Simply stated, hematology analyzers determine the quantity of each type of cell that makes up the patient’s blood. Only recently has the role and importance of each blood component been understood. As blood travels throughout the body, it provides nourishment to each cell and removes its waste. Theoretically, there is no change in the body that is not reflected by some change in the blood. Hematologists are working to identify more markers for particular events.

Blood 101
Whole blood primarily comprises three types of cells homogeneously suspended in a fluid called plasma. Plasma constitutes a little more than half of the blood’s volume and is mostly water with dissolved salts and proteins such as albumin, antibodies, and clotting proteins. In addition, plasma contains a number of hormones, electrolytes, sugars, fats, minerals, and vitamins.

The remainder of the blood’s volume comprises the cellular components that hematology analyzers examine. A complete blood count (most commonly known as a CBC, full blood count [FBC], full blood exam [FBE], or simply a blood panel or hemagram) is ordered by a medical practitioner to provide detailed information about these cells. The most populous of the three are the red blood cells (RBC) or erythrocytes. RBCs are filled with hemoglobin (HgB) and give blood its characteristic color—bright red for arterial blood and dark red for venous blood. HgB bonds with oxygen in the lungs and transports its “cargo” to the rest of the body. After delivering the oxygen, the RBCs carry carbon dioxide back to the lungs where it is exchanged for more oxygen, and the process begins again.

White blood cells (WBCs) are fewer in number (about 1 WBC for every 660 RBCs) and appear in one of five main types, each serving a specific purpose in the body. WBCs can be thought of as the body’s army, since they fight infections and other foreign invaders. Each type of WBC—granulocytes (such as neutrophils, eosinophila, and basophils), lymphocytes, and monocytes—performs a specific defensive function.

The third cellular component of blood is the platelets (Plt) or thrombocytes. They are smaller than RBCs or WBCs and constitute the body’s protective mechanism against continuous bleeding. When bleeding, these cells gather at the site, become sticky, and clump together to form a plug that helps stop the bleeding.

Hematology analyzers count cellular components, perform some mathematics, and provide results for interpretation. For example, an elevated WBC count can indicate the presence of a bacterial infection, while a decrease in RBCs can indicate anemia. The numbers, mix, and ratios of these cells provide critical information to the clinician about the patient’s overall health and insight into specific conditions. With a basic understanding of the cells that constitute “whole” blood, we can move on to how the cells are counted.

Current Technology
Before cells can be counted, a blood sample must be obtained from the patient and prepared for analysis. First, a phlebotomist draws a blood sample using a test tube coated with an anticoagulant to keep the blood from clotting. In the laboratory, the blood tube is continuously agitated (not shaken) to prevent the components from settling during any delays (performing tests on other samples, STAT lab tests, etc.) encountered in the process.

There are three methods of counting blood cells: manual, semi-automated, and fully automated, in two different designs. All hematology analyzers fall into either the semi- or fully automated methods. The first, and the most traditional method is a manual count by direct observation. Manual counts are still performed when the exact number of certain cells is required. This is typically performed for abnormally shaped cells that are difficult to count automatically, such as those that occur with certain diseases.

The second is a semi-automated method using quantitative buffy coat (the layer containing platelets and granulocytes) analysis (QBCA). This method obtains
hemocrit (Hct), WBC, and Plt counts from both venous and capillary blood using a less-labor-intensive process than a fully manual count. The blood is drawn into a special, stain-coated tube about the size of a microhematocrit tube. The tube is then sealed, incubated, and centrifuged using a special plastic float. The float settles between the RBCs and the plasma, separating their layers and (because the diameter of the float is slightly smaller than the inside diameter of the tube) expands the length of the buffy coat. The presence of the stain causes the buffy coat layers to fluoresce different colors. The laboratory technician manually examines the tube using a magnifier and a special light. The length of each layer of the tube, including the separated buffy coat, is measured as precisely as possible using a micrometer device. Particular cell counts (RBC, WBC, Hct, Plt, etc.) are determined using a conversion factor applied to the layer lengths. Other calculations, based upon measured values, can be manually performed by the laboratory technician as needed.

