube being drawn, the PT or APTT tests no longer require a discard tube. All other coagulation tests still require a discard tube as a precaution.

A discard tube is still required for all coagulation tests (including PT, APTT) using a butterfly if it is the first or only tube being drawn. A discard tube is used to remove air in the tubing and avoid under filling of the citrate tube.

Inversion of Tubes

5-10 inversions after collecting tubes can prevent clotting and the delays that recollection can bring.

It is especially important in small capillary tubes used for CBC’s that if not mixed periodically during collection, clots can form causing rejected specimens and recollection delays.

Micro clots may go undetected and alter test results compromising patient care.
**Anticoagulant Ratio to Blood**

The most sensitive tube for under filling is the light blue or Sodium Citrate Tube for Coagulation Studies.

Citrate tubes must be filled to at least 90% of stated capacity or the test will be falsely lengthened. False results can mislead the physician into adjusting the patient’s anticoagulant dosage to a degree that risks serious complications.

Small volume tubes should be used for difficult veins or when limited volume of blood is anticipated.

*Collected tubes that do not reach the manufacturers minimum volume requirement subjects the patient to diagnosis, medication, or treatment based upon erroneous results.*

When EDTA is under filled, excess anticoagulant causes the red blood cells to shrink affecting Hematology Tests.

**Blood Cultures**

The volume requirements for adult blood cultures is 20 cc’s of blood evenly distributed between two bottles/ vials not to exceed 12 cc per vial.

The bacteria that can infect blood can be low as 1 organism per mL of blood. The more adequately filled the specimen, the better chance of recovering the organism.

If you only obtain 12 ccs of blood, inject all 12 cc into the aerobic bottle. The media can support the growth for all aerobic organisms and most anaerobes.

Do not exceed the volume of blood recommended by the bottles manufacturer.

Over filling can cause some instruments to give false positive results due to interference of excessive white blood cells.

**Serum Separator Tubes**

NCCLS (CLSI) recommends that gel separator tubes not be used for Progesterone or Tricyclic Antidepressants unless the manufacturer or your lab’s internal studies establish stability.

**Preanalytical Error that Occur After Collection**

Specimens should be centrifuged within 2 hours of collection to avoid prolonged contact between serum/ plasma and cells.

Avoid prolonged contact between serum/plasma and cells by using gel separator tubes and centrifuge within 2 hours of collection or by physically separating serum/plasma into properly labeled transfer tubes.

Proper centrifugation prevents falsely elevated potassium results.
Analytes Affected by Prolonged Contact with Cells

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Prolonged Contact with Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionized Calcium</td>
<td>B12</td>
</tr>
<tr>
<td>Potassium</td>
<td>LDH</td>
</tr>
<tr>
<td>ALT</td>
<td>Albumin</td>
</tr>
<tr>
<td>AST</td>
<td>CO2</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Chloride</td>
</tr>
<tr>
<td>Glucose</td>
<td>HDL Cholesterol</td>
</tr>
<tr>
<td>Folate</td>
<td>Total Protein</td>
</tr>
</tbody>
</table>

Once serum/plasma is removed, it must be kept at room temperature for no longer than 8 hours without refrigeration.

It should be refrigerated no longer than 48 hours without freezing.

Re-spinning a gel tube to get more serum/plasma is not appropriate. The specimen received from the second spin is not the same as the first. It has been allowed to remain in contact with red cells for a prolonged period of time and should not be used.

**Plunger –type Separators**

Use with caution when storing serum/plasma

Create an air barrier between the separator and the cells.

Review manufacturer’s documentation.

<table>
<thead>
<tr>
<th>Test</th>
<th>Room Temperature</th>
<th>Refrigerator</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBC</td>
<td>24 hrs</td>
<td>24 hrs</td>
</tr>
<tr>
<td>Sed Rate</td>
<td>4 hrs</td>
<td>12 hrs</td>
</tr>
<tr>
<td>Retic</td>
<td>6 hrs</td>
<td>72 hrs</td>
</tr>
<tr>
<td>aPTT</td>
<td>4 hrs</td>
<td>4 hrs</td>
</tr>
<tr>
<td>PT</td>
<td>24 hrs</td>
<td>24 hrs</td>
</tr>
</tbody>
</table>

**Protection from Light**

Bilirubin can deteriorate up to 50% after one hour of exposure.

Protect from light by collecting in amber colored tubes, wrapping specimen in foil, or by placing in a light tight container for transfer to the laboratory.

**Clotting of the Specimen**

Must allow specimens adequate time to clot. Some may take up to 30 minutes to clot.

*Remember accurate results begin with you!*
OBJECTIVES

At the conclusion of this laboratory session, the student will be able to:

1. Demonstrate an ability to properly perform the following:
   a. control serological pipettes
   b. use a pipette bulb
   c. use a micropipet

2. EVALUATION: The student must be able to accurately and precisely pipette 3 mL of distilled water into a series of five (5) 12 x 75 mm test tubes using a serological or Mohr pipette. Then add 50 μL, 100 μL or 200 μL of a colored dye solution into these five test tubes. Then read these solutions on the Gilford III Stasar instruments obtaining Absorbance results that are within 0.010 Absorbance Value of the mean value of the Absorbance readings.
Rotation A
MACRO AND MICRO PIPETTING PROCEDURE

1. Label five (5) 12 x 75 mm test tubes with numbers 1 through 5.

2. Carefully pipette 3 mL of distilled water with the appropriate graduated pipette into each of the labeled test tubes.

3. Using a 50 μL, 100 μL or 200 μL semi-automatic pipette, secure a plastic disposable tip to the end of the pipette with your hand.

4. Carefully aspirate the colored dye solution, wipe off the tip with a Kimwipe without pulling out any of the colored dye solution, and add the pipetted volume to the FIRST labeled test tube.

5. Remove and dispose of the plastic tip into a biohazard container and repeat steps #3 - #5 for the other test tubes.

6. Mix each of the five test tubes by placing Parafilm over the top of each test tube and invert 5 times.

7. With the aid of the instructor, read each of the solutions on the Gilford Stasar III Spectrophotometer to obtain the Absorbance Values for each solution at the wavelength of 515 nm.

8. Calculate the mean value of the five Absorbance Values.

9. Ensure each solution’s Absorbance Value is within 0.010 Absorbance Value of the mean Absorbance Value.
ROTATION B
ERYTHROCYTE SEDIMENTATION RATE (ESR)

OBJECTIVES

At the end of this first laboratory session, the student should be able to:

1. explain the principle of and accurately perform the Westergren ESR procedure.
2. state at least 2 patient conditions, which would elevate or decrease the test result.
3. recall and list several technical factors, which affect the results of test.

QUOTAS

- Perform two (2) Westergren erythrocyte sedimentation rate (ESR) tests on unknown samples of EDTA blood.

MANUAL MICROHEMATOCRIT

OBJECTIVES:

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

1. explain the principle of and accurately perform manual microhematocrits
2. identify two patient conditions, which would elevate or decrease a patient’s hematocrit.
3. list several technical factors that could influence a patient’s microhematocrit test result.

QUOTAS

- Perform the manual microhematocrit procedure on three (3) Quality control samples and five (5) unknown patient samples.
ROTATION B
ERYTHROCYTE SEDIMENTATION RATE METHODS

PRINCIPLE:

When anticoagulated blood is allowed to stand undisturbed, the RBCs will normally settle out to the bottom of the tube. By definition, the ESR is the distance in millimeters that RBCs fall per unit of time, which is usually one hour. Various factors will affect the ESR, such as RBC size and shape, plasma fibrinogen and globulin levels, as well as mechanical and technical factors.

ERYTHROCYTE SEDIMENTATION RATE (ESR)
(WESTERGREN METHOD)

PROCEDURE:

1. Pipette 0.5 mL of 0.85% NaCl (isotonic saline) into a 13 x 100 mm glass test tube (labeled appropriately).
2. Pipette 2.0 mL of room temperature, well-mixed EDTA blood into the saline. Use the plastic disposable biological pipettes with cotton plugs!
3. Parafilm the test tube and mix at least 10 times by inversion.
4. Using a vacuum bulb, aspirate the diluted sample upward into the Westergren glass tube, until the cotton plug is saturated.
5. Wipe off the glass tube, and place into a vertical settling rack, placing Parafilm underneath the tube.
6. After placing tube(s) in the rack, allow them to settle undisturbed for EXACTLY 60 minutes.
7. At 60 minutes, read the position of the top of the sedimented RBCs in millimeters.
8. Remove the Westergren glass tube(s) from the settling rack, and discard into a sharp’s container.

