Introduction

The complete blood count (CBC) with leukocyte differential count is one of the most frequently requested hematologic tests in medical laboratories ***(Pratumvinit et al., 2013).*** The accurate and timely delivery of differential white blood cell (WBC) count results by the hematology laboratory is crucial in many clinical settings, including acute infections, hematologic malignancies, and the administration of chemotherapy ***(Sireci et al., 2010).***

Automated blood cell counters offer leukocyte, red blood cell (RBC), and platelet (PLT) counts and a 5-part leukocyte differential count. In addition, some instruments provide a nucleated RBC count. Hematology instrument differentials provide only limited information about cell morphologic features using various algorithms to generate abnormal cell flags and are often unable to reliably classify abnormal and immature cells ***(Briggs et al., 2011).***

Despite the great precision, high accuracy, and expandability of automated hematologic analyzers, manual slide review is still necessary to identify some morphologic abnormalities that may be relatively unremarkable in automated methods ***(Pratumvinit et al., 2013).*** A film is reviewed to provide information additional to or missing

from the analyzer report or to confirm results provided by the analyzer ***(Briggs et al., 2011).***

The rate of slide review differs according to the type of hematology analyzer and criteria for slide review, as well as patient characteristics including age, disease, and disease severity. In general, analyzer flags constitute the major proportion of criteria for manual review. The usefulness of instrument-generated flags depends on their sensitivity and specificity ***(Kim et al., 2012).***

With continuing pressure on laboratory resources and the need for faster turnaround times (TAT), it is essential to reduce the number of manual blood film reviews and manual differential counts. The challenge is to reduce the number of blood films examined without missing important diagnostic information ***(Briggs et al., 2011).***

Each laboratory has its own criteria about when to perform manual smear review following automated blood count analysis. The laboratory productivity of CBC is inversely related to the number of manual differential count review rates; in addition, the rate of smear review is variable in each institution ***(Pratumvinit et al., 2013).***

In 2005, the International Consensus Group for Hematology Review established guidelines composed of 41

rules for action after automated analysis of a blood sample that had false negative (FN) and false positive (FP) rates of 2.90% and 18.60%, respectively ***(Barnes et al., 2005).***

The number of manual differentials performed daily by clinical hematology laboratories of many tertiary care medical centers can be quite large. Determining acceptable rates for performing manual blood smears is important to ensure the quality of the reported complete blood count results, but reported rates are highly variable. In a study of 263 institutions serving both inpatients and outpatients, the 10th–90th percentile smear review rates were 9.9%–50% ***(Novis et al., 2006)***. The variability was probably due to the patient mix and to differences in criteria for triggering manual reviews ***(Froom et al., 2009).***

Aim of the Work

- To validate the currently used criteria for manual smear review following automated blood cell analysis in Ain Shams University main laboratory.
- Compare the performance of the criteria suggested by The International Consensus Group for Hematology Review (2005) with the criteria used in the laboratory.

Chapter (1)
Automated Cell Counters

The complete blood count is a very common test which provides important information about the types and numbers of cells in blood. Currently the CBC is performed with complex and expensive hematology analyzers located in central and or satellite laboratories, which require trained and accredited professionals to operate ***(Rao et al., 2007).*** Automated laboratory hematology analyzers provide cell counts, flags, cell plots (instrumental morphology) and distributional histograms with greater precision and accuracy than that provided by manual determinations ***(Froom et al., 2009).***

I. Manual blood counts

During the first half of the twentieth century, the complete blood count was performed using exclusively manual techniques:

1. Blood cell counts (red cells, white cells, platelets) were performed using appropriately diluted blood samples and a ruled counting chamber (hemocytometer).
2. Hemoglobin concentration (Hb) was analyzed colorimetrically by the cyanmethemoglobin method.
3. The hematocrit (Hct) (packed cell volume) was measured by high speed centrifugation of a column of blood, either

in a specially designed tube (the Wintrobe tube), or in sealed microcapillary tubes (i.e., the "spun" hematocrit, often obtained by fingerstick blood collection).

