INTRODUCTION

The advent of automation in the diagnostic laboratory and increasing dependence on machine generated results for analytical tests highlight the importance of laboratory quality management programmes ***(Hoffbrand et al., 2009)***.

The needs of clinical chemistry established the original patterns of internal and external quality control methodology and the design of control materials and calibrators. Although automated hematology analyzers share these principles, they also have unique characteristics that require a specialized approach to quality control ***(Koepke, 1999)***.

Laboratory quality management programmes are designed to detect, reduce, and correct deficiencies in a laboratory's internal analytical process prior to the release of patients results ***(Lewis and De la Salle, 2006)***.

An established programme of quality management is essential to ensure that the results of the tests are reliable, reproducible and as accurate as possible, to achieve the necessary level of good practice and to

be reassured that the standard is constantly maintained ***(Lewis and Kumari, 2000)***.

A Quality assurance programme includes internal quality control, external quality assessment and standardization. It must also ensure adequate control of the pre analytic and post analytic stages from specimen collection to the timely dispatch of an informative report ***(Lewis and De la Salle, 2006)***.

Internal Quality Control (IQC) is intended to ensure that measurements are sufficiently precise day to day or batch by batch within established limits. It is based on monitoring the procedures which are actually used for the tests in the laboratory There are several methods of IQC which complement each other ***(Hoffbrand et al., 2009)***.

Regulatory agencies require the use of Quality control (QC) materials to assess the validity of results on patients samples. The results of sequential (daily) measurements of the quality control material (at least two levels of control material every 24hrs) are plotted on a Levey-Jennings control chart and Westgard rules are applied, to detect a random or a systematic error during the function of the hematology analyzer (***www.streck.com/.../Streck-Paper-QC for Hematology Instrumentation 2007***).

Other methods of IQC include:

- Duplicate tests on a proportion of the patient specimens.
- Delta check, comparing current test results with previous results.
- Consistency of mean values of patient data.
- Correlation check (e.g., blood film features or sensibility of interrelated parameters).

(*Lewis and Kumari, 2000)*

Present analyzers appear to have an unrealized potential for automated internal performance checks. Such tests, while not replacing the functional tests provided by whole blood or stabilized blood methods, could adjunctively enhance the control of precision and accuracy. One of these is automatically calculating the moving average of RBCs indices and by this the patients' blood samples can themselves be used to check for both calibration and hematology analyzer drift *(**www.streck.com/.../Streck-Paper-QC for Hematology Instrumentation 2007***).

A Participation in an external Q.C. program (EQAS) or proficiency testing program (PT) offers the most practical means of monitoring overall work performance in comparison with instrument, method,

and/or reagent-based peer group data. A laboratory may choose to participate in one or more national and/or regional QC programs, depending upon the range of tests it performs and the requirements of accreditation and regulatory agencies. Most of the accreditation agencies require participation in programs covering at least all of the routinely or frequently performed tests by the laboratory ***(www.proficiency.org/Portals/0/PJLAAcred, 2011)***.

Quality assurance provides a foundation for the confidence that is placed in laboratory results and their use in the diagnosis and treatment of diseases. Establishing continuous internal and external quality assessment systems in hematology, together with continuing education for laboratory personnel and the availability of automated instrumentation, will lead to achievement of optimum laboratory quality ***(Kibak, 2008)***.

AIM OF THE WORK

This work aims to:

- Establish internal and external quality control programmes for complete blood picture parameters at Ain Shams University Laboratories (Demerdash Hospital) as a preparatory step for laboratory accreditation according to the ISO standard ISO 15189, 2007.
- Monitor the laboratory performance as regards the complete blood picture parameters.
- Identifying problems during the routine work and initiating a corrective and preventive action.
- Establishing key performance indicators which can be used to monitor laboratory performance for complete blood picture testing.
- Calculating the turn around time for the CBC sample at Ain Shams University Laboratories (Demerdash Hospital).

AUTOMATED BLOOD CELL COUNTERS

During the first half of the twentieth century, the complete blood count (CBC), one of the commonly ordered laboratory tests, was performed using exclusively manual techniques:

- Blood cell counts (red cells, white cells, platelets) were performed using appropriately diluted blood samples and a ruled counting chamber (hemocytometer).
- Hemoglobin concentration was analyzed colorimetrically by the cyanomethemoglobin method.
- The hematocrit (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).
- 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.

