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The automated hematology analyzers

Automatyczne analizatory hematologiczne

INTRODUCTION

The complete blood count (CBC) and differential leukocyte count (DLC) are the backbone of any laboratory evaluation, not only used by general practitioners, but also by other medical specialties. They provide valuable information about the blood and to some extent about the bone marrow, which is the blood-forming tissue. The CBC and DLC are used to diagnose anemia, to identify acute and chronic illnesses, bleeding tendencies, and white blood cell disorders, for example leukemia. During the last quarter century, blood cell analysis has progressed from the use of labor-intensive manual procedures to the use of highly automated instruments. Modern hematology analyzers provide new additional parameters enabling earlier and more precise diagnosis. Moreover, they incorporate flow cytometry, robotics and expert system technologies. Therefore, it is crucial that obtained parameters are clear for clinicians, and the results completely used.

Development of automation - electrical impedance methods

Hematology blood cell analyzers have become increasingly sophisticated during the past decade. Until the mid-1950s, laboratories performed hematology testing manually, thus making it time consuming and labor intensive. The first automated analyzer was introduced in 1956 by Wallace Coulter. This was a single channel instrument that revolutionized the tedious and imprecise manual chamber counting methods [1]. The first hematology analyzers were based upon the principle of impedance (i.e. resistance to current flow). In this method a small sample of the blood is aspirated into a chamber and diluted with an isotonic saline solution. The analyzer prepares two dilutions: one lysed and the other unlysed. After the first dilution is measured, the white blood cells (WBC) and hemoglobin (Hb) values are displayed on the screen of the instrument; meanwhile, the analyzer processes the second dilution, which measures red blood cells (RBC), hematocrit (Hct), mean corpuscular volume (MCV) and platelet count (PLT) [2, 11]. A small portion of the diluted fluid in each bath is allowed to flow past a small aperture. An electrical current is produced in each aperture by two electrodes, one on the inside and the other on the outside of the aperture. The saline solution is responsible for conducting current between the electrodes. The cells move through the aperture, then displace a volume of electrolyte equal to its size. The cell acts as an electrical resistor, and impedes the flow of current. This produces a voltage pulse, the magnitude

of which is proportional to the size of the cell. Instrument electronics are adjusted to discriminate voltage pulses produced by different cells (called thresholds). For example, the threshold for counting a RBC is equivalent to a cell volume of 36 fl or higher. Voltage pulses that are equivalent to volumes of 2–20 fl are counted as platelets. Although the methods directly determining RBC, WBC and PLT counts differ, the 50-year-old impedance procedure continues to be the most frequently used, either alone or in combination with the others. Small hematology analyzers based on this method are found in small laboratories, and are also used in hospitals as the backups for larger analyzers [2, 11].

Hemoglobin, hematocrit, RBC count and RBC parameters

Automated counting methods for RBC have been based originally on electrical impedance or later on light-scattering techniques. The hemoglobin concentration is measured optically using the solution in the WBC bath. The lysing agent contains potassium cyanide that reacts with the hemoglobin to form cyanmethemoglobin. The color intensity, measured in separate cuvette, is read spectrophotometrically at 540 nm and is proportional to the concentration of hemoglobin. Some instruments use a modified cyanmethemoglobin procedure, and others use cyanide-free colorimetric determinations [5]. The Hct is a test that measures the volume of blood in percent that is comprised of the RBC. Automated cell counters calculate the Hct by multiplying the RBC count by the MCV. The three main RBC indices are used to determine the average size and hemoglobin content of the RBC, and they help to determine the cause of anemia. MCV is the average size of the red blood cells expressed in femtolitres (fl). The MCV is measured by electronic cell counters, usually by dividing the summation of the cell volumes by the RBC count. Mean corpuscular hemoglobin (MCH) is the average amount of hemoglobin inside an RBC expressed in picograms. The MCH is calculated by dividing the hemoglobin concentration by the RBC count. Mean corpuscular hemoglobin concentration (MCHC) is the average concentration of hemoglobin in the RBC. It is calculated by dividing the hemoglobin by the Hct. Some analyzers calculate additional parameters to screen for various types of anemia. The mean of the RBC distribution histogram, based on electrical impedance, is the MCV, and the coefficient of variation, or sometimes the standard deviation, is the red cell distribution width (RDW). The RDW provides some insight and quantification into the variation in red cell size, or anisocitosis [2].

Some analyzers (e.g. Advia, Siemens) have generated characteristic red cells cytograms, representing the red cell volume and hemoglobin concentration (V/HC). On the V/HC cytogram , Hb concentration is plotted along the x-axis and the cell volume along the y-axis. It is easy to interpret, for the blood samples with normal red cells show most RBC to fall in the central square of cytogram, because of their normal size and Hb content [5].