Fully automated hematology analyzers employ two principal methods of counting blood cells: volumetric impedance and light-scatter technique. Both employ variations in the handling of samples before the count, such as automatic dilution and separation of samples into aliquots. One design dilutes the entire sample and then divides it into aliquots, while the other divides the sample and then dilutes each as required. In either case, the methodology requires the addition of a lysing agent to remove the RBCs, so that the WBC count can be per-

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### The Origin and Evolution of Hematology Analyzers

Manual counts were once the only way to obtain CBC, RBC, WBC, etc., but since they were quite laborious to perform and had little clinical value, they were seldom requested. In the manual method, the laboratory technician prepared a blood smear using a special microscope slide containing an etched reticle. Alternatively, the blood smear was made on a regular slide but was examined using a specially equipped microscope with a counting reticle built into the instrument. In either case, the technician observed the blood smear, counted each of the different cells, and simultaneously recorded the numbers of cells using a mechanical blood cell count recorder. The advantage to this method is that it provides the best identification of irregularly shaped blood cells. The disadvantages are many—inaccurate counts caused by human error brought on by fatigue and eyestrain, the long time it takes to perform the manual count, the simple possibility of pressing the wrong buttons on the mechanical blood cell count recorder, etc. With all the disadvantages, this was the sole method of counting blood cells until the mid-1950s.

Although the volumetric impedance principle is simple to understand, Wallace Coulter did not discover it until after World War II, developing it as a way to ensure solids in paint were of uniform size. It was not applied to counting blood cells and patented until 1953. Coulter always had an interest in electronics and worked for General Electric x-ray as a sales and service engineer. Remembering the laborious process used to count blood cells during his hospital visits as a GE engineer, he began applying his newly discovered principle to counting red blood cells. In 1958, he cofounded the Coulter Corporation with his brother. Due to the aggressive defense of Coulter Corporation’s patents, virtually all automatic blood cell counters were not only of the volumetric impedance method, but from then until

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### Table 1. Common hematology terminology.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Term</th>
</tr>
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<tbody>
<tr>
<td>Hct</td>
<td>Hematocrit</td>
</tr>
<tr>
<td>HDW</td>
<td>Hemoglobin distribution width</td>
</tr>
<tr>
<td>Hgb</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>L</td>
<td>Lymphocyte</td>
</tr>
<tr>
<td>LI</td>
<td>Lobularity index</td>
</tr>
<tr>
<td>M</td>
<td>Monocyte</td>
</tr>
<tr>
<td>MCH</td>
<td>Mean corpuscular hemoglobin</td>
</tr>
<tr>
<td>MCHC</td>
<td>Mean corpuscular hemoglobin concentration</td>
</tr>
<tr>
<td>MCV</td>
<td>Mean (average) corpuscular volume</td>
</tr>
<tr>
<td>MPV</td>
<td>Mean platelet volume</td>
</tr>
<tr>
<td>Plt</td>
<td>Platelet</td>
</tr>
<tr>
<td>QBCCA</td>
<td>Quantitative buffy coat analysis</td>
</tr>
<tr>
<td>RBC</td>
<td>Red blood cell or red blood cell count</td>
</tr>
<tr>
<td>RDW</td>
<td>Red cell distribution width</td>
</tr>
<tr>
<td>WBC</td>
<td>White blood cell or white blood cell count</td>
</tr>
</tbody>
</table>
formed with minimal interference. A second aliquot is further diluted before making RBC and Plt counts. Some designs have the Hgb determination made on a third aliquot while others use one of the other aliquots.

The principle of volumetric impedance, patented by Wallace Coulter in 1953, is the oldest automated method of counting blood cells. Simply put, the principle utilizes two chambers, each containing a single electrode, separated by a small, round aperture. Both chambers contain a conductive solution and a low constant current flows between the electrodes. At rest, nothing happens since both chambers are at equal pressure. To begin the cell count, the sample is first mixed with a diluent (usually the same conductive solution already in each chamber) and introduced to one chamber while a simultaneous vacuum is drawn on the other chamber. Because of the pressure difference, the mixture of conductive fluid and blood begins to flow through the aperture to the other chamber. Since the chamber has a small current flowing through it, as each cell passes through the aperture, a slight change in conductivity is observed. Because RBCs, WBCs, and Plts are different sizes, each cell passing through the aperture creates a spike of a different amplitude. By measuring the amplitude and determining voltage-measuring windows, cells of varying size are identified and counted as they pass through the aperture. Given a constant known volume of blood and diluents, it is easy to calculate the number of various cells per microliter (cubic millimeter) of whole blood. Additionally, using built-in microcomputer technology, other blood parameters are also calculated and reported.