Expected Values: Sedimentation rates increase gradually with age.

Males: 0 - 10 mm/hr
Females: 0 - 20 mm/hr
ROTATION B
MANUAL MICROHEMATOCRIT PROCEDURE

PRINCIPLE:

The height of packed cells in a capillary tube of heparinized or oxalated blood after centrifugation expresses the proportionate volume of packed cells in the blood sample.

PROCEDURE:

1. Use special heparinized capillary tubes for capillary blood and **plain tubes** for **EDTA samples**.
2. Fill the capillary tube about 2/3 full and seal one end with clay or rubber cap. Patient samples must be **in duplicate**. Hold the filled tube horizontally and seal by placing the dry end into the clay or rubber cap.
3. Balance the tubes in the centrifuge with the plugged ends facing the outside away from the center, touching the rubber gasket. Record the numerical position of each sample on your worksheet.
4. **Tighten the head cover** on the centrifuge **before closing the top**. The tubes will be thrown out place if this step is omitted.
5. Centrifuge for 5 minutes (10,000-15,000 rpm), or as labeled on the centrifuge.
6. Read the hematocrit in the microhematocrit reader. Do not include the buffy coat in the hematocrit reading. All tubes should be read within 10 minutes to avoid the movement of red cells back into the plasma.
7. Duplicates should be within 1% of one another. Results should be within 3% of the Hospital results.

Normal Values:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>42 - 54 %</td>
</tr>
<tr>
<td>Female</td>
<td>36 - 48 %</td>
</tr>
</tbody>
</table>

NOTE: If any hemolysis is present after spinning hematocrit, repeat. Normal hematocrit values vary with the age of the patient.
OBJECTIVES

At the end of this first Hematology rotation, the student should be able to:

1. explain the principle of and accurately perform the following test procedures:
   a. Manual WBC Count
   b. Manual Platelet Count

2. for each test listed in #1, state at least 2 patient conditions, which would elevate or decrease the test result.

3. recall and list several technical factors in each procedure, which affect the results of tests performed in this rotation.

QUOTAS

- Perform two (2) Manual WBC counts on unknown EDTA blood samples.
- Perform two (2) Manual Platelet counts on unknown EDTA blood samples.
- Perform one (1) Manual Platelet Quality Control Sample within established range.
ROTATION C
MANUAL UNOPETTE WBC COUNT PROCEDURE

OBJECTIVES:

Given the necessary hematologic equipment and two blood specimens, the student will perform a manual white blood cell count on each specimen, obtaining results within the acceptable limits of ± 15% from the instructors results. All results, which fail to meet this acceptable limit, will be repeated until results are deemed acceptable. All results must be reported in the correct format to be considered acceptable.

NOTE: Meeting the objectives for the WBC counts and platelet counts will take a great deal of patience and determination. It is the student’s responsibility to arrange practice time outside of the normally scheduled laboratory time should it be necessary.

PROCEDURE

EQUIPMENT

1. Kimwipes
2. Counting Chamber
3. Coverglass
4. EDTA Blood Unknowns
5. Unopette Reservoir
   1.98 ml of diluent
   Ammonium oxalate 11.45 gm
   Sorensen’s Phosphate Buffer 1.0 gm
   Thimerosal 0.1 gm
   Purified Water qs to 1 liter
6. Unopette Capillary Pipette - 20 μL
7. Petri Dish
8. Filter Paper
9. Microscope
10. Cell Counter
11. Isopropyl Alcohol - 70%
12. Pencil
13. Work Sheet
14. Blood Sample Mixers
15. Gloves

PROCEDURE

1. Mix the EDTA blood unknown by completely inverting for 10 minutes prior to use. Make sure the EDTA sample comes to room temperature before continuing.

2. Place the Unopette Reservoir on a flat surface. Grasping the Reservoir in one hand, take the Unopette Capillary Pipette assembly in the other hand and push the tip of the pipette shield firmly through the diaphragm in the neck of the Reservoir, then remove.

3. Remove the shield from the pipette assembly with a twisting action.
4. Make sure you have your gloves on. Invert the EDTA blood unknown 10 to 12 times and remove the stopper.

5. Holding the pipette almost horizontally, touch the tip of the pipette to the blood unknown as you also tip the blood unknown tube back horizontally. The pipette will fill by capillary action. Filling is complete and will stop automatically when the blood reaches the end of the capillary bore in the neck of the pipette.

6. Wipe the excess blood from the outside of the capillary pipette, making sure that no sample has been removed from the pipette.

7. Squeeze the reservoir slightly to force out some air. **DO NOT EXPEL ANY LIQUID.** Maintain the pressure on the Reservoir.

8. Cover the opening of the overflow chamber of the pipette with your index finger and seat the pipette securely in the Reservoir neck.

9. Release the pressure on the Reservoir. Then remove your finger from the pipette opening. Negative pressure will draw blood from the pipette into the Reservoir.

10. Squeeze the Reservoir gently two or three times to rinse the capillary bore, forcing the diluent up into, but not out of, the overflowing chamber, releasing pressure each time to return the mixture into the Reservoir.

11. Place your index finger over the upper opening and gently invert several times to thoroughly mix the blood with the diluent.

12. Let the Reservoir mix on the Blood Sample Mixers for **10 minutes** to allow the red blood cells to lyse or hemolyze.

13. Remove the counting chamber and the coverglass from the Petri dish. Clean the ruled area of the counting chamber and the coverglass with isopropyl alcohol and wipe dry with a Kimwipe.

14. Set the coverglass on the counting chamber. Do **NOT** misplace the coverglass!

**CHARGING THE COUNTING CHAMBER**

1. Convert to a dropper assembly, by withdrawing the pipette from the Reservoir and reseating securely in the reverse position.

2. Invert the Reservoir to resuspend the WBCs.

3. To clean the capillary pipette bore, invert the Reservoir, gently squeeze the sides and discard the first 3 to 4 drops into a Kimwipe.

4. While holding the longest edges of the coverglass, carefully charge the counting chamber by gently squeezing the sides of the Reservoir to expel the fluid until the counting chamber is properly filled. Fill both counting areas with the same Reservoir for a duplicate.
5. Place the counting chamber into a Petri dish, which contains a water moist piece of filter paper for **2 minutes** to permit the WBCs to settle.

**COUNTING THE WHITE BLOOD CELLS**

1. Place the objective of the microscope on low power (4X).

2. Wipe the bottom of the counting chamber with a Kimwipe and place the counting chamber on the stage of the microscope and focus so that the counting area is seen.

   NOTE: The condenser of the microscope must be lowered. This diminishes the light. If the light is not diminished, you will not be able to see the cells.

3. Locate the 9 large squares of the ruled area. These are the 9 squares, which will be counted.

4. Make sure that you have good distribution of cells.

5. Place the objective of the microscope on low power (10X).

6. Count the cells and remember to count all the cells in each of the 9 large squares.

   NOTE: Cells touching the top and left boundary lines are counted. Those cells touching the bottom and right boundary lines are NOT counted.

7. Count each side of the counting chamber for duplication. The duplications of your unknowns should be within 10% of the first count.

8. Add each of the nine large squared areas together to get the total. (9 "W" squares). If there is a variation of more than 5 cells in any of the two "W" squares, your distribution was not good and should be repeated. Remember that if your dilution was good, you can use the diluted sample; mix the sample, expel a few drops and reload the counting chamber.

9. When you are finished counting, remove the counting chamber from the microscope and clean the counting chamber and the coverglass with 70% isopropyl alcohol, dry with a Kimwipe, and place them back into the Petri dish.

10. Place your unknown blood sample back into the refrigerator. Throw your Unopette Reservoir in the Biohazard Container. Wipe down your work area with 10% Bleach.