The platelet count

The platelet count (PLT) is most often measured by impedance counting. Using this principle PLT and RBC which are both analyzed in the same channel, are discriminated according to their volume and volume histograms are generated next. For PLT, the histogram generates a log curve if the distribution of PLT volume fits that of a (log) normal distribution: eventually all particles located under the fitted curve are considered as PLT. Mean PLT volume ranges from 6 to 10 fl, but impedance-type counters analyze particles ranging from 2 to 20 fl and, according to the fitted curve, the upper threshold that discriminates

PLT from RBC may either be at 36 fl or may vary automatically depending on the characteristics of individual blood sample (Sysmex). Instrument flags are triggered for cases corresponding to inability to separate clearly PLT from RBC [10].

On laser-type analyzer (Abbott, Bayer, others), each particle passes through a laser beam and scatters light that is detected by a photodiode. The amount of light scattered (at one, two or even four angles for some analyzers), is proportional to the area and therefore to the volume of the particle. PLT are indentified on a scatter histogram based on their volume and refractive index values. Some analyzers (e.g. Abbott) provide up to three counts on the same dilution if required, corresponding to optical, impedance, and immunological counts (CD61). Other analyzers (e.g. Sysmex) may determine, if required, an optical PLT count together with the reticulocyte count, after the use of RNA fluorescent stain [10].

An abnormal mean platelet volume or platelet histogram indicates that morphological platelet abnormalities are present and the platelets should be observed from a stained blood film to characterize the abnormality. Manual evaluation is performed when the PLT is very low, platelet clumping is observed, or abnormally large (giant) platelets are present. Often these abnormalities and others, such as cryoglobulinemia, cell fragmentation (hemolysis), and microcytic RBC are signaled by abnormal RBC and PLT indices and abnormal population flags [1].

It is important to note that the most often abnormal result (about 1.0-2.0% for hospitalized patients) is pseudothrombocytopenia. Spurious thrombocytopenia occurs in several circumstances related to the presence of ethylenediamine tetra-acetic acid (EDTA) used as the anticoagulant. Mechanism of EDTA-dependent platelet agglutination is related to circulating antibodies directed against normally hidden epitopes in the glycoprotein alpha IIb/beta IIIa complex from PLT membrane exposed only in the presence of EDTA. Other spuriously low PLT counts may be related to EDTA, including PLT rosetting around white blood cells (satellitism) and PLT-WBC aggregates [10]. Spurious increase of PLT count may be related to several situations, including fragmented red blood cells, cytoplasmic fragments of nucleated cells, cryoglobulins, bacteria or fungi, and lipids. Flags generated in several of these situations alert the operator on possible abnormal findings and may identify the problem. Analysing only PLT parameters is not sufficient: in many situations the WBC differential scattergram is of crucial help for flagging [1, 10, 11].

Differential leukocyte count (DLC)

Automated differential leukocyte count (DLC) implementation is a more complicated process and has been through several different technologies. The first automated analyzer used cell volume to provide a three-part differential analysis: neutrophil, lymphocyte and monocyte cell counts [2, 6]. The voltage pulses produced by the WBC depend upon the size of the cell and its nuclear density. Therefore, the pulses may be analyzed to differentiate between the types of WBC found. For example, lymphocytes are the smallest WBC and comprise the lower end of the size scale. Monocytes, prolymphocytes, and immature granulocytes comprise the central area of the WBC histogram, and mature granulocytes comprise the upper end. Many small hematology instruments use the three-part differential WBC separation because it is inexpensive and generally reliable. These analyzers are intended for low-volume laboratories, or as a backup for larger laboratories. It should be remembered that identification of monocytes, immature myeloid cells and nucleated red blood cells (NRBC) by those instruments is difficult. In general, a peripheral smear review is necessary to validate the automated differential generated by the instrument [1, 3, 6, 11].

Current hematology instruments combine laser technology, impedance, radio frequency, direct current, optimized temperatures and volumes, and staining in various ways to maximize sensitivity and specificity for the automated DLC. As a result of improvements in the technologies, the accuracy, precision and standardization of leukocyte differentials have increased substantially. Examples of multichannel counters which have been introduced since 1995 include those produced by Coulter-Beckman Electronics, Sysmex, Abbott, ABX Diagnostics and Bayer-Technicon (now Siemens) [2, 6].

