CLS417 - Clinical HEMATOLOGY II
Automated Unit

I. Definition of Automated Unit
A. Perform quality control checks and maintenance of the automated cell counter.

B. Specimen handling:
   1. Evaluate sample acceptability by checking labeling, quantity, & quality.
   2. Prepare blood smears as needed.
   3. Demonstrate correct handling of specimens and contaminated equipment.
   4. Set-up and report erythrocyte sedimentation rates.

C. Operate the automated cell counter for patient workload - perform alternative testing (e.g., dilution) as needed to correct and/or verify “problem” results.

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

E. Perform CBCs with manual differential meeting established criteria, including WBC & PLT estimates, evaluation of cell morphology, and checking data correlation of CBC parameters with smear findings:
   1. Perform leukocyte differentials, recognizing normal and abnormal differential variations.
   2. Perform WBC estimates from the blood smear which are in agreement with the automated WBC ± 20%.
   3. Perform platelet estimates from the blood smear which are in agreement with the automated PLT count ± 20% if > 50,000/cmm and ± 10,000 if < 50,000/cmm.

F. Practical will include CBCs with manual differential and WBC/PLT estimates.

NOTE: Refer to your site’s instrument handout and/or procedure manual for specific information regarding instrument principle(s), reagents/components, reference ranges, critical values, linearity limits, etc.

II. Objectives regarding Specimen Handling and Erythrocyte Sedimentation Rates
A. Specimen Handling
   1. Describe specimen requirements for each test performed, including expiration times.

   2. Assess sources of collection error that may invalidate laboratory determinations, including corrective action for pre-analytic errors.

   3. Explain the proper procedure to follow in the event of an accident (e.g., spill), including the location of the MSDS manual.

B. Erythrocyte Sedimentation Rates, Westergren method
   1. Discuss the principle, acceptable specimens, normal values, sources of error, and clinical significance of the manual Westergren sedimentation rate.

   2. Describe technical and physiologic variables (e.g., fibrinogen concentration) that influence the Westergren sedimentation rate.

   3. Discuss those conditions/disease states where elevations of the ESR are common.

NOTE: You are responsible for information regarding the manual Westergren ESR procedure (reference method). Most automated ESR methods take less than 1 hour and sedimentation is determined optically. Be familiar with the automated ESR method performed at your institution.
III. Objectives regarding Quality Control, Theory and Operation of Automated Cell Counters

A. Quality Control and Maintenance

1. Discuss the importance of a good quality control program in the clinical laboratory, including QC methods utilized.

2. Perform calculations necessary for quality control:
   a. Means, modes, and medians of sets of lab data.
   b. Standard deviation (SD) and coefficient of variation (CV)

3. Define the following:
   a. accuracy, precision and reliability
   b. sensitivity and specificity

4. Describe a normal frequency (Gaussian) distribution of values.

5. Describe a Levy-Jennings quality control chart, including standard deviations from the mean (confidence limits/intervals) expressed in percentage of values.

6. Analyze QC data (i.e., Levy-Jennings chart) to determine:
   a. Violations of Westgard rules, e.g., 1-2s.
   b. Presence of a shift or trend
   c. Random or systematic error

7. Describe criteria used for selection of control materials to monitor the validity of a test system.

8. Evaluate control results to detect values that fall outside acceptable limits or that fail to exhibit appropriate positive or negative results, taking corrective action.

9. Describe the frequency of routine quality control monitoring on the automated cell counter, including situations that require additional monitoring.

10. Analyze deviations in patient or QC values that may be the result of instrument malfunction, describing corrective action(s).

11. Interpret control results to differentiate a control problem from a reagent/instrument problem when two or more control levels are run.

12. Differentiate standards used for calibration from controls, including indications for calibration.

13. *Explain the process used when assaying a new lot number of control material to replace current QC material.*

14. *Explain the use of X-B analysis (Bull's moving averages) as a method of monitoring instrument performance to determine the need for recalibration of the instrument.*

15. Recognize acceptable background counts/system checks when performing daily preventive maintenance.

B. Theory and Operation of the Automated Cell Counters

1. Describe the theory of the Coulter electronic impedance principle of cell counting and sizing.

2. Describe the theory of the light scatter principle of cell analysis using flow cytometry.
3. Define cell coincidence.

4. Explain the advantage of hydrodynamic focusing to induce laminar flow of cells through the sensing zone of the flow cell.