11. Be sure to have the instructor/LIA initial your completed cell count/ worksheet
CALCULATIONS

1. If the counts on both sides of the chamber are within 10%, average the two counts together.

2. Perform the following calculation on the average number of WBCs counted in the 9 'W' squares:

   \[
   \frac{\text{Number of WBCs counted in 9 squares} \times \text{Dilution (100)} \times \text{Depth (10)}}{\text{Number of Large Squares Counted (9)}}
   \]

3. Units = WBCs/ mm\(^3\) or WBCs / μL

4. Remember to place your results in the correct scientific notation for White Cell Counts.

5. Clinical Significance of WBC Count:

<table>
<thead>
<tr>
<th>Increased Concentration of Leukocytes</th>
<th>Decreased Concentration of Leukocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial Infection</td>
<td>Viral Infection</td>
</tr>
<tr>
<td>Acute Leukemia</td>
<td>Radiation/ Chemotherapy/ Drug-Induced</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Aplastic Anemia</td>
</tr>
<tr>
<td>After Strenuous Exercise</td>
<td>Megaloblastic Anemia</td>
</tr>
<tr>
<td>Anxiety or Stress</td>
<td>Myelodysplastic Syndromes</td>
</tr>
<tr>
<td>Myeloproliferative Disorders</td>
<td></td>
</tr>
</tbody>
</table>
ROTATION C
MANUAL UNOPETTE PLATELET COUNT PROCEDURE

OBJECTIVES:

Given the necessary hematologic equipment and two blood specimens, the student will perform a manual platelet count on each specimen, obtaining results with the acceptable limits ± 15% from the instructors results. All results, which fail to meet this acceptable limit, will be repeated until the expected result is achieved. All results must be reported in the correct format to be considered acceptable. Students will perform one QC platelet count on quality control material, obtaining results within the acceptable limits as written on the quality control material insert. These results will be recorded on the manual count worksheet. Accurate QC results should be obtained before performing platelet counts on patient unknowns.

PROCEDURE

EQUIPMENT

1. Kimwipes
2. Counting chamber
3. Coverglass
4. EDTA blood unknowns
5. Unopette Reservoir
6. Unopette Capillary Pipette - 20 µL
7. Petri dish
8. Filter paper
9. Microscope
10. Cell counter
11. Isopropyl alcohol - 70%
12. Pencil
13. Work sheet
14. Blood Sample Mixers
15. Gloves

PROCEDURE

1. Mix the EDTA blood unknown sample by inverting for 10 minutes prior to use. Make sure the EDTA sample comes to room temperature before continuing.

2. Place the Unopette Reservoir on a flat surface. Grasping the Reservoir in one hand, take the Unopette Capillary Pipette assembly in the other hand and push the tip of the pipette shield firmly through the diaphragm in the neck of the Reservoir, then remove.

3. Remove the shield from the pipette assembly with a twisting action.

4. Make sure you have your gloves on. Invert the EDTA blood unknown 10 to 12 times and remove the stopper.
5. Holding the pipette almost horizontally, touch the tip of the pipette to the blood unknown as you also tip the blood unknown tube back horizontally. The pipette will fill by capillary action. Filling is complete and will stop automatically when the blood reaches the end of the capillary bore in the neck of the pipette.

6. Wipe the excess blood from the outside of the capillary pipette, making sure that no sample has been removed from the pipette.

7. Squeeze the reservoir slightly to force out some air. *DO NOT EXPEL ANY LIQUID.* Maintain the pressure on the Reservoir.

8. Cover the opening of the overflow chamber of the pipette with your index finger and seat the pipette securely in the Reservoir neck.

9. Release the pressure on the Reservoir. Then remove your finger from the pipette opening. Negative pressure will draw blood from the pipette into the Reservoir.

10. Squeeze the Reservoir gently two or three times to rinse the capillary bore, forcing the diluent up into, but not out of, the overflowing chamber, releasing pressure each time to return the mixture into the Reservoir.

11. Place your index finger over the upper opening and gently invert several times to thoroughly mix the blood with the diluent.

12. Let the Reservoir mix on the Blood Sample Mixers for 10 minutes to allow the red blood cells to lyse or hemolyze. Platelets in the diluent are viable for 3 hours.

13. Remove the counting chamber and the coverglass from the Petri dish. Clean the ruled area of the counting chamber and the coverglass with the isopropyl alcohol and wipe dry with a Kimwipe.

14. Set the coverglass on the counting chamber. Do *NOT* Misplace the Coverglass!

**CHARGING THE COUNTING CHAMBER**

1. Convert to a dropper assembly, by withdrawing the pipette from the Reservoir and reseating securely in the reverse position.

2. Invert the Reservoir to resuspend the platelets.

3. To clean the capillary pipette bore, invert the Reservoir, gently squeeze the sides and discard the first 3 to 4 drops into a Kimwipe.

4. While holding the longest edges of the coverglass, carefully charge the counting chamber by gently squeezing the sides of the Reservoir to expel the fluid until the counting chamber is properly filled. Fill both counting areas with the same Reservoir for a duplicate.

5. Place the counting chamber into a Petri dish, which contains a water moist piece of filter paper for 10 minutes to permit the platelets to settle.
COUNTING THE CELLS

1. Place the objective of the microscope on low power (10X).

2. Wipe the bottom of the counting chamber with a Kimwipe and place the counting chamber on the stage of the microscope and focus so that the counting area is seen. (The Center RBC squares.)

   NOTE: The condenser of the microscope must be lowered. This diminishes the light. If the light is not diminished, you will not be able to see the platelets.

3. Place the objective of the microscope on high dry power (40X).

4. Platelets are counted in all of the 25 small RBC squares.

5. If there is a greater variation than 10% in your duplicate, the count must be repeated. Use the same Reservoir, just mix, expel a few drops, and reload the counting chamber.

6. Place your results on your answer work sheet. Remove the counting chamber from the microscope. Clean the counting chamber and the coverglass with 70% isopropyl alcohol, dry with a Kimwipe, and place them back into the Petri dish.

7. If you are satisfied with your count, you can throw the Reservoir away in the Biohazard Container. Please wipe your work area down with 10% bleach. Complete the work sheet and hand it into the instructor when ALL counts are satisfactory.

CALCULATIONS

1. If duplicate counts are within 10% of one another, average the two counts together.

2. Perform calculation as follows:

\[
\text{Number of Platelets counted in all 25 "R: squares} \times \text{Dilution (100)} \times \text{depth (10)}
\]

\[
\text{Number of Large Squares Counted (1)}
\]

3. Units = Platelets/ mm\(^3\) or Platelets/µL

4. Remember to place your results in the appropriate scientific notation for Platelet Counts.

5. Clinical Significance:

<table>
<thead>
<tr>
<th>Increased Platelets</th>
<th>Decreased Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic Leukemia (Myeloproliferative Disorder)</td>
<td>Idiopathic Thrombocytopenic purpura</td>
</tr>
<tr>
<td>Polycythemia Vera</td>
<td>Disseminated Intravascular Coagulation</td>
</tr>
<tr>
<td>Following splenectomy</td>
<td>Aplastic anemia</td>
</tr>
</tbody>
</table>
OBJECTIVES

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

1. explain the principle of and accurately perform the following test procedures:
   a. reticulocyte stain
   b. manual reticulocyte count from stained smears
   c. make and stain an acceptable differential smear

2. state at least two patient conditions that would elevate or decrease a reticulocyte count.

3. recall and list several technical factors in each procedure listed in #1, which affect the results of the tests performed in this rotation.

4. identify those factors that will influence the thickness or thinness of a wedged blood smear

5. identify the components of Wright Stain.

6. identify the proper pH of Wright's Buffer

7. recall the colors of neutrophils, lymphocytes, monocytes, eosinophils, and red blood cells when evaluating an acceptable blood smear.

QUOTAS

- Perform one (1) Reticulocyte Count when given an unknown EDTA blood specimen.
- Perform four (4) Reticulocyte Counts from prepared smears.
- Prepare three (3) acceptable blood smears from unknown EDTA blood specimens.
- Prepare one (1) acceptable blood smear with Wrights stain – check under the microscope for acceptable staining criteria.
PRINCIPLE:

Reticulocytes are immature RBCs that contain remnant cytoplasmic RNA and organelles such as mitochondria and ribosomes. Reticulocytes are visualized by staining with supravital dyes such as new methylene blue and brilliant cresyl blue that precipitate RNA and organelles, forming a filamentous network of reticulum. The reticulocyte count is a means of assessing erythropoietic activity of the bone marrow.

Procedure:

1. Put 5 drops of New Methylene Blue N stain into a labeled 12 x 75 mm test tube using a transfer pipette.

2. Mix the EDTA unknown blood samples for 5 minutes prior to use.

3. Add 5 drops of the EDTA unknown blood sample into the labeled test tube using a transfer pipette.

4. Mix the stain-blood mixture.

5. Allow the mixture to stand 10 minutes.

6. Remix the stain and blood, adding 1-2 drops of 22% albumin to the mixture if desired. (The addition of the albumin may increase the quality of the final slide.) Place 1 drop of the mixture on a glass slide and spread in a manner similar to that for preparing a differential film.

7. Make two acceptable blood films for each unknown sample.

8. Allow slides to air dry.

Counting the Reticulocytes:

1. Place the slide on the stage of the microscope, and focus under low power to assess the distribution of RBCs and quality of the stain.