The Coulter-Beckman STKS, like other instruments, includes a 5-parameter DLC reportable in both relative percentage and absolute numbers. The analyzer uses the volume, conductivity and light scatter (VCS) technology to generate the DLC. The V measures cell volume and number by impedance, the C measures the nuclear size and density of each cell with high-frequency current, and the S scattering from a laser source measures internal structure, granularity, and surface characteristics of cells as well as provides information on the shape and structure of individual cells [2, 6, 8].

The Sysmex hematology analyzers simultaneously measure both cell size and intracellular information by using the radio frequency/direct current method (RF/DC). This method uses direct current combined with radio frequency current to count and classify cell types. Choosing the appropriate frequency allows for detection of certain WBC features. Another channel detects immature WBC, where a specific reagent lyses the cytoplasm of normal WBC, but leaves immature WBC intact to be counted [2, 3].

The Abbott hematology analyzers use both impedance and laser optical technology. Placed in a single file by hydrodynamic focusing and laminar flow, cell stream through a flow cell for counting and analysis. WBC are counted and classified by laser light-scattering data, using a multi-angle polarized scatter and separation technique. The angle of scattering is a function of cell size, refractive index, nuclear shape, nuclear-cytoplasmic ratio, and granularity. One of the recent Cell-Dyn instrument uses optical scatter and fluorescence technology with an argon laser to count NRBC and to separate DLC parameters. The next Cell-Dyn instrument offers reticulocyte, CD4/CD8, and CD64 counts [2, 6].

The Advia (Bayer, now Siemens) DLC uses light scatter readings of cell size and peroxidase activity after exposure to a peroxidase - chromogen reagent to enumerate the neutrophils, eosinophils, monocytes, and lymphocytes. The basophils are counted in a different flow cytometric operation; a lysing reagent is added, stripping the other WBC of their cytoplasm, and leaving the basophils intact and countable. Another cluster of abnormal WBC, designated as large unstained cells (LUC), sometimes results. These are peroxidase-negative cells that are either large lymphocytes, virocytes, or blast cells [2, 6].

The hematology analyzer of ABX Diagnostics offers a 5-part automated DLC count by combining impedance, light transmission, cytochemistry, and fluoro-flow cytometry into their Double Hydrodynamic Sequential System. This approach combines a primary focused flow to measure impedance and a second focused flow to detect light [2].

Generally, analyzers reduced the need to manually verify abnormal or flagged patient results by combining different methods to maximize sensitivity and specificity. This has improved the accuracy in counting low-incidence cell such as NRBC, percentage of hypochromic cells (% HYPO), and various immature cells, thus expanding the list of tests done by hematology analyzers. The development of other tests, such as the reticulocyte, CD4, CD8, and CD64 counts will obscure the line between the immunology and hematology testing environments [5, 6].

The automated hematology instruments provide an accurate and precise total neutrophil count but do not report band numbers. In some clinical situations this information is very necessary, for example band neutrophil counts are helpful for the detection of neonatal sepsis. Instrument flagging for samples with increased band has been characterized as unreliable, and one way for the physician to obtain a band count is to request a manual review. It may be manual scan of film in which a technologist reviews at least 10 microscopic fields to confirm the absence of significant abnormality bands or more precise, time-consuming differential count [8].

When leukocyte aggregation occurs, the hematology analyzer produces spurious and low WBC count. A demonstrated increase in the VCS parameters (Coulter VCS technology), such as neutrophil volume distribution width (NDW) may be used as an adjunct indicator for leukocyte aggregation along with the unstable WBC counts or unexplained low WBC counts [9]. The manual differential is the accepted reference method for evaluating an automated DLC, but poses problems in evaluating the automated monocyte and basophil results. The correlation between the automated and manual differential for neutrophils, lymphocytes, and eosinophils is described as good, but because of low numbers in manual counts, there is poor agreement between the manual and automated basophil and monocyte counts [1, 6].

For the most accurate and reproducible hematological results, whole blood specimens should be analyzed as soon as possible after collection. Because the size and scatter properties of white cells change as a blood sample ages, automated analysis of the differential become more unreliable with time. New technologies for the performance of automated differentials have reduced many of the problems associated with automated differential counts dependent on the scatter properties only. On a 24-hour old sample, the MPV and MCHC should be excluded from the report. Because the HCT, MCV and RDW parameters are consistently elevated due to the sample's age, even at 24 hours, those results should also be excluded [4].