5. Explain the use of thresholds/discriminators.

6. Describe the general characteristics of histograms and scatterplots (scattergrams).
   a. Evaluate histograms and scatterplots to determine acceptable cell distribution.
   b. Recognize the location of various cell populations or subpopulations.

7. Identify the cell parameters on the automated cell counter that are:
   a. Directly measured
   b. Derived from histograms or scatterplots
   c. Calculated from measured or derived parameters

8. Explain the method (absorbance, optical, and/or impedance) used to obtain each of the following instrument parameters:
   a. WBC
   b. RBC
   c. HGB
   d. PLT
   e. Retics

9. Discuss the principle(s) utilized by automated differential systems:
   a. VCS technology (Beckman Coulter)
   b. Flow cytometry with Fluorescence and RF/DC analysis (Sysmex)
   c. Flow cytometry with Cytochemistry (Siemens, formerly Bayer)
   d. MAPSS technology (Abbott Cell-Dyn)

   **View the Automated Systems Powerpoint** located on Blackboard, Auto Unit.

10. Correlate normal and abnormal RBC size with the RDW value.

11. Correlate normal and abnormal PLT size with the MPV value.

12. **Explain the significance and use of the immature retic fraction (IRF).**

13. Describe sample flow during the instrument operating cycle including the reagents used and their purpose, as well as the instrument components utilized.

14. Evaluate patient results by following established policies, instrument linearity limits, and performing data correlation checks.
   a. List instances in which the hemoglobin and hematocrit values may not match.

15. Recognize critical patient values.

16. Compare patient results with previous results (delta check), taking action if needed.

17. Notify appropriate personnel of results and/or investigate treatment when required, documenting actions.

18. Calculate the following:
   a. RBC indices (MCV, MCH, MCHC)
   b. Corrected WBC count in the presence of nucleated red cells
   c. Absolute number of each WBC type

19. Distinguish between conventional and SI (System of International) units of measure.

20. **Discuss the protocol for establishing reference ranges for a particular analytic method.**
21. Evaluate any instrument messages or parameter flags/invalid data, taking corrective action as needed.

22. Discuss the following causes for obtaining inaccurate determinations on automated cell counters, selecting corrective solutions and/or alternative testing methods:
   a. Overrange parameters (WBC, RBC or PLT)
   b. Lipemia
   c. Bilirubinemia
   d. Lyse resistant red cells/"hard to lyse" rbcs
   e. Hyperproteinemia/rouleaux
   f. MCHC value greater than 36.0%
   g. RBC agglutination
   h. Microcytic red cells/schistocytes
   i. Platelet clumping or satellitism
   j. Anucleate cytoplasmic fragments
   k. Fibrin strands/partially clotted samples
   l. Giant platelets
   m. Nucleated red cells
   n. Hemolyzed samples
   o. Aspiration errors
   p. Contaminated/diluted samples
   q. Old samples
   r. Red cell inclusions or parasites

23. Describe instances when manual counts (e.g., Platelet, WBC or Retic) may be necessary.

IV. Objectives regarding Hemoglobinometry and manual Microhematocrits

A. Hemoglobinometry
   1. Describe the principle and accuracy of the following methods of hemoglobin determination:
      a. Cyanmethemoglobin (or modifications), including hgb forms measured
      b. Copper sulfate
   2. Evaluate possible sources of error when measuring hemoglobin photometrically.

B. Evaluate a manual spun hematocrit determination, taking into account the principle, specimen acceptability, mechanical and technical variables.

C. Discuss the effect of a falsely elevated HGB result or a falsely low HCT result on the MCHC.

V. Objectives regarding CBC’s with Differential

A. Explain the procedure for performing a manual leukocyte differential, including sources of error.

B. Recognize the normal differential percentage ranges (including age variations) of each type of leukocyte.

C. Correlate cell count parameters with blood smear findings, taking corrective action if correlation is unacceptable.

D. Correlate differential results with patient age and clinical information, taking appropriate action when necessary.
   1. Explain when a 200 cell differential and/or pathologist review may be needed.