2. Place a small drop of immersion oil onto the slide and move the immersion oil objective into place and focus with the fine adjustment knob until the cells come into view.

3. Select an area where the red blood cells are evenly distributed.

4. Now, using two cell counters, start counting all the red blood cells and reticulocytes. Move the slide as you would for counting a differential WBC count. One counter is for red blood cells, the second counter is for reticulocytes.

5. When a reticulocyte is noted, it should be counted on both the red cell counter and the reticulocyte counter.
6. Count 500 red blood cells keeping track of the number of reticulocytes present. Remember, reticulocytes are counted on both counters.

7. The two reticulocyte smears should yield reproducible results within 10% of each other.

8. Add the total reticulocytes from each slide together for the calculation below.

9. An instructor must count your slide after you. Do not dispose of your stain or slides until the instructor has verified your results.

10. When you are finished counting the reticulocyte smear, remove the slide from the stage of the microscope and wipe the immersion oil objective with a Kimwipe to remove the excess immersion oil.

11. Throw the stain-blood mixture and your slides into a Sharps Container.

Calculation:

\[
\frac{\text{\# Reticulocytes counted}}{\text{Total 1000 cells counted}} \times 100 = \% \text{ Reticulocytes}
\]

Normal Values:

- Adults: 0.5 – 1.5 %
- At Birth: 2.0 - 6.0 %

Clinical Significance of a Reticulocyte Count:

<table>
<thead>
<tr>
<th>Increased Reticulocyte Count</th>
<th>Decreased Reticulocyte Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sickle Cell Anemia</td>
<td>Iron Deficiency Anemia</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>Megaloblastic Anemia</td>
</tr>
<tr>
<td>PNH</td>
<td>Aplastic Anemia</td>
</tr>
<tr>
<td>Malaria</td>
<td>Radiation</td>
</tr>
<tr>
<td>Autoimmune Disease</td>
<td></td>
</tr>
</tbody>
</table>
BLOOD SMEAR PREPARATION AND WRIGHT STAINING PROCEDURE

EQUIPMENT:

1. Microscope Slides
2. EDTA Blood Specimens
3. Plain (blue ringed) Micro Capillary Tubes
4. Kimwipes
5. Test Tube Rack
6. Tube Mixer
7. Pencil
8. Coplin Jars
9. Absolute Methanol
10. Wright Stain
11. Wright’s Buffer
12. Timer
13. Distilled Water

PROCEDURE

1. Place your EDTA blood specimens on a tube mixer for at least 10 minutes to allow the sample to adequately mix and come to room temperature.

2. Remove three (3) plain microscope slides and lay them down on a paper towel with the frosted end side UP.

3. Take the EDTA blood specimen from the tube mixer and place a Kimwipe over the top of the tube stopper and twist and pull to remove the stopper. Place the stopper down and take one plain (blue ringed) micro capillary tube and fill the tube with blood by tipping the specimen tube and inserting the micro capillary tube.

4. Place a drop of the blood specimen from the capillary tube on each of three (2) microscope slides near the frosted end of the slide.

5. Place a microscope slide with no blood [Spreader Slide] on it on an approximate 30° angle on the middle of one of the microscope slides with blood on its end. Move the spreader slide backwards until the spreader slide comes in contact with the drop of blood and the blood has spread the width of the spreader slide.

6. Holding the spreader slide at an approximate 30° angle, push the spreader slide at a constant, relatively fast speed the complete length of the slide.

7. Wave the blood smeared slide in the air to completely dry the blood film.

8. Evaluate the blood smear for the following:
Position of the feathered edge on the slide. [Should be ½ to ¾ position on the slide.]
Thickness of the blood film.
Ridges or not smooth appearance.

9. Label the slide with the appropriate information by using a pencil and writing on the frosted end of the slide.

10. Repeat until you have three acceptable blood smears.

11. See the instructor for blood smear evaluation PRIOR to the Wright Staining of the blood smears.

12. Dip each of the prepared blood smears into the Coplin Jar with the Fixative to adhere the blood onto the glass slide (fix). Dip 5 times quickly and allow to drain slightly before moving to the next Coplin jar.

13. Dip the slide five times in the Wrights stain. Allow to drain slightly before moving to Coplin jar three.

14. Dip the slide five times in the third Coplin jar (Wrights buffer). Allow to drain slightly before rinsing with distilled water.

15. Dip the slide into the beaker of distilled water to remove access stain.

16. Let air dry by standing the slides frosted end up, until completely dry.

17. Wet a Kimwipe with Distilled Water and wipe off the back of each stained slide.

18. Microscopically examine the slides using the oil immersion objective (100x) for proper Wright Staining characteristics.
OBJECTIVES

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

1. explain the principle of and accurately perform normal differentials
2. identify the normal relative percentages for those white blood cells found in normal peripheral blood.
3. recall the normal reference values for a Total WBC count.
4. calculate the absolute lymphocyte count from a patient’s relative lymphocyte count and total WBC count.
5. calculate the platelet estimate on patient differential data.
6. perform a complete differential (Differential Practicum) with the instructor with 97% accuracy.

QUOTAS

- Perform ten (10) normal differentials on prepared slides in duplicate.
- Perform an complete differential (Differential Practicum) with the instructor within one week of the conclusion of this rotation.
NORMAL DIFFERENTIALS PROCEDURE

1. There are 10 unknown NORMAL differentials labeled A through J in five black slide boxes.

2. Remove a microscope from the cabinet and set it up as follows:
   a. Remove the cover
   b. Turn the light source ON
   c. Turn the condenser so it is up just below the stage
   d. Open the iris on the condenser
   e. Turn the objective to lower power (10X)

3. Place one of the unknown slides on the microscope stage and focus under low power (10X). Locate the area, which shows good cellular distribution.

4. When in focus, move the objective out of the way and place a drop of oil on the slide.

5. Move the 100 X-oil immersion objective into place.

6. Using ONLY the fine focus adjust knob, bring the image or blood cells into focus.

7. Scan the blood film to locate the feather edge area. The cells should have a uniform appearance. If you get too deep into the blood film, the cells lose their normal morphologic appearance.

8. Review the CBC information on the worksheet. Note if the values are normal, increased, or decreased.

9. Evaluate the CBC information by correlating what you should see on the differential smear.

10. Perform a platelet estimate. Average the number of platelets in ten fields and multiply by 20,000. This estimate should approximate the direct platelet count.

11. Start at the top of the blood film after locating the proper area, and begin to locate and classify the white blood cells. Movement of the stage should be as follows:

    feather edge

12. After 100 cells are classified, the counter alarm should ring. Record your results on the worksheet. Make sure that all cells add up to 100 cells counted.

13. Observe the Red Cell morphology as you perform the 100-cell differential. Compare the cells observed on the slide to the indices results reported from the Cell-counting instrument. The two should correlate. If the red cell morphology is normal, you should record normocytic, normochromic on your worksheet for red cell morphology. Otherwise note abnormal morphology in the spaces provided on the worksheet.
14. Either take another slide of same unknown or use a different area of the slide for the duplicate. Your duplicate should match with the original count.

15. When you are finished with the slide, wipe dry with a Kimwipe. Place the unknown slide(s) back in their proper slide box.

16. Wipe the oil off the oil immersion objective using a Kimwipe. Place the low power objective (10X) in place before covering and storing.

17. Performing a differential gives a “relative” cell count in terms of percentages of total leukocytes represented by specific cell types. An “absolute” cell count provides the number of cells per given volume of blood (such as Liter) and provides a much more accurate assessment of the actual number of each cell type in the peripheral blood. To estimate an absolute count, the following calculation is used:

\[
\% \text{ of a certain cell type from differential (converted into decimal form)} \times \text{Total WBC Count} = \text{Absolute count (units are the same as the total cell count)}.
\]

To find the normal range for the absolute cell counts, simply take the normal range for each type of cell and calculate an absolute count on the lowest and highest number in that range. This will give you the normal range for a particular cell.

UNKNOWN RESULTS CAN BE CHECKED WITH THE INSTRUCTOR

AT THE COMPLETION OF ALL UNKNOWNS, PLEASE MAKE AN APPOINTMENT WITH THE INSTRUCTOR FOR THE FINAL UNKNOWN NORMAL DIFFERENTIAL EVALUATION. SEE BELOW!

EVALUATION:

At the end of ROTATION D [HEMATOLOGY II] when all ten (10) normal differentials have been completed, the student must make an appointment with the instructor to do an unknown normal differential on the conference microscope. Satisfactory performance is identifying correctly 97 cells out of the 100 cells counted. If the student fails to complete this objective successfully, the student will receive an ‘F’ for a final course grade.