The Automated Technology – good and bad points

Hematology instrument operations consist of computation, CBC analysis, and other analyses such as DLC. On many large analyzers, the onboard computer does computation, analysis; it controls all hardware and software operations, and contains a database of stored patient data and quality control results [2, 6]. Some instruments have incorporated sophisticated features such as delta-checking of data from the same patient, and the displaying of electronic cell-sizing data via multi-color scattergram plots. The blood cells scattergrams provide a wealth of information about the DLC, however, these scattergrams have not gained popularity among clinicians. Generally, an experienced hematology technologist can link an abnormal WBC scattergram to various clinical conditions or interfering substances. In addition to reviewing scattergrams, the technologist must rely on the discriminatory flagging of suspect WBC parameters by the instrument. These are subroutines developed by each vendor in an attempt to accurately detect WBC abnormalities. In addition to these in vivo abnormalities, other abnormal results can be produced by a variety of factors. Pseudoleukopenia can result from prolonged specimen storage at room temperature in excess of 24 hours, or exposure to excessive temperatures. Pseudoleukopenia also may occur secondary to cell clumping, which may be antibody related, EDTA dependent or, rarely, secondary to mucopolysaccharide produced by tumors, especially adenocarcinomas [2, 10]. Erroneous

leukocyte counts can be seen in leukemic patients undergoing chemotherapy. In such situations, the cells often are more fragile than normal and disintegrate during their pressurized passage through the instrument counting orifice.

New hematology systems include tests, such as reticulocyte counts and flow cytometric CD4/CD8 counts, and CD64. Enhancements in flow cytometry make it possible for these new instruments to also count immature (blast) cells and NRBC [2].

Since the number and characteristic of the reticulocytes in the peripheral blood reflect the activity of the bone marrow, reticulocyte counting has become a fundamental part of the evaluation of patients with hematopoietic disease. In contrast to microscopic counting, automated techniques of reticulocyte enumeration are more precise, accurate, objective, and cost-effective, since 30 000 or more cells can be accurately evaluated in very short period of time. A variety of RNA-specific fluorescent dyes have been utilized for automated reticulocyte enumeration and some hematology analyzers utilize optical light scatter analysis to perform reticulocyte analysis on specimens stained with new methylene blue or other dyes. Measurements of reticulocyte and other parameters are still under investigation, but there is extensive evidence that these parameters are useful in the accurate classification of anemia patients, and monitoring patients receiving rEPO or recovering from chemotherapy or bone transplantation. Although economic consideration limit dedicated reticulocyte analyzers to laboratories with large reticulocyte sample volumes, the recent trend to incorporate reticulocyte enumeration into routine capacity of the hematology analyzers will make automated reticulocyte analysis increasingly common [7].

Some large instruments have expert systems computer-based programs to automate the labor-intensive application of complex review criteria. Expert systems can be programmed to initiate reflex testing, return specimens, or make and stain slides according to user-defined rules. Flagging properties have also been incorporated into these analyzers to alert the operator to the existence of abnormal cell morphology and the presence of abnormal cells which the analyzer is unable to count, as well as certain sample characteristics, for example platelet clumps, which may cause incorrect results to be produced [1]. Early automated differential analyzers suffered from excessive flagging of samples for manual review, with some instruments being virtually unusable because they flagged a high proportion of either normal or slightly abnormal specimens. Over the years, as the capabilities and performance of automated analyzers have improved, the respective roles of the automated analyzer and the complementary procedures have changed. It is recognized that the automated systems are superior for counting of WBC, RBC and PLT and for differential counting of WBC for well-characterized (matured) cell types, whereas visual microscopy is superior for differentiating cell based on nuances of cytological features, especially for immature cells [1]. Microscopic smear review is the main complementary procedure , the decision as to whether smear review is necessary for each sample plays a major role in hematology laboratory costs, productivity and speed of reporting [1, 6]. There is little uniformity among different laboratories of criteria for action. Recognizing the long-standing need for generally accepted guidelines (rules), which could be applied to criteria for review of CBC and differential results from automated hematology analyzers, Barnes and co-workers [1] described 41 rules of the automated analyzer and complementary procedures - manual action, most commonly smear review. They include rules for first-time samples analysis, as well as rules for repeating samples within 72 hours from their taking from a patient, for example, if any plates PLT <100 x109/l or >1000 x109/l and first time analysis, then reviewing a slide is required [1] .

To conclude, the automated hematology analyzers provide valuable information regarding common hematological conditions. It is important that operators and end users have an understanding of the parameters while interpreting their numerical and graphical data. This can enhance the diagnostic utility of automated data.

The automated hematology analyzers are characterized by a good sensitivity and specificity, reliability, high-quality patient results and safety. In general, their performance has been excellent and is now generally accepted by the medical community.

