Section 8

HEMATOLOGY TESTS

LEARNING OBJECTIVES

When you complete this section, you will be able to:

1. **Recognize blood components that are often tested**
2. **Indicate types of technologies used in testing**
3. **List the tests that comprise a complete blood count**
4. **Recognize normal values for the cellular elements in blood**

A WORD ABOUT TESTS

Because few laboratory tests are disease-specific, a single abnormal test value is not considered diagnostic of a disease state. Many variables affect almost every test; these include:

- The patient's state of stress
- Lack of patient compliance with procedure (e.g., fasting)
- Improper sample handling
- Improper lab procedures
- Lack of accuracy in the procedure or instrument used

Reason for Repeating Tests. Lab tests are repeated to confirm an abnormal finding. When used to determine the prognosis of a disease or to monitor the progress of therapy, tests that are repeated over time can indicate a trend, e.g., the disease is growing worse or getting better.

HOW BLOOD TESTS ARE DONE

Hematology tests include a wide variety of laboratory studies, ranging from coagulation factors to various cell evaluations. The tests discussed here are those that are most likely to be done with a hematology analyzer.

The Sample. A sample of whole blood is taken, usually from a vein. Amounts differ according to the number and types of tests to be run and the testing instruments to be used. Typically, RBCs are counted and lysed (broken down); then WBCs are measured.

Dilutions. Because blood clots quickly, the measured blood sample is diluted with either a lysing agent or an anti-clotting agent, depending on the test(s) to be completed. A lysing agent destroys the RBCs and allows counting of WBCs. The dilution is an important step in preparing samples for testing for several reasons.

First, concentrations of the anticoagulant must be adequate for the volume of blood. Insufficient dilution may allow formation of small clots that lower cell counts; excessive dilution can cause cells to shrink or swell. Anticoagulants that are widely used are EDTA (ethylenediaminetetraacetic acid) and heparin. EDTA (purple-top vacutainer) is used often for routine cell counts.

Second, even relatively large blood samples do not provide a sufficient quantity to flow through an analyzer for measurement. Blood must be mixed with a diluent that will allow the cells to be evenly suspended in sufficient liquid to flow at a constant rate for measurement.

Originally, blood counts were performed manually by visualizing the cells through a microscope and counting after diluting and pipeting a small volume onto a pre-defined glass slide containing etched counting areas. For identifying and counting the WBC subpopulations, a blood sample was smeared on a slide and stained, then placed under a microscope. The hematologist or technician identified and counted the cells in a systematic prescribed method, classifying the first 100 cells seen and assigning a percentage figure to each type found. Although generally accurate, the process was very technologist-dependent and time-consuming.

Now cells are almost always counted electronically by an analyzer utilizing specific technologies and methods. When abnormalities are suspected or determined, however, a hematologist or technician will still do an actual visual count and identification of the cells.

Counts and other tests should be run within 3-4 hours of obtaining blood samples (within 1-2 hours for platelet counts).

CELL COUNTING TECHNOLOGIES

Automated hematology has been used in large laboratories for many years. In the late '70s it became easy and cost-effective enough to move into physician office practices as well.

Automated technologies include the following:

Impedance technology. This technology was originally known as the Coulter principle. There are several variations and adaptations to the impedance principle in use today from a variety of commercial manufacturers.

Impedance technology is based on the fact that blood cells are poor conductors of electricity. Cells are diluted with an electrolyte, directed to a moving stream, and pass through a small orifice (opening) within a transducer (detection device). As each cell passes through the small opening of the aperture, an electrical field exists in the transducer; and when the cells pass through the aperture orifice (small opening), the increase in electrical impedance (resistance) is measured **(Figure 9)**.