The student should be able to evaluate the CBC results as well. Note if values are increased, decreased, or normal. The student should be able to discuss with the instructor how changes in the CBC values manifest in the differential results.
DIFFERENTIAL PRACTICUM:

The Differential Practicum will cover all aspects of performing a differential. The student will correlate the CBC Instrument results with morphology on the differential. The student will correctly identify 97 out of 100 WBC’s with the Instructor on the teaching microscope. RBC Morphology will be discussed and correlated with indices results. The student should be able to note which CBC results are increased, decreased, or normal. The Differential Practicum should be completed within 1 week of completing D-Rotation. **Failure to complete this objective results in a final course grade of “F”.**

My Differential Practicum Appointment is: ____________________________________________

Instructor: ______________________________________________________________________

------------------------------------------------------------------------------------------------------------------------

(To be given to the Instructor):

______________________________________ will complete the Differential Practicum on
______________________________________ 2008
OBJECTIVES

During the laboratory session, students will perform MACROSCOPIC AND MICROSCOPIC URINALYSIS including:

- Description of the macroscopic appearance of the urine specimen
- Chemical analysis of the urine specimen using multi-test urinary dipsticks
- Performance of manual confirmatory tests when indicated by urinary dipstick results
- Microscopic examination of urinary sediment for cellular elements, crystals, casts, and other findings.
- Quality control testing on dipstick, refractometer and confirmation tests.

RECORDING RESULTS:

1. CHEX-STIX, Manual Refractometer QC Results, and the Confirmation Tests QC Results will be recorded on the Routine Urinalysis Quality Control Log Sheet.

2. Macroscopic Patient Unknowns will be recorded on the Routine Urinalysis Log Worksheet.

3. Confirmation Test results for patient unknowns will be recorded on the Confirmation Test Log Sheet. This is found on the backside of the Routine Urinalysis Log Worksheet.

4. Microscopic Patient Unknowns will be recorded on the Microscopic Urinalysis Log Sheet. The correct format for reporting results is found on the backside of this worksheet. All reported results should follow the appropriate format.

QUOTAS

- Perform Macroscopic, Chemical Analysis, and Microscopic Analysis on four urine specimens
- Perform Quality Control on Dipstick, Refractometer, and Confirmation Test(s)

It is essential that an Instructor check your results before leaving the laboratory each week. If you need to repeat a procedure, it must be repeated before the end of the week or the specimen will be discarded. If you fail to repeat the procedure on the provided specimen, you will have to repeat the entire lab on a different day on new specimens.
ROTATION F: ROUTINE MACROSCOPIC URINALYSIS PROCEDURES

CHEMISTRY STRIP QUALITY CONTROL PROCEDURE:

1. Place 12 mL of distilled water in a clear, plastic graduated KOVA centrifuge tube.
2. Remove a CHEK-STIX from the bottle and place the strip into the 12 ml. of distilled water and cap tightly.
3. Invert the tube gently back and forth for 2 minutes.
4. Allow the tube to stand for 30 minutes at room temperature.
5. Invert one more time. Remove and discard the CHEK-STIX.
6. Remove an MULTISTIX 10 SG reagent strip from the bottle and replace the cap.
7. Completely immerse the reagent areas of the strip in the CHEK-STIX urine solution and remove immediately to avoid dissolving out the reagents.
8. While removing the strip, run the edge of the strip against the rim of the KOVA graduated centrifuge tube to remove excess urine. Hold the strip in a horizontal position to prevent possible mixing of chemicals from adjacent reagent areas and/or contaminating the hands with urine.
9. Compare the reagent areas to the corresponding Color Chart on the bottle label at the time specified. HOLD THE STRIP CLOSE TO THE COLOR BLOCKS AND MATCH CAREFULLY. Avoid laying the strip directly on the Color Chart, as this will result in the urine soiling the chart.

READING TIMES ARE CRITICAL FOR THE DIFFERENT TESTS!

<table>
<thead>
<tr>
<th>Test</th>
<th>Reading Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>30 seconds</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>30 seconds</td>
</tr>
<tr>
<td>Ketones</td>
<td>40 seconds</td>
</tr>
<tr>
<td>Specific Gravity</td>
<td>45 seconds</td>
</tr>
<tr>
<td>Blood</td>
<td>60 seconds</td>
</tr>
<tr>
<td>pH</td>
<td>immediately to 60 seconds</td>
</tr>
<tr>
<td>Protein</td>
<td>immediately to 60 seconds</td>
</tr>
<tr>
<td>Urobilinogen</td>
<td>60 seconds</td>
</tr>
<tr>
<td>Nitrite</td>
<td>60 seconds</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>2 minutes</td>
</tr>
</tbody>
</table>

NOTE: Color changes that occur after 2 minutes are of no diagnostic significance.
10. Record your CHEK-STIX results on the ROUTINE URINALYSIS QUALITY CONTROL log sheet.

11. Discard the MULTISTIX 10 SG reagent strip into a biohazard container.

REFRACTOMETER QUALITY CONTROL PROCEDURE

1. Turn the Refractometer Lamp ON by pressing in the black switch in the back of the meter next to the power cord.

2. Make sure the plastic cover plate is placed over the measure prism.

3. Using a plastic transfer pipette, place a drop of DISTILLED WATER on the top of the plastic cover plate so that the sample flows by capillary action under the cover plate in contact with the measuring prism.

4. Place your finger on the plastic cover plate and press gently but firmly. This spreads a minimal volume of sample in a thin even layer over the prism.

5. If necessary, bring the scale seen in the eyepiece into best focus by rotation of the eyepiece.

6. Make the URINE SPECIFIC GRAVITY T/C reading from the lower left scale. Record your results on the ROUTINE URINALYSIS QUALITY CONTROL log sheet.

   **Expected Results**
   
   Distilled Water  \[1.000 \pm 0.001\]

7. Use a Kimwipe moistened with distilled water for wiping the prism and wipe dry with a second Kimwipe before the next sample is added to the prism.

8. Process the following Quality Control solutions on the Refractometer and record their results on the ROUTINE URINALYSIS QUALITY CONTROL log sheet:

   **Expected Results**
   
   CHEK-STIX  \[1.000 - 1.015\]
   
   5% NaCl Solution  \[1.022 \pm 0.001\]
   
   9% Sucrose Solution  \[1.034 \pm 0.001\]

**NOTE:** If your results do NOT fall within the appropriate ranges, please notify the instructor.
CONFIRMATION TESTS QUALITY CONTROL PROCEDURES:

Use the CHEK-STIX solution for quality control material for the following confirmation tablet tests: CLINITEST, ACETEST, ICTOTEST, and SULFOSALICYLIC ACID.

1. **CLINITEST Tablet Test**
   - Using a plastic disposable transfer pipette, place 10 drops of distilled water into a 16 x 100 mm test tube. Using a second plastic disposable transfer pipette add 5 drops of the quality control solution. Mix well.
   - Place a test tube in a test tube rack. Open 1 foil CLINITEST TABLET and without touching the tablet, drop the tablet into the test tube and watch while the complete boiling reaction takes place. Do NOT shake the test tube during boiling, or for the following 15 seconds after the boiling has stopped.
   - **DO NOT TOUCH THE BOTTOM OF THE TEST TUBE! THE CHEMICAL REACTION THAT OCCURS PRODUCES A GREAT AMOUNT OF HEAT!!**
   - After the end of this 15 second waiting period, shake the test tube gently to mix the contents. Compare the solution’s color to the color chart. Record your results in mg/dL units as follows on the Routine Urinalysis Quality Control Log Sheet:
     
     | Percentage | mg/dL  |
     |------------|--------|
     | 0%         | 0.00   |
     | 0.25%      | 250    |
     | 0.5%       | 500    |
     | 0.75%      | 750    |
     | 1%         | 1000   |
     | 2% or more | 2000   |

2. **ACETEST Tablet Test**
   - Without touching the tablet with your fingers, remove 1 tablet and place it on a clean Kimwipe.
   - Using a plastic disposable transfer pipette, add 1 drop of quality control solution directly on top of the tablet.
   - Allow the reaction to occur for 30 seconds, then compare the color change of the tablet to the color chart provided on the package insert.
   - Record your results as negative, small, moderate, or large amount of ketone (specifically acetoacetate) on the Routine Urinalysis Quality Control Log Sheet.
3. **ICTOTEST Tablet Test**

- Remove from the package 1 white absorbent test mat square and place it on a clean Kimwipe.
- Using a plastic disposable pipette, add 10 drops of the quality control solution to the center of the white absorbent test mat square.
- Without touching the ICTOTEST tablet with your fingers, place 1 tablet on the center of the absorbent test mat square.
- Using a second plastic disposable transfer pipette, place 1 drop of distilled water on the tablet and wait for 5 seconds. Then place a second drop of distilled water on the tablet so that the water runs off the tablet onto the mat.
- Observe the color of the mat around the tablet at 60 seconds. Match the color change on the white absorbent mat square with the package insert’s color chart.
- Record your results as either negative or positive on the Routine Urinalysis Quality Control Log Sheet.