4. The white blood cell differential was obtained by examining and enumerating by class (e.g., granulocytes, lymphocytes, monocytes) 100 to 200 individual white blood cells on a suitably stained blood smear ***(George, 2010).***

In 1932, Wintrobe developed a set of calculated indices that estimated erythrocyte size and hemoglobin content based on the red blood cell count, hemoglobin concentration, and hematocrit. These indices included:

a. Mean corpuscular volume (MCV): the volume (in femtoliters, fL) of the average circulating red blood cell.

$$MCV = \frac{Hct}{RBC} \times 10 \text{ (millions/ microL).}$$

b. Mean corpuscular hemoglobin (MCH): the hemoglobin content in pictograms (pg) of the average circulating red blood cell.

$$MCH = \frac{Hb}{RBC} \times 10 \text{ (millions/ microL).}$$

c. Mean corpuscular hemoglobin concentration (MCHC): the hemoglobin concentration within circulating red blood cells (grams of hemoglobin per 100 mL of packed red blood cells).

$$MCHC = \frac{Hb}{Hct} \times 100 \text{ (percent)}$$ ***(George, 2014).***

Disadvantages of manual cell counting:

1. Cell identification error:

a. It is subjective, labor-intensive, and statistically unreliable (only 100-200 cells are counted).

b. It is imprecise with reported coefficients of variation (CV) ranging from 30 – 110 %.

c. This is mostly associated with distinguishing lymphocytes from monocytes, bands from segmented forms and abnormal cells (variant lymphocytes from blasts). The monocytes tend to be underestimated and the lymphocytes tend to be overestimated ***(Kamath, 2011).***

2. Slide cell distribution error:

a. Experience is needed to make technically adequate smears consistently.

b. Non-uniform distribution of WBCs over the smear, with larger leukocytes concentrated near the edges and lymphocytes scattered throughout.

c. There is a non-uniform distribution of red blood cells as well, with small crowded red blood cells at the thick edge and large flat red blood cells without central pallor at the feathered edge of the smear ***(Kamath, 2011).***

3. **Statistical sampling error:** Strict adherence to correct procedures for specimen collection is critical to accuracy of any test ***(Kamath, 2011).***

II. Evolution of automated cell counters

In 1956, Walter and Joseph Coulter patented a device developed in their basement that used an electric impedance aperture to count red cells and white cells called the coulter impedance aperture. In the 1960s, the first multichannel automated hematology instruments appeared. Anticoagulated whole blood samples were aspirated into the apparatus and automatically aliquoted and diluted into RBC and WBC counting chambers (baths) for cell counting and sizing with impedance apertures, and into a spectrophotometric cuvette for Hb determination using the cyanmethemoglobin method ***(George et al., 2014).***

In the 1970s, improvements of the cell counting apertures led to more accurate cell sizing and the ability to enumerate platelets in whole blood samples. The 1970s saw the introduction of cell counting by light scattering technology. In the 1980s began the three part differential which differentiate

leukocyte subsets in liquid suspension, rather than on a stained smear. Flow-through automated differentials were first performed using standard electrical resistance or impedance technology ***(George et al., 2014).***

Five part differentials were first introduced in the early 1990s. Different instrument manufacturers all report at a minimum the basic five leukocyte subsets (neutrophils, eosinophils, basophils, lymphocytes, and monocytes) and have flags to detect the presence of abnormal cells (eg, blasts, nucleated RBC, reactive lymphocytes, lymphoma cells, mononuclear granulocytes) ***(George et al., 2014).***

III. Principles of automated cell counters

Number of principles for cell counting and differential analysis have been utilized which are electrical impedance principle, optical detection principle (light scattering), fluorescence flow through cytometry, radio frequency (RF) ***(Lehner et al., 2007).***

1. Electrical impedance technology

Impedance measurement (Coulter principle) was historically the first principle of measurement. This is based on the measurement of changes in resistance during cell passing through small defined opening between 2 electrodes. Each cell suspended in a conductive liquid (diluent) acts as an insulator ***(Beckman Coulter, 2001).***

Particles such as blood cells are non conductive but are suspended in an electrically conductive diluent. As a dilute suspension of cells is drawn through the aperture, the passage of each individual cell momentarily increases the impedance (resistance) of the electrical path between two electrodes that are located on each side of the aperture ***(Kratz, 2010)***. As shown in Figure 1, this produces a voltage pulse whose number indicates the blood cell count while its magnitude is proportional to the size of the cell ***(Lehner et al., 2007).***