The other automated method uses light-scatter, which is based on the principle that a single-file stream of cells will scatter light in proportion to the size of each cell. This is the basis of flow cytometry. A blood cell counter employing this technology injects a diluted blood sample into the center of a flowing sheath of fluid. Through mechanical design and by regulating flow pressures and rates, the blood sample stays in the center of the stream and becomes so slender that the blood cells pass single-file in the center of the fluid sheath through the sensing zone. As the stream of hydrodynamically focused cells passes through a beam of light in the sensing zone, the individual blood cells in the stream break the light beam. Although the sensing zone of the modern light-scatter hematology analyzer employs more than a simple single beam of light, the principle is the same. Instead of measuring the number of times the light beam is broken by the individual cells in the stream, blood cell counters depend on the amount of light scattered by the passing cells and measure it ahead of the stream. Scattered light, rather than direct light, provides a better measure of cells

the 1970s they carried the name “Coulter Counter.”

Meanwhile early in the 1960s, now having a relatively quick and easy way to count blood cells, Alexander Vastem began counting the various cells contained in whole blood of greyhounds and began to establish reference ranges that are still used today. Concurrent with the establishment of these ranges, other researchers began to correlate particular observations of blood component counts with various disease states, and were even able to begin tracking the remediation of certain disease processes through repeated counts.

By the late 1960s, with Coulter’s early patents expiring, their continuing research helped stretch the performance envelope of their analyzers, patenting incremental improvements. By the early 1970s, new capabilities included platelet counts, 7-parameter CBC analyzers, and 3-part differential leukocyte counters. In the 1980s 10-parameter CBC analyzers entered the marketplace and by the 1990s further advancements in differentials emerged using combinations of the volumetric impedance principle and other emerging technologies, including lasers.

For the three decades that the Coulter Corporation dominated the market, their competitors did not rest on their laurels. Competing firms actively sought to develop new technologies to quantify the components of whole blood. Newly developed (1968) flow cytometry technology was adapted to the task of counting blood cells and analyzers began hitting the market to compete with the well-established Coulter products. Since it was still developing technology and the volumetric impedance based counters were considered the “gold standard” of automated cell counters, it met with mixed success. Although competitively priced, some analyzers required more manual sample preparation. The instruments’ operation were also more complex, thus requiring the attention of the more experienced laboratory technicians. Even today, nearly every modern hematology analyzer depends in some way on the Coulter principle.
because cells of different volume scatter light in proportion to their size. Since each type of cell has a different size and volume, different cells cause light to be scattered at different angles. By measuring the scattered light at different angles, different cells can be counted. Because of the different optical characteristics of each cell, today's blood cell counters use a helium-neon (He-Ne) laser to measure RBC, RBC indexes, Plt, Hct, etc. and a tungsten-halogen light beam to measure Hgb, WBC, and WBC differentials. Both are focused on the cell stream in the counting chamber. Optical sensors pick up the scattered light impulses at each angle and count and report the number of each cell type counted. Like other automated hematology analyzers, they also calculate other blood cell parameters based on the cell counts.

Some hematology analyzers employ both automated methods in their design in separate analytical channels, with each contributing results to the overall output. Of the four discreet methods of counting blood cells, automated hematology analyzers utilizing the volumetric impedance principle are the most common, followed by the light-scatter-method. The manual count is the least utilized, but it is useful for counting special cells, and blood smears should still be microscopically examined for parasitic infections and inclusion bodies. However, in some developing nations, manual counts may still be the norm.

Managing Hematology Analyzers

For Joint Commission accreditation and College of American Pathologists (CAP) laboratory certifications, automated hematology analyzers must be included in a hospital's maintenance database. Even if the instrument is leased or part of a reagent agreement that includes original equipment manufacturer (OEM) maintenance, much of the routine maintenance (cleaning/flushing, running standards and calibrants, phased tubing replacement, etc.) is performed by the laboratory technician. If the maintenance is contracted out, the accreditation authorities still hold the facility accountable for instrument maintenance. If the in-house biomed manager is responsible for all medical device maintenance management, he or she must also maintain the hematology analyzer maintenance records for the two-year retention period dictated by CAP. If the laboratory is not included in the in-house program, or it maintains separate maintenance records (which is often the case), some accommodation must be made locally. If the instrument is maintained in-house, the minimum requirement to provide thorough maintenance services is comprehensive service literature delineating the complete preventive maintenance procedure; both plumbing and circuit descriptions are mandatory. The ideal situation would additionally include brand/model-specific training.

Regulations

Under the Clinical Laboratory Improvement Amendments (CLIA), most hematology analyzers described in this article fall under the moderately complex regulatory category. Users of equipment in this category are required to have minimal scientific and technical knowledge and training to perform their duties accurately. The test steps are either automatically executed (which is the case with most analyzers) or easily controlled, and minimal interpretation and technician assessment is required. These requirements and constraints pertain to operation of the laboratory, testing facilities, and personnel performing the tests. Neither CLIA nor CAP nor the U.S. Food and Drug Administration (FDA) attempt to regulate or establish credentials for individuals maintaining these analyzers. While CAP has maintenance criteria that must be followed to attain certification, neither they nor FDA specifies who will maintain laboratory equipment. Only the Joint Commission, which is not a regulatory agency, addresses maintainer qualifications and credentials.