4. **3% Sulfosalicylic Acid Test For Protein**

- Add approximately 3 mL. of Quality Control Solution to a 16 x 100 mm test tube. Using the dispenser, add 3 mL of 3% SSA. Invert twice and let the mixture sit for 10 minutes. Grade the turbidity as follows:

  - **Negative:** No Turbidity (Appx. 0.005 g/dL)
  - **Trace:** Perceptible Turbidity (Appx. 0.020 g/dL)
  - **1+:** Distinct Turbidity but no discrete granulation (Appx. 0.050 g/dL)
  - **2+:** Turbidity with granulation but no flocculation (Appx. 0.20 g/dL)
  - **3+:** Turbidity with granulation and flocculation (Appx. 0.50 g/dL)
  - **4+:** Clumps of precipitated protein or solid precipitate (Appx. 1.0 g/dL or more)

  Record your results on the Routine Urinalysis Quality Control Log Sheet
PATIENT/UNKNOWN SAMPLE PROCEDURE:
1. Make sure the sample is well mixed by swirling the urine specimen in the container before continuing.

2. Immediately pour over 10 ml. of the urine into a labeled disposable KOVA graduated centrifuge tube.

3. Determine the URINE COLOR and URINE APPEARANCE.
   Examples: “yellow and cloudy”
   “ dark yellow and clear”
   “ light yellow and hazy”

4. Report the presence of any unusual URINE ODOR or abnormal amounts of COLORED FOAM.

5. Perform the URINE SPECIFIC GRAVITY on the REFRACTOMETER as outlined in the REFRACTOMETER QUALITY CONTROL PROCEDURE. Record your results in the appropriate place on the ROUTINE URINALYSIS WORKSHEET.

6. Invert the urine sample to mix and perform the MULTISTIX 10 SG reagent strip tests according to the procedure outline under the CHEMISTRY STRIP QUALITY CONTROL PROCEDURE. Record your results on the ROUTINE URINALYSIS WORKSHEET.

7. Cap the urine-filled KOVA tubes and centrifuge the specimens for 5 minutes at 1500 rpm’s.
   NOTE: This speed using the Damon/IEC centrifuge provides 400 RCFs.

8. Perform the CONFIRMATION TESTS on the clear urine supernatant if the following MULTISTIX TESTS are positive:

<table>
<thead>
<tr>
<th>MULTISTIX TESTS</th>
<th>CONFIRMATION TESTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>3% Sulfosalicylic Acid Test</td>
</tr>
<tr>
<td>Glucose</td>
<td>CLINITEST TABLET</td>
</tr>
</tbody>
</table>

   NOTE: ALL PATIENTS UNDER 2 YEARS OLD are tested for reducing substances using the CLINITEST TABLET.

   | Ketone          | ACETEST TABLET     |
   | Bilirubin       | ICTOTEST TABLET    |

9. If the MULTISTIX Protein result is positive, confirm this result using the 3% SSA Test Procedure. This is performed exactly as outlined in the CONFIRMATION TESTS QUALITY CONTROL PROCEDURES, using the clear centrifuged supernatant from the patients specimen instead of the AMES CHEK-STIX solution.

10. If the MULTISTIX Glucose result is positive, confirm this result using the CLINITEST TABLET Procedure. This is performed exactly as outlined in the CONFIRMATION TESTS QUALITY CONTROL PROCEDURES, using the clear centrifuged supernatant from the patient specimen instead of the AMES CHEK-STIX solution.
11. If the MULTISTIX Ketone result is positive, confirm this result using the ACETEST TABLET Procedure. This is performed exactly as outlined in the CONFIRMATION TESTS QUALITY CONTROL PROCEDURES, using the clear centrifuged supernatant from the patient specimen instead of the AMES CHEK-STIX solution.

12. If the MULTISTIX Bilirubin result is positive, confirm this result using the ICTOTEST TABLET Procedure. This is performed exactly as outlined in the CONFIRMATION TESTS QUALITY CONTROL PROCEDURES, using the clear centrifuged supernatant from the patient specimen instead of the AMES CHEK-STIX solution.

13. After all confirmation tests have been completed and the urine supernatant is no longer needed, insert a special KOVA PETTER transfer pipette into the KOVA centrifuge tube and seat the KOVA PETTER into the bottom of the centrifuge tube.

14. Pour the urine supernatant out into the sink. Exactly 1 mL. of urine supernatant will be held back with the urine sediment.

15. Mix the urine sediment with the supernatant by squeezing the KOVA PETTER transfer pipette bulb.

16. Continue on with the Microscopic Urinalysis Procedure directly following this page.
ROTATION F: ROUTINE MICROSCOPIC URINALYSIS PROCEDURES

1. Remove a binocular microscope from the cabinet. Place the 10x objective into position and LOWER THE CONDENSER.

2. If you want to use SEDI-STAIN, place ½ of the urine sediment into a separate small 10 x 75 mm test tube and add one drop of the SEDI-STAIN to the test tube. To mix, take a separate plastic transfer pipette and mix the stain with the sediment.

3. Mix the urine sediment with the KOVA PETTER and place a small drop of the first specimen into the position #1 on the KOVA SLIDE.

4. Scan the EDGES and the ENTIRE SLIDE AREA of the first specimen using the 10x objective and record those items seen under low power. Record your results on the MICROSCOPIC URINALYSIS log sheet using the “SCALE FOR REPORTING RESULTS OF EXAMINATION OF URINARY SEDIMENT” on the next page.

5. Place the 40x objective into position.

6. Scan approximately 10 - 12 fields and record those items seen under high power. Record your results on the MICROSCOPIC URINALYSIS log sheet using the “SCALE FOR REPORTING RESULTS OF EXAMINATION OF URINARY SEDIMENT” on the next page.

7. Place an “X” over the coverglass of position #1 with a crayon marker so it will not be confused with an empty position.

8. Place the 10x objective into position. Mix the urine sediment with the KOVA PETTER and place a small drop of the second specimen to position #2 on the KOVA SLIDE and repeat steps #4-7.

9. Place an “X” over the cover glass of position #2 with a crayon marker so it will not be confused with an empty position.

10. Continue with the other urine sediments. Discard the Kova Slide when all positions have been filled and read.

11. **Have an instructor check your work** before you put away your microscope for the day.
# Scale for Reporting Results of Examination of Urinary Sediment

## RBCs and WBCs: cells/HPF

<table>
<thead>
<tr>
<th>Count</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td></td>
</tr>
<tr>
<td>2-5</td>
<td></td>
</tr>
<tr>
<td>5-10</td>
<td></td>
</tr>
<tr>
<td>10-25</td>
<td></td>
</tr>
<tr>
<td>25-50</td>
<td></td>
</tr>
<tr>
<td>50-99</td>
<td></td>
</tr>
<tr>
<td>&gt;100</td>
<td></td>
</tr>
</tbody>
</table>

## Casts and Abnormal Crystals / LPF

<table>
<thead>
<tr>
<th>Count</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>neg</td>
<td></td>
</tr>
<tr>
<td>0-2</td>
<td></td>
</tr>
<tr>
<td>2-5</td>
<td></td>
</tr>
<tr>
<td>5-10</td>
<td></td>
</tr>
<tr>
<td>10-25</td>
<td></td>
</tr>
<tr>
<td>25-50</td>
<td></td>
</tr>
<tr>
<td>&gt;50</td>
<td></td>
</tr>
</tbody>
</table>

## Epithelial Cells and Normal Crystals

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rare</td>
<td>1 in every 5 high power fields</td>
</tr>
<tr>
<td>Occasional</td>
<td>1 in every high power field</td>
</tr>
<tr>
<td>Few</td>
<td>2-5 in every high power field</td>
</tr>
<tr>
<td>Moderate</td>
<td>5-10 in every high power field</td>
</tr>
<tr>
<td>Many</td>
<td>&gt; 10 in every high power field</td>
</tr>
</tbody>
</table>

## Amorphous Material:
- light, moderate, or heavy

## Mucus (Mucous):
- negative, light, moderate, or heavy amounts

## Bacteria or Yeast:
- negative, light, moderate, or heavy amounts

## Normal Crystals:
- identify type and number per high power field (see above table)

### NORMAL VALUES:
- **RBCs:** 0-5 / hpf
- **WBCs:** 0-10 / hpf
- **Renal Tubular Cells:** 0-2 / hpf
- **Hyaline Casts:** 0-2 / lpf
- **Granular Casts:** 0-1 / lpf

(Specific for this procedure!)
ROTATION G:
ROUTINE URINALYSIS

OBJECTIVES

During the laboratory session, students will perform MACROSCOPIC AND MICROSCOPIC URINALYSIS including

- description of the macroscopic appearance of the urine specimen
- chemical analysis of the urine specimen using multi-test urinary dipsticks
- performance of manual confirmatory tests when indicated by urinary dipstick results
- microscopic examination of urinary sediment for cellular elements, crystals, casts, and other findings.
- quality control testing on dipstick, refractometer and confirmation tests.