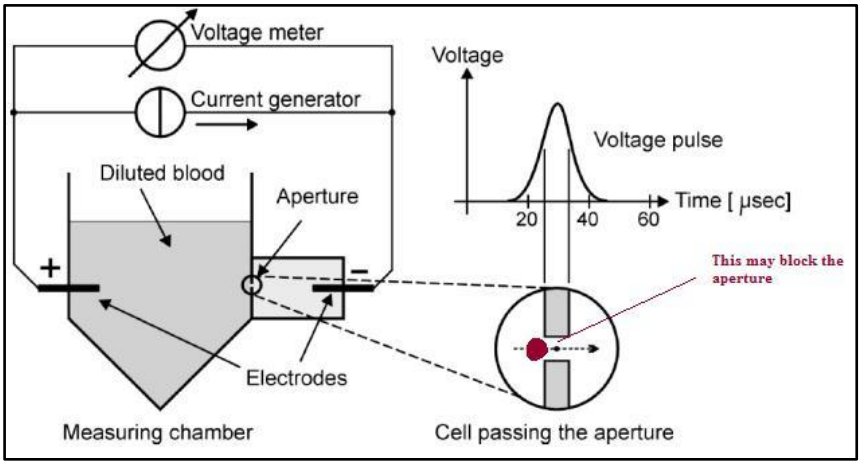

Figure 1: Impedance technology in automatic cell counting ***(Kratz, 2010).***

2. Optical Scatter Technology

Scattering a laser beam by each individual cell is measured. A diluted blood specimen passes in a steady stream through which a beam of laser light is focused, as each cell passes through the sensing zone of the flow cell, it scatters the focused light. Scattered light is detected by a

photodetector and converted to an electric impulse, number of impulses generated is directly proportional to the number of cells passing through the sensing zone in a specific period of time as shown in Figure 2 ***(Kratz, 2010).***

The application of light scatter means that as a single cell passes across a laser light beam, the light will be reflected and scattered, the patterns of scatter are measured at various angles (forward scatter and right angle 90°). Scattered light provides information about cell structure, shape, and reflectivity. These characteristics can be used to differentiate the various types of blood cells and to produce scatter plots with a five-part differential ***(Kratz, 2010).***

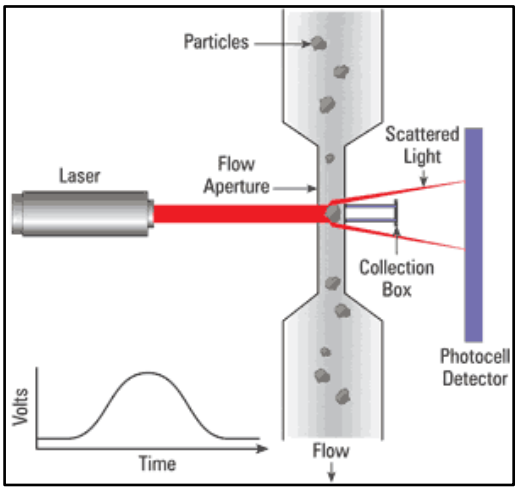

Figure 2: Light scatter method of automatic cell counting ***(Kratz, 2010).***

3. Flow Cytometry

Flow cytometry is defined as the simultaneous measurement of multiple physical characteristics of a single cell as the cell flow in suspension through a measuring device. Flow cytometry combines the technologies of fluid dynamics, optics, laser, computers, and fluorochrome-conjugated monoclonal antibodies that rapidly classify groups of cells with heterogeneous mixtures ***(McCoy, 2003).***

The principle of flow cytometry is based on the fact that cells are stained in suspension. Flow cytometry has specially come to donate the use of fluorescence measurement, usually with a laser light source which is the most common light source used in flow cytometers because of the properties of intensity, stability, and monochromatism ***(McCoy, 2003).***

One of the major advantages of flow cytometry is that more than one measurement can be made on every cell during the few milliseconds that the cell spends passing through the laser beam. Each cell can be optically measured for the intensity of scattered light ***(Kelliher, 2001).***

The cellular light scatter patterns can be used to identify cells. Both intrinsic and extrinsic properties of cells can be analyzed by flow cytomety. The intrinsic properties include forward and right angle light scatter, which correlate with size and granularity of cell, respectively. This data

output does not require addition of dyes or stains for detection. In contrast, extrinsic properties rely on binding of various probes to the cells ***(Dunphy, 2003).***

In addition, fluorescence can be used to measure extrinsic features (e.g., specific protein expression and nucleic acid content) by adding reagents (e.g., fluorescent stains and antibodies). Virtually all flow cytometric assay use fluorescent stains. Fluorescent dye used in flow cytometry bind or react specifically with the cellular component (e.g., reticulocytes (retics), peroxidase enzyme, or DNA content) ***(Turgeon, 2010).***