(Ward, 2000)

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

- Mean corpuscular volume (MCV)—the volume (in femtoliters, fL) of the average circulating red blood cell.
- Mean corpuscular hemoglobin (MCH)—the hemoglobin content (in picograms) of the average circulating red blood cell.
- Mean corpuscular hemoglobin concentration (MCHC)—the hemoglobin concentration within circulating red blood cells (grams of hemoglobin per 100 mL of packed red blood cells).

(*George et al., 2012*)

These three indices were calculated manually, as follows:

- MCV (fL)= 10*x*HCT (percent) ÷ RBC (millions/ microL).
- MCH (pg/red cell)= HGB (g/dL)*x*10 ÷ RBC (millions/ microL).

  MCHC (g/dL)= HGB (g/dL)*x*100 ÷ HCT (percent).

(*George et al., 2012*)

These early methods were laborious, time intensive and have certain limitation such as:

- Manual WBCs, RBCs and platelets count by hemocytometer are very imprecise with reported Coefficients of Variation (CV) ranging from 30-110%.
- Experience is needed to make technically adequate smears consistently.
- Non-uniform distribution of WBCs over the smear, with larger leukocytes concentrated near the edges and lymphocytes scattered throughout.
- 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.
- Manual cell counters subjective, labor-intensive, and statistically unreliable (only 100-200 cells are counted).
- Cell identification errors in manual counting which is mostly associated with distinguishing lympho-cytes 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.

(Buttarello et al., 2004)

Principles of automated blood Counter

Number of principles for cell counting and differential analysis have been utilized. These are:

- Electrical Impedance principle.
- Optical detection principle (light scattering).
- Radio frequency.
- Fluorescence flow through cytometry.

(Lehner et al., 2007)

Electrical Impedance

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. The cells have low conductivity than diluent.

Later on in the late 1940s, Wallace H. Coulter developed a technology for counting and sizing particles using impedance measurements. The technology was principally developed to count blood cells quickly by measuring the changes in electrical conductance as cells suspended in a conductive fluid passed through a small orifice. Presently, over 98% of automated cell counters incorporate this technology, which is referred to as the Coulter Principle ***(Beckman Coulter, 2001)***.

In a ***Coulter Counter*** instrument, a tube with a small aperture on the wall is immersed into a beaker that contains particles suspended in a low concentration electrolyte. Two electrodes, one inside the aperture tube and one outside the aperture tube but inside the beaker, are placed and a current path is provided by the electrolyte when an electric field is applied **(Fig. 1)** ***(Beckman Coulter, 2001)***.

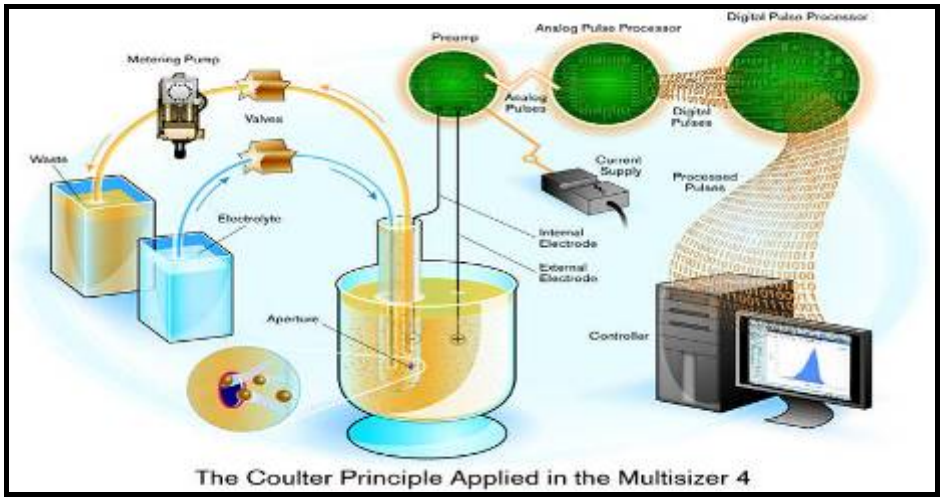

Fig. (1): Schematics of a COULTER COUNTER ***(Beckman Coulter, 2001)***.

As a blood cell is carried through the aperture, it displaces some of the conducting fluid and increases the electrical resistance. This produces a corresponding change in potential between the electrodes, which lasts as long as the cell takes to pass though the aperture; the height of the pulses produced indicates the volume of the cells passing through. The pulses

can be displayed on an oscillograph screen. The pulses are led to a threshold circuit provided with an amplitude discriminator for selecting the minimal pulse height, which will be counted. The height of the pulses is used to determine the volume of the red cells ***(Briggs and Bain, 2011)***.