RECORDING RESULTS:

1. CHEX-STIX, Manual Refractometer QC Results, and the Confirmation Tests QC Results will be recorded on the Routine Urinalysis Quality Control Log Sheet.

2. Macroscopic Patient Unknowns will be recorded on the Routine Urinalysis Log Worksheet

3. Confirmation Test results for patient unknowns will be recorded on the Confirmation Test Log Sheet. This is found on the backside of the Routine Urinalysis Log Worksheet.

4. Microscopic Patient Unknowns will be recorded on the Microscopic Urinalysis Log Sheet. The correct format for reporting results is found on the backside of this worksheet. All reported results should follow the appropriate format.

QUOTAS

- Perform Macroscopic, Chemical Analysis, and Microscopic Analysis on six (6) urine specimens
- Perform Quality Control on Dipstick, Refractometer, and Confirmation Test(s)

It is essential that an instructor check your results before leaving the laboratory each week. If you need to repeat a procedure, it must be repeated before the end of the week or the specimen will be discarded. If you fail to repeat the procedure on the provided specimen, you will have to repeat the entire lab on a different day on new specimens.

PROCEDURE:
Refer to pages 106-113 in this Syllabus.
OBJECTIVES

During the laboratory session, students will perform MACROSCOPIC AND MICROSCOPIC URINALYSIS including:

- description of the macroscopic appearance of the urine specimen
- chemical analysis of the urine specimen using multi-test urinary dipsticks
- performance of manual confirmatory tests when indicated by urinary dipstick results
- microscopic examination of urinary sediment for cellular elements, crystals, casts, and other findings.
- quality control testing on dipstick, refractometer and confirmation tests.

RECORDING RESULTS:

1. CHEX-STIX, Manual Refractometer QC Results, and the Confirmation Tests QC Results will be recorded on the Routine Urinalysis Quality Control Log Sheet.

2. Macroscopic Patient Unknowns will be recorded on the Routine Urinalysis Log Worksheet.

3. Confirmation Test results for patient unknowns will be recorded on the Confirmation Test Log Sheet. This is found on the backside of the Routine Urinalysis Log Worksheet.

4. Microscopic Patient Unknowns will be recorded on the Microscopic Urinalysis Log Sheet. The correct format for reporting results is found on the backside of this worksheet. All reported results should follow the appropriate format.

QUOTAS

- Perform Macroscopic, Chemical Analysis, and Microscopic Analysis on eight (8) urine specimens
- Perform Quality Control on Dipstick, Refractometer, and Confirmation Test(s)

It is essential that an Instructor check your results before leaving the laboratory each week. If you need to repeat a procedure, it must be repeated before the end of the week or the specimen will be discarded. If you fail to repeat the procedure on the provided specimen, you will have to repeat the entire lab on a different day on new specimens.

PROCEDURE:

Refer to pages 106-113 in this Syllabus.
OBJECTIVES

At the end of this laboratory session, the student will be able to:

1. prepare a Urine Total Protein standard curve.

2. determine the Urine Total Protein concentration on two (2) quality control samples and compare their results with acceptable quality control ranges.

3. determine the Urine Total Protein concentrations on one (1) unknown sample and the 24-hr urine sample in duplicate and compare the duplicate results with the quality control data to ensure reliability.

4. resolve quality control, patient sample, and standard curve errors by repeating the procedure until valid results are obtained.

5. explain the principle of the Urine Total Protein procedure.

6. determine the Absorbance of Urine Total Protein samples using a Gilford Stasar III spectrophotometer.

7. recall the wavelength at which Urine Total Protein Absorbances are made.

8. plot the Standard Absorbance Values versus Standard Urine Total Protein Concentrations on linear graph paper.


10. recognize those Standard data points that are non-linear.

11. state the procedure for collecting a 24-hour urine specimen.
ROTATION I
QUANTITATIVE URINE TOTAL PROTEIN PROCEDURE

PRINCIPLE: Protein, in low concentrations, reacts with a pyrogallol red-molybdenum complex to form a violet-color complex. This color is read spectrophotometrically at 600 nm. The addition of a combination of surfactants renders the reactivity of human albumin and globulins approximately equal, enabling this procedure to be used for both urine and CSF protein analysis.

CLINICAL SIGNIFICANCE: The kidney glomeruli behave as ultrafilters for the plasma proteins. Normally, only a small amount of albumin is excreted. Increased protein in urine can be found with increased glomerular permeability, defective tubular reabsorption, increased concentration in plasma of abnormal low molecular weight protein, and abnormal secretion of protein into the urinary tract.

REFERENCE RANGE: Urine Total Protein

1 - 14 mg/dL [Random Urine]
20 - 150 mg/24 hrs

MATERIALS REQUIRED:

- Microprotein Reagent
- Microprotein Standard Set (5, 25, 50, 100, and 200 mg/dL)
- Quality Control Material: (2) Normal and Abnormal
- Unknown Samples:
  Students will perform this procedure on their own 24-hour urine collection, and a second unknown urine sample that will be provided.
- 12 x 75 mm test tubes
- 10 mL Mohr pipette
- 20 µL semi-automated pipette
- Marking pen
- Gilford Stasar III Spectrophotometer
PROCEDURE:

1. Mix the 24-urine sample well. **Measure the total volume and record on the worksheet.** This value will be used to calculate the total protein amount for the sample at the end of the lab. Pour off an aliquot of the 24-hour urine sample to be used during lab. (Urine cup will be provided).

2. Pick up twelve (12) 12 x 75 mm test tubes and label accordingly:

<table>
<thead>
<tr>
<th>Tube</th>
<th>Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube 1</td>
<td>Blank</td>
</tr>
<tr>
<td>Tube 2</td>
<td>5 mg/dL</td>
</tr>
<tr>
<td>Tube 3</td>
<td>25 mg/dL</td>
</tr>
<tr>
<td>Tube 4</td>
<td>50 mg/dL</td>
</tr>
<tr>
<td>Tube 5</td>
<td>100 mg/dL</td>
</tr>
<tr>
<td>Tube 6</td>
<td>200 mg/dL</td>
</tr>
<tr>
<td>Tube 7</td>
<td>QC N</td>
</tr>
<tr>
<td>Tube 8</td>
<td>QC Ab</td>
</tr>
<tr>
<td>Tube 9</td>
<td>Your Name (24 hr specimen #1)</td>
</tr>
<tr>
<td>Tube 10</td>
<td>Your Name (24 hr specimen duplicate)</td>
</tr>
<tr>
<td>Tube 11</td>
<td>Unknown Patient Name</td>
</tr>
<tr>
<td>Tube 12</td>
<td>Unknown Patient Name duplicate</td>
</tr>
</tbody>
</table>

Place in order in the test tube rack.