Risk Management Issues

The risk management issues with hematology analyzers are no greater than with other clinical laboratory equipment. The main risks include inaccurate results, which could result in delay of treatment or inappropriate treatment. The equipment manager should always err on the side of caution when assessing risk.

Troubleshooting

No electromechanical device operates correctly all of the time. Each design has its advantages and problems. Analyzers based upon or employing volumetric impedance occasionally experience aperture clogging as well as counting errors when the specimen is cloudy. Some samples from patients with uremia, leukemia, and those who are immunosuppressed can result in false low WBC counts caused by cell damage occurring in the aperture. Additionally, RBCs can clump if the specimen is cold, resulting in very high mean corpuscular hemoglobin concentration (MCHC) values. Most sample feed prob-
lems can be traced to minute vacuum leaks in the maze of plastic tubing, so it behooves the laboratory technician or biomed to replace tubing at specified intervals as part of scheduled maintenance, regardless of the condition of the tubing.

Light-scattering designs can suffer from misalignment of the laser or light beam, debris in the sample stream, and electronic noise. Furthermore, they tend to record very high cell counts inaccurately. Lastly, using the wrong or expired diluent, flush, etc., can introduce problems where no instrument malfunction exists. Fortunately, most of these problems are within the purview of the laboratory technician to prevent or correct. The biomed will rarely encounter these problems, but must be aware of them when dealing with inexperienced technicians. Outside of the aforementioned problems, the electronics used in modern hematology analyzers are remarkably stable; trouble-free; and are closely monitored, both operationally and for quality control purposes, by computer software that continually evaluates instrument performance.

Service Training and Equipment
Although the lab technician receives extensive training in performing operator maintenance, biomeds may occasionally be required to service hematology analyzers. The failures usually seen by in-house maintainers are hard failures; that is, the permanent failure of a substantial part of the instrument. In these instances, device-specific training is strongly recommended. Lacking this, knowledge of the test methodology and lab equipment experience coupled with good service literature is the next recommendation; technical phone support from the manufacturer can also be helpful. Even with model-specific training, good service literature is helpful. Lacking both, the in-house biomed can diagnose and repair most obvious problems like unserviceable power supplies, open and shorted power transistors, burnt out motors, etc.

Future Development
To protect personnel from both HIV and hepatitis, instrument designs are automating the transfer of the blood sample from the collection tube to the instrument. Modern and future designs automate this process to reduce or eliminate aerosols that may occur when removing the cap of the collection tube. Traditionally, the lab technician removed the cap of the collection tube and either withdrew an aliquot of the sample and injected it into the analyzer or raised the open collection tube up to a sampling tube. Safer designs require the operator only to insert the still-capped collection tube into a “sample carrier” which is automatically drawn into the instrument. Once inside, the instrument automatically pierces the cap, withdraws the required sample size, and begins the processing.

Incremental improvements in both the analysis and control software are regularly fielded by the manufacturer. As more instruments become software controlled, better integration between the various counting methods and systems results in improved histogram and cytogram displays, improved differentiation of WBCs, and higher throughput. Instrument manufacturers are continually working to improve their linearity and expand the reportable ranges. The next big step is likely to be in the analyzer’s ability to perform extended differential counts—the ability to not only identify but to count blast cells, immature granulocytes, and atypical lymphocytes, since those counts must still be performed manually.

Looking into the very distant future, one can see the integration of various whole blood testing instruments into complete “systems” to provide a more encompassing “blood profile” of the patient to aid in diagnosis and treatment monitoring.

For More Information
- The Merck Manual of Medical Information: www.merck.com/mmhe
- Public Law 100-578: www.fda.gov/cdrh/clia/pl100-578.pdf
- ECRI Healthcare Product Comparison System for desired analyzer: www.ecri.org
- College of American Pathologists (CAP): www.cap.org
- U.S. Food and Drug Administration: www.fda.gov
- Clinical Laboratory Improvement Amendments (CLIA): www.cms.hhs.gov/clia

Robert Dondelinger, CBET-E, MS, is the senior medical logistician at the U.S. Military Entrance Processing Command in North Chicago, IL. An internationally certified biomedical electronics technician, he entered the U.S. Army in 1970 and retired from active duty in 2002.