SUMMARY

The complete blood count (CBC) and differential leukocyte count (DLC) are the backbone of any laboratory evaluation, not only used by general practitioners, but also by other medical specialties. The CBC and DLC are used to diagnose anemia, to identify acute and chronic illnesses, bleeding tendencies, and white blood cell disorders. During the last quarter century, blood cell analysis has progressed from the use of labor-intensive manual procedures to the use of highly automated instruments. Modern hematology analyzers provide new additional parameters enabling earlier and more precise diagnosis. Moreover, they incorporate flow cytometry, robotics and expert system technologies.

Sensitive flags now allow the identification of abnormal cells or spurious counts, but only the most sophisticated hematology analyzers have optimal flagging and more simple analyzers, especially those without a WBC differentiation scattergram, do not possess the same sensitivity for detecting anomalous results. Therefore it is crucial that obtained results are clear for clinicians, and the parameters obtained completely used.

The study presents the current state of knowledge about hematology analyzer, concerning principles of their operation, their advantages and limitations, as well as the provided parameters.

Keywords: automated hematology analyzer, complete blood count, differential leukocyte count

STRESZCZENIE

Morfologia krwi obwodowej i różnicowanie leukocytów stanowią podstawę w badaniach laboratoryjnych zarówno dla lekarza pierwszego kontaktu, jak również dla lekarzy innych specjalności. Wykorzystywane są w diagnostyce anemii, w rozpoznawaniu ostrych i przewlekłych schorzeń, diagnostyce krwawień oraz w zaburzeniach układu białokrwinkowego. W ostatnim ćwierćwieczu dokonał się ogromny postęp w badaniu komórek krwi, od używania metod manualnych, aż do stosowania wysoko zautomatyzowanych aparatów. Nowoczesne analizatory hematologiczne dostarczają nowych dodatkowych parametrów pozwalających na wcześniejszą i dokładniejszą diagnozę. Ponadto, wyposażone są w cytometrię przepływową, robotykę i eksperckie systemy technologiczne. Jedynie najbardziej zaawansowane analizatory posiadają wrażliwe systemy flagowania, pozwalające na identyfikację komórek nieprawidłowych i zafałszowanego zliczania, zaś prostsze analizatory nie posiadają takiej czułości w wykrywaniu nieprawidłowości. Dlatego kluczowe jest, aby uzyskane parametry były zrozumiałe dla klinicysty, a otrzymane wyniki w pełni wykorzystane. Praca przedstawia stan wiedzy na temat analizatorów hematologicznych, zasad ich działania, zalet i ograniczeń oraz dostarczanych parametrów.

Słowa kluczowe: analizatory hematologiczne, morfologia krwi, różnicowanie leukocytów

REFERENCES

1. Barnes P. W., McFadden S. L., Machin S. J., et al.: The international consensus group for hematology review: suggested criteria for action following automated CBC and WBC differential analysis. Lab Hematol., 11, 2005.
2. Chapman M.: Hematology analyzers offer new technology and user-friendliness. Lab Med, 31, 3, 2000.
3. Gopal S., Rappaport E. S., Koss W., et al: Applicability of the Sysmex pocH-100i analyzer for satellitate laboratories. Labmedicine, 36, 8, 2005.
4. Hill V. L., Simpson V. Z., Higgins J.M., et al.: Evaluation of the performance of the Sysmex XT-2000i hematology analyzer with whole blood speciments stored at room temperature. Labmedicine 40, 12, 2009.
5. Kakkar N., Makkara M.: Red cell cytograms generated by an Advia 120 automated hematology analyzer: characteristic patterns in common hematological conditions, Labmedicine, 40, 9, 2009.
6. Koenn M. E,, Kirby B. A., Cook L. L., et al.: Comparison of four automated hematology analyzers. Clin Lab Sci, 14, 4, 2001.
7. Riley R. S., Ben-Ezra J. M., Tidwell A.: Reticulocyte enumeration: past and present. Lab Med, 10, 32, 2001.
8. Senzel L. B., Spitzer E. D., DeCristofaro J. D.: A streamlined laboratory process for obtaining band counts in hospitalized neonates. Labmedicine, 41, 5, 2010.
9. Yang D., Guo X., Chen Y., et al.: Leukocyte aggregation in vitro as a cause of pseudoleukopenia. Labmedicine 39, 2, 2008.
10. Zandecki M., Genevieve F., Gerard J., et al.: Spurious counts and spurious results on hematology anayzers: a review. Part I: platelets. Int J Lab Hematol., 29, 1, 2007.
11. Zandecki M., Genevieve F., Gerard J., et al.: Spurious counts and spurious results on hematology anayzers: a review. Part II: white blood cells, red cells, hemoglobin, red cell indices and reticulocytes. Int J Lab Hematol., 29, 1, 2007.