3. Bring reagent, standards, controls and unknown samples to room temperature.

4. Using a 10 mL Mohr pipette, dispense 1.0 mL of Microprotein Reagent into each labeled tube.

5. With a 20 µL semi-automated pipette, add 20 µL of each of the following to your labeled tubes:

<table>
<thead>
<tr>
<th>Tube</th>
<th>20 uL of</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>distilled water</td>
</tr>
<tr>
<td>5 mg/dL</td>
<td>5 mg/dL Standard</td>
</tr>
<tr>
<td>25 mg/dL</td>
<td>25 mg/dL Standard</td>
</tr>
<tr>
<td>50 mg/dL</td>
<td>50 mg/dL Standard</td>
</tr>
<tr>
<td>100 mg/dL</td>
<td>100 mg/dL Standard</td>
</tr>
<tr>
<td>200 mg/dL</td>
<td>200 mg/dL Standard</td>
</tr>
<tr>
<td>QC N</td>
<td>Normal Quality Control Sample</td>
</tr>
<tr>
<td>QC AB</td>
<td>Abnormal Quality Control Sample</td>
</tr>
<tr>
<td>Your 24 hr sample</td>
<td>24 hour urine sample from the aliquot</td>
</tr>
<tr>
<td>24-hr duplicate</td>
<td>24 hour urine sample from the aliquot</td>
</tr>
<tr>
<td>Unknown Patient</td>
<td>Unknown patient sample provided</td>
</tr>
<tr>
<td>Unknown duplicate</td>
<td>Unknown patient sample provided</td>
</tr>
</tbody>
</table>

Mix each tube well using the vortex.

6. Incubate the tubes for 10 minutes at 37°C in the incubator.
7. With an instructor present, set the wavelength of the spectrophotometer to 600 nm, using the wavelength control knob.

8. Adjust the temperature setting to 37° C with the temperature control knob.

9. Set the sample time control knob to 2. This determines the length of time the instrument uses vacuum to aspirate the sample through the cuvette.

10. Insert the tip of the aspiration tubing into the reagent blank test tube, raising the test tube until the aspiration tube reaches the bottom.

11. **Quickly depress** the sample actuator bar, allowing the instrument to aspirate sample. If the bar is pressed too far, continuous aspiration will result.

12. Withdraw the tube and adjust zero control for a reading of 0.000 on the LED readout.

13. Without a tube in place, depress the sample actuator bar completely, holding it “in” for at least 5 seconds. This purges the sample into the waste collection container and replaces the sample in the cuvette and lines with air.

14. Read and record the absorbance of all of the Standard, QC, and unknown tubes. Purge with air for at least 5 seconds between samples to reduce carry-over within the flow-through cuvette.

15. When all samples have been read, purge approximately 10 mL of “Flow-kleen” through the cuvette, allowing the cuvette to soak for 10 minutes. Follow with 10 seconds of purging air, then purging with distilled water. If you are the last person to use the spectrophotometer, perform the end of day cleaning procedure. Set the temperature control back to 25C. Shut off the vacuum pump and then the spectrophotometer. Empty the collection container into the sink.

16. Turn the linear graph paper sideways – using the longer side for concentration and the shorter side for absorbance.

17. Plot your Standard concentrations against their respective absorbance results, and draw the "best fit" line. Use this plot to then determine the concentrations of your QC and unknown samples.

18. Check your QC results to see if they are acceptable. Obtain the acceptable range from the instructor.

19. Calculate the final total protein per 24 hours for your unknown samples as follows:

   \[ \text{Unknown Total Protein (mg/dL)} \times 0.01 = \text{mg / mL} \]

   \[ \text{Unknown Total Protein (mg/mL)} \times \text{Urine Total Volume (mL / 24 hrs)} = \text{mg / 24 hrs} \]

20. You will not be able to calculate the unknown patient per 24 hour because you do not have the urine total volume value.
OBJECTIVES

At the end of this Body Fluid rotation, the student should be able to:

1. explain the principle of and accurately perform the following test procedures:
   a. Manual Eosinophil Count
   b. Body Fluid Count

2. for each test listed in #1, state at least 2 patient conditions, which would elevate or decrease the test result.

3. identify the area of the counting chamber used for body fluid cell counts

4. describe the procedure used to differentiate between a large number of WBCs and RBCs seen in a body fluid cell count.

5. calculate RBCs, WBCs, and Eosinophils in mm$^3$ or uL from cell count data

QUOTAS

- Perform two (2) Manual Eosinophil counts on unknown EDTA blood samples.
- Perform one (1) Body Fluid count on unknown body fluid sample
ROTDATION J
EOSINOPHIL COUNT PROCEDURE

PRINCIPLE:
This method is based on the accepted procedure for eosinophil enumeration using modified Pilot's solution containing phloxine B as a selective stain for eosinophils. Propylene glycol, which is incorporated in the diluent to act as a vehicle for the phloxine B stain due to its viscosity and low evaporation rate, renders erythrocytes relatively nonrefractile so that they will not interfere with visual counting procedures.

Fresh whole blood is added to the diluent, which preserves all leukocytes but stains only eosinophils. The diluted specimen is added to a hemacytometer according to accepted technique. Under the 10X objective (100X magnification), eosinophils appear bright orange-red and are clearly distinguishable from neutrophils, basophils, lymphocytes and monocytes, which do not stain.

Reagents:
1. Unopette Reservoir containing 0.775 mL of diluent mixture:
   - Phloxine B 1 gm
   - Propylene glycol 500 mL
   - Distilled water qs to 1 liter
2. Unopette Capillary Pipette 25 µL capacity

Procedure:
1. Puncture Diaphragm: Using the protective shield on the capillary pipette, puncture the diaphragm of the reservoir as follows:
   a. Place reservoir on a flat surface. Grasping reservoir in one hand, take pipette assembly in other hand and push tip of pipette shield firmly through diaphragm in neck of reservoir, then remove.
   b. Remove shield from pipette assembly with a twist.

2. Add sample: Fill capillary with fresh whole blood and transfer to reservoir as follows:
   a. Holding pipette almost horizontally, touch tip of pipette to blood. Pipette will fill by capillary action. Filling is complete and will stop automatically when blood reaches neck of pipette.
   b. Wipe excess blood from outside of capillary pipette, making certain that no sample is removed from capillary bore.
   c. Squeeze reservoir slightly to force out some air. Do not expel any liquid. Maintain pressure on reservoir.
   d. Cover opening of overflow chamber of pipette with index finger and seat pipette securely in reservoir neck.
e. Release pressure on reservoir. Then remove finger from pipette opening. Negative pressure will draw blood into diluent.

f. Squeeze reservoir gently two or three times to rinse capillary bore, forcing diluent into, but not out of, overflow chamber, releasing pressure each time to return mixture to reservoir.

CAUTION: If reservoir is squeezed too hard, the specimen may be expelled through the top of the overflow chamber, resulting in contamination of the ringers.

g. Place index finger over upper opening and gently invert several times to thoroughly mix blood with diluent. Eosinophil count should be performed within one (1) hour.

3. Charge Hemacytometer: Mix diluted blood thoroughly by inverting reservoir (2g above) to resuspend cells.

a. Convert to dropper assembly by withdrawing pipette from reservoir and reseating securely in reverse position.

b. To clean capillary bore, invert reservoir, gently squeeze sides and discard first three or four drops.

c. Carefully charge hemacytometer with diluted blood by gently squeezing sides of reservoir to expel contents until chamber is properly filled.

d. Place hemacytometer on moistened filter paper in Petri dish. Cover Petri dish and allow to stand 10 minutes to permit cells to settle. (Moistened filter paper retards evaporation of diluted specimen while standing.)

4. Count and Calculate: With Neubauer hemacytometer, an eosinophil count is performed as follows:

a. Using 100X magnification (10 X Objective), count eosinophils on both sides of the counting chamber.

[i.e., 9 ‘W’ squares on one side and 9 ‘W’ squares on the other side = 18 ‘W’ squares]

**Dilution**

\[
\text{EDTA Sample Volume} + \text{Diluent Volume} = \text{Total Volume} \\
[25 \mu L = 0.025 \text{ mL}] + [0.775 \text{ mL}] = [0.8 \text{ mL}] \\
\frac{0.025 \text{ mL EDTA Sample Volume}}{0.800 \text{ mL Total Volume}} = \frac{1}{X} ; \quad X = 32
\]
**Calculation**

Number of Eosinophils counted in 18 “W” squares X Dilution (32) X depth (10)

Number of Large (“W”) squares Counted (18)

*Normal Values:* Adults: 0 - 450 Eosinophils/mm$^3$
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 if possible.

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 semi automated 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 semi automated 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 semi automated 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 semi automated 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)

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:

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

2. 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.)

3. 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.

4. 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.

5. 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:**

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

   \[
   \frac{\text{Total # of cells counted} \times \text{DCF} \times 10}{\text{# of large (W) squares counted (mm}^3\text{)}} = \text{#cells per mm}^3 (\mu\text{L})
   \]

**REMEMBER:** Body Fluid cell counts are reported out as whole numbers per mm$^3$, regardless of how big the number is. Scientific notation format ($x 10^3 / \text{mm}^3$, $x 10^6 / \text{mm}^3$, etc.) is used only for cell counts performed on whole blood samples.
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>