By measuring the number of pulses and their amplitudes, one can obtain information about the number of particles and the volume of each individual particle. The number of pulses detected during measurement is the number of particles measured, and the amplitude of the pulse is proportional to the volume of the particle. Because this is a single particle measurement process, it yields the highest resolution that any particle characterization technique can achieve. The particle diameter can be determined at the resolution of voltage or current measurement which can be very accurately using current electronics technology. The distribution amplitude can be determined to the accuracy of a single particle ***(Beckman Coulter, 2001)***.

Since most measurements aim at obtaining particle counting or size distribution, the recorded pulse height is converted to particle size using the calibration constant and placed into one of the pre-set size bins. The particle size distribution and counting

are the cumulative result of all pulses measured ***(Beckman Coulter, 2001)***.

In the Coulter Principle instrumentation, the change in electric resistance due to passages of particles through the aperture is determined using fast electronic circuitry. The detected signals are instantaneously digitized at a rate of a few million times per second into digital signals. The digital signal is then recorded for every pulse in the form of pulse parameters, i.e., timing, height, width of pulses, etc. the output histogram is a display of the distribution of the cell volume and frequency. Each pulse on the x-axis represents size in femtolitre (fl); the y axis represents the relative number of cells (Fig. 2) ***(La Porta, 2002)***.

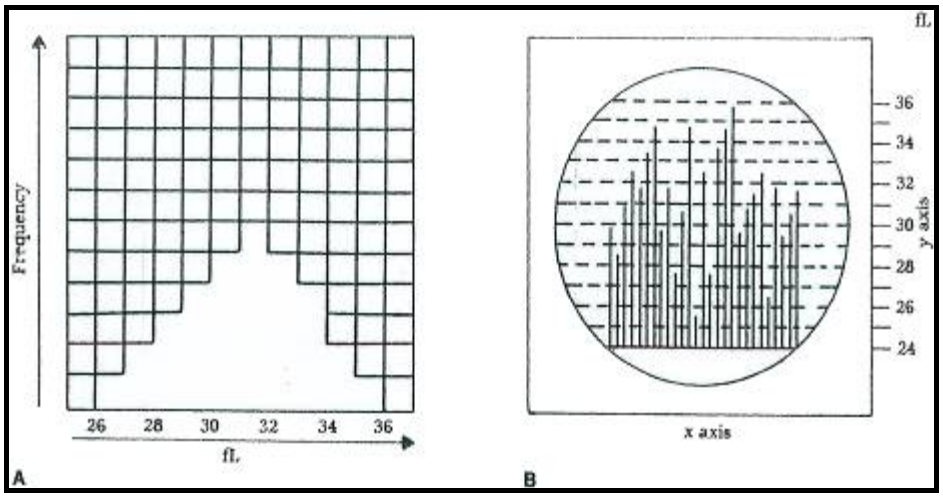

Fig. (2): Cell counting: impedance principles. The number of pulses on the oscilloscope screen indicates the number of particles passing through the aperture. The height (amplitude) of each pulse reflects the volume of each cell. A: Histogram distribution of normal erythrocytes. B: Oscilloscope appearance as erythrocytes pass through the cell counting aperture and produce an electrical resistance. *(Adapted with perimission from Pierre R. Seminars and Case Studies: The Automated Differential, Hialeah, FL: Coulter Electronics, 1985: 4)*.

Optical detection principle (light scattering):

In optical or hydrodynamic focusing method of cell counting and cell sizing, laser light is used and a diluted blood specimen passes in a steady stream through which the beam of laser light is focused. As each cell passes through the sensing zone of the flow cell ***(La Porta, 2002)***.

Scattered light is detected by a photo detector and converted into an electrical pulse. The number of pulses generated is directly proportional to the

number of cells passing through the sensing zone in a specific period ***(La Porta, 2002)***.

The light will be reflected and scattered .the patterns of scatter are measured at various angles. Scattered light provides information about cell structure, shape, and reflectivity. These characteristics can be used to differentiate the various types of white blood cells and to produce scatter plots with a five part differential ***(Orsulak, 2003)***.

Angles of light scatter

Various angles of light scatter can aid in cellular analysis these are:

1. Forward light scatter 0°. This is diffracted light, which relates to the volume of the cell.
2. Forward low-angle light scatter 2° to 3°. This characteristic can relate to size or volume.
3. Forward high angle 5° to 15°. This type of measurement allows for description of the refractive index of cellular components.
4. Orthogonal light scatter 90°. The result of this application of light scatter is the production of data based on reflection and refraction of internal components, which correlates with internal complexity.

(Gonder and Mell, 2002)