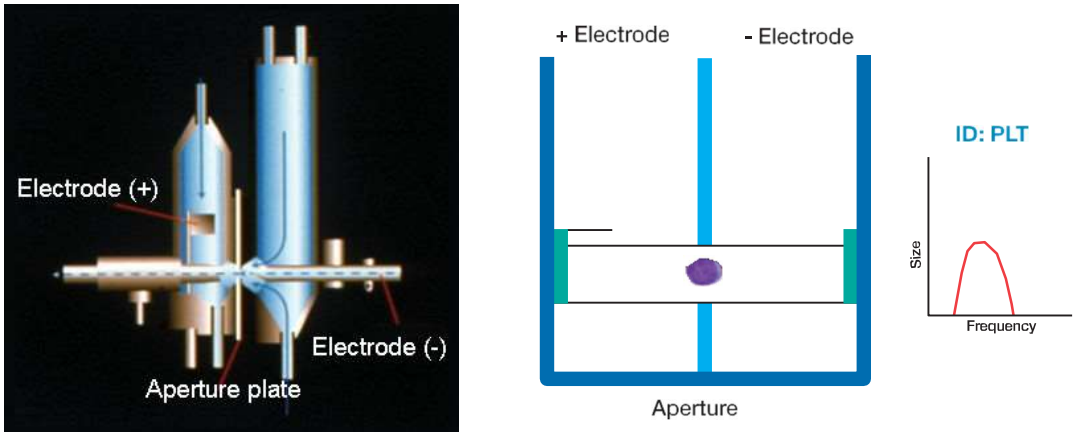

Figure 9. Simple impedance detection device (transducer).

Impedance technology provides a one-dimensional method for enumeration of the formed cellular elements as well as for cell sizing due to the resulting impedance signal being proportional to the cell's (event) size.

Different approaches to size discrimination (thresholding) and the cell dilution allow for accurate enumeration of RBCs, WBCs, and PLTs even though the absolute numbers (of cells) and size vary broadly within the blood. Displays of the cellular measurements are usually provided in a graphical form called a histogram (representing number of events versus relative size of the events). **(Figure 10)**

Histogram

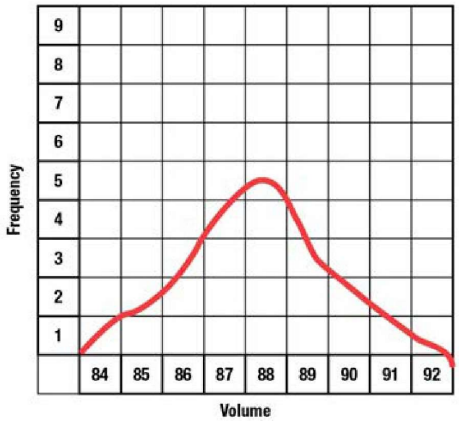

Figure 10. One-dimensional histogram, frequency of events versus cell volume.

Optical principles. Automated systems are now based on flow-through (also called flow cytometry) optical technologies that identify cells on the basis of light scatter properties broadly equating to the cell's physical characteristic differences.

Blood cell recognition is optimized by diluting specifically to the number of cells present in the blood so as to count the number of cells appropriate for statistical accuracy.

A focused light beam illuminates a small area of a flow cell. Cells are rapidly injected in single file (hydrodynamic focusing) through the illuminated area (interrogation zone) where the cells intersecting the light beam scatter light in all directions in a manner that is measurable and unique to each cell type **(Figure 11)**.