The scattered light passes through a variety of fibers and lenses and is then measured by photomultiplier tubes, which convert; light signal into electronic signals for computer analysis. Light scattered along the axis of the laser beam is forward scatter (FSC), and light scattered perpendicular to the axis is side scatter (SSC) or "orthogonal scatter". Forward scatter is roughly proportional to cell size, side scatter to cytoplasmic granularity. Granulocytes have a much larger side scattered light signal than do lymphocytes ***(Dunphy, 2003).***

4. Radio Frequency

The high voltage electromagnetic current is used to detect cell size, based on the cellular density. The radio frequency pulse is directly proportional to the nuclear size

and density of a cell. Radio frequency or conductivity is related to the nuclear cytoplasmic ratio, nuclear density, and cytoplasmic granulation ***(Aller, 2009).***

Sample analysis cycle

In a typical automated blood cell counter, the blood sample is aspirated and separated into two portions: one is lysed and diluted to permit measurement of hemoglobin concentration and leukocyte enumeration, and the other is diluted without lysis to enable counting and sizing of red cells and platelets. Automated hematology instruments lately rely heavily on analysis of light scattered at different angles from an incident laser beam striking passing cells ***(Ryan, 2010).***

Cell count, volume, and internal structure can be determined by multivariate analysis of these data. Modern automated instruments use multiple parameters (including light scatter measured at different angles, electrical impedance, myeloperoxidase staining, electrical conductivity, or differential resistance to lysis) to identify and enumerate the five major morphologic leukocyte types in blood: neutrophils, basophils, eosinophils, lymphocytes, and monocytes ***(Ryan, 2010).***

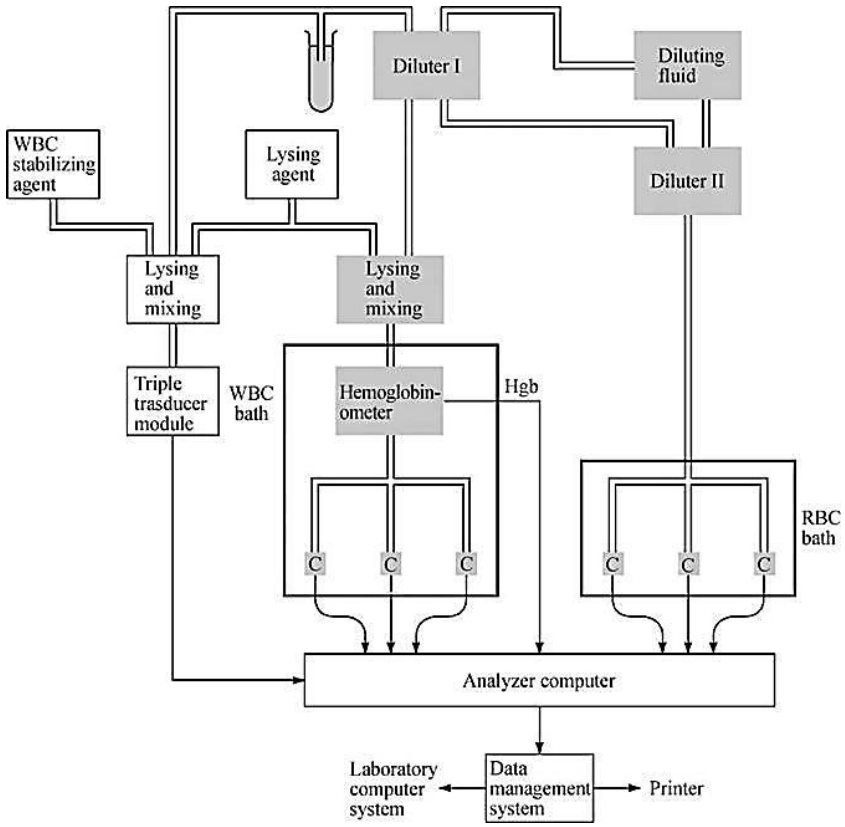

Figure 3: Block diagram of the hematology analyzer Coulter ***(http://intranet.tdmu.edu.ua/).***

A. Red Blood Cell/Platelet Chamber

1. Red blood cell and platelet counts

The sample is divided into aliquots (Figure 3). The first aliquot is delivered to the RBC/platelet dilution chamber where blood cells are diluted in an electrically conductive diluent. Within the dilution chamber, there are an external electrode and three apertures each of which has an internal electrode. A steady stream of diluent flows behind each