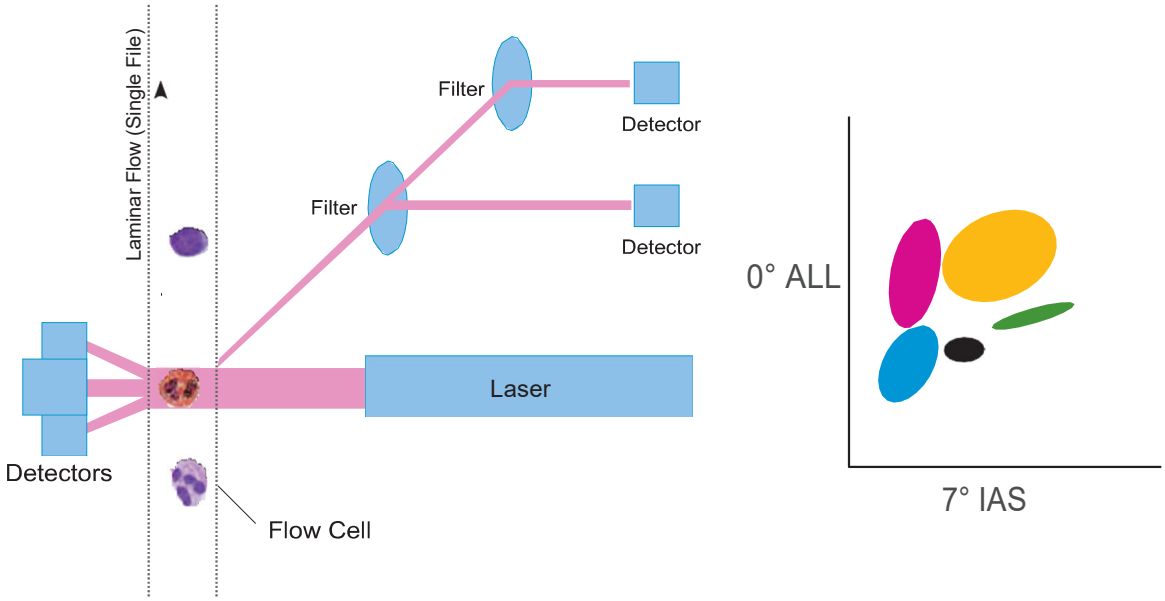

Figure 11. Optical light scatter technology with two-dimensional graphic scatterplot.

The resulting light scatter information can be collected at multiple locations incident to the light beam providing a multi-dimensional analysis of the unique light scatter properties from each cellular event. Analysis and displays of the optical light scatter measurements are provided in a two-dimensional graphical form called a scatterplot **(Figure 11)**. Multiple scatterplots may be generated for complete visualization of all the cellular components including sub-populations of each cell line.

Radio Frequency and Multi-Color Fluorescence Emission Detection Methods.

In addition to impedance and optical flow cytometry, other electrical signals and advanced pre-analysis, rapid staining methods have further advanced identification of cellular characteristics and subsequent cellular sub-populations.

Radio Frequency (RF) involves applying a high energy electrical field across an impedance aperture resulting in wave transformations that characterize internal structure, mostly related to WBC structure.

Multi-Color Fluorescence emission has provided the greatest recent advancements in cell identification. Fluorescent flow cytometry is a measurement technology developed by specifically staining internal components of cells (RNA/DNA) or tagging a specific antigenic site of a cell with a fluorochrome conjugated antibody that fluoresces at higher wavelengths when illuminated by a specific wavelength light source (usually a laser diode). Specificity of cell lineage identification has been advanced with these methods, providing greater insight in hematology disease identification and management.

HEMATOLOGY TESTS

Complete Blood Count (CBC)

A complete blood count (CBC) is the most widely performed test in the clinical laboratory. Many CBCs are done as routine screens – tests that provide general information about the patient's status. Most CBCs include the following cellular measurements:

- WBC count
- WBC differential, 3-part: lymphocytes, mid-cells, granulocytes **OR** 5-part: lymphocytes, monocytes, neutrophils, eosinophils, basophils
- RBC count
- Hemoglobin (Hb)
- Hematocrit (Hct)
- RBC indices (MCV, MCH, MCHC)
- Platelet count

Normal ranges (also called reference ranges) depend on geographic location, the patient's sex, and age; ranges are used as guidelines and vary based on specific populations.

White Blood Cell (Leukocyte) Count

This is a count of the number of WBCs present in a known volume of blood. Automated systems often count as many as 20,000 WBCs to ensure accuracy and precision. The WBC count is further identified by the major WBC sub-populations (WBC differential). WBC counts and the WBC differential are generally measured by optical technologies or a combination of optical, impedance, radio frequency, or multi-color fluorescence in modern hematology analyzers.

Less sophisticated hematology analyzers provide WBC sub-populations as 3-part WBC differentials reporting a percentage and absolute value for lymphocyte, mid-cells, and granulocyte populations. Advanced hematology analyzers minimally provide a 5-part WBC differential reporting percentages and absolute values for lymphocytes, monocytes, neutrophils, eosinophils, and basophils. The modern hematology analyzer also indicates (flags) suspected abnormalities, from which the technologist may perform a microscopic smear review or an actual manual differential count to ensure accuracy of the reported results **(Table 7)**.

WBC VALUES normal range, total WBCs: 4.5-11.0 X 10³/µl (adult population)*		
Normal ranges, differential leukocyte counts:		
3-part differential	Lymphocytes Mid-Cells Granulocytes	1.0-4.8 x 103/µL 0-0.9 x 103/µL 1.8-8.1 x 103/µL
5-part differential	Lymphocytes Monocytes Neutrophils Eosinophils Basophils	1.0-4.8 x 103/µL 0-0.8 x 103/µL 1.8-7.7 x 103/µL 0-0.5 x 103/µL 0-0.2 x 103/µL
Other normal WBC values	Band neutrophils (%) Segmented neutrophils (%)	0-6% 40-70%

Table 7. WBC values.
*Reference ranges should be established by each laboratory.

Red Blood Cell (Erythrocyte) Count. This is a count of the number of RBCs present in a known volume of blood. Automated systems often count as many as 20,000-50,000 RBCs to ensure precision. The RBC count is also used to calculate the RBC indices.

RBC COUNT normal range: 4.00-5.9 X 10⁶/µl*	
High	**Low**
Polycythemia, severe dehydration, trauma, surgery, burns	Anemia, hemorrhage, excessive fluid intake

Table 8. RBC count.
*Reference ranges should be established by each laboratory.

Hematocrit (Hct). This is the volume of RBCs in a whole blood sample expressed as a percentage (%).

HCT VALUES normal range: 36%-53%	
High	**Low**
Polycythemia, severe dehydration, trauma, surgery, burns	Anemia, hemorrhage, excessive fluid intake

Table 9. Hct Values
*Reference ranges should be established by each laboratory.

Hemoglobin (Hb). Hemoglobin values may be obtained in several ways. The most common method adds potassium cyanide (or similar compounds) to convert Hb to cyanmethemoglobin, which is measured with a spectrophotometer.

The results, recorded as grams per deciliter (g/dL), indicate the oxygen-carrying capacity of the RBCs.

HB VALUES normal range: 12.0-17.5 g/dl*	
High	**Low**
Polycythemia, severe dehydration, trauma, surgery, burns	Anemia, hemorrhage, excessive fluid intake

Table 10. Hb Values.
*Reference ranges should be established by each laboratory.

RBC INDICES

The RBC indices –mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) – indicate the volume and character of hemoglobin and, therefore, aid in the differential diagnosis of the type of anemia present.

Mean Cell Volume (MCV). The MCV is the average volume of RBCs; it is calculated from the hematocrit (volume of packed red cells) and the erythrocyte count. This is the most important RBC index in the differential diagnosis of anemias.

MCV VALUES normal range: 80.00-100 fl*	
High	**Low**
Pernicious anemia, tapeworm infestation, certain medications	Anemia, hemorrhage, excessive fluid intake

Table 11. MCV Values.
*Reference ranges should be established by each laboratory.

Today's modern hematology analyzers actually do cell sizing, therefore, the MCV is actually a measured parameter and Hct is calculated from the MCV.

Mean Cell Hemoglobin (MCH). This is a calculation of the ratio of hemoglobin to the erythrocyte count. The formula for the calculation is:

MCH = Hb (g/dL)/RBC (x 106/µL) x 10

MCH VALUES normal range: 26-34 pg*
Low
Anemias, iron deficiency, liver disease, hemorrhage

Table 12. MCH Values.
*Reference ranges should be established by each laboratory.

Mean Cell Hemoglobin Concentration (MCHC). This calculation of the ratio of hemoglobin to hematocrit indicates the concentration of hemoglobin in the average red cell. The calculation is:

MCHC = [Hb (g/dL)/Hct (%)] x 100

MCH VALUES normal range: 31-37 pg*
Low
Anemia due to inadequate RBC formation, pernicious anemia, tapeworms, certain medications

Table 13. MCHC Values.
*Reference ranges should be established by each laboratory.

Reticulocyte Count. For a count of these young RBCs, a few drops of blood are smeared on a slide, stained with methylene blue, and counterstained with Wright's stain before being counted under a microscope. One thousand RBCs are counted and the number of cells having a blue-stained reticulum are expressed as a percentage. Now, most automated systems provide reticulocyte counts using various stains and dyes.

RETICULOCYTE COUNT normal range: 0.5%-1.5% of rBCs*	
High	**Low**
Reticulocytosis indicates bone marrow activity in response to: blood loss, therapy for anemia, hemolytic anemia in pregnancy, high altitudes	Low or normal counts in anemic patients indicate failure of bone marrow, infection, inflammation, aplastic anemia, severe iron deficiency, megaloblastic anemia

Table 14. Reticulocyte count.
*Reference ranges should be established by each laboratory.

Platelet Count. Because their normal numbers are so high, platelets were often estimated. Now, automated systems provide platelet counts from a variety of methods (i.e., impedance, optical, and immuno-platelet).

PLATELET COUNT normal range: 140-440 x 103/µl*	
High	**Low**
Rheumatoid arthritis, many cancers, hemorrhage, polycythemia, some anemias	Diseases of the spleen, leukemias, aplastic anemia, alcoholism, severe infections, cardiac surgery, blood transfusion

Table 15. Platelet count Values.
*Reference ranges should be established by each laboratory.