Feature Article

Measurement Modalities of the HORIBA Medical ABX Pentra DX120 Hematology System

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The HORIBA Medical ABX Pentra DX120 is a complete hematology platform featuring Complete Blood Count (CBC), Nucleated Red Blood Cell (NRBC) count, and Reticulocyte (Retic) count at an approximate throughput of 120 CBC samples per hour. Multiple measurement modalities are used by the system including cell counting by electronic impedance variation, hemoglobin measurement by absorbance spectrophotometry, White Blood Cell (WBC) population differential by optical light scatter and impedance measurements, and measurement of Reticulocytes and NRBC using thiazole orange stain and orthogonal fluorescence with an argon laser.

ELECTRONIC IMPEDANCE VARIATION

The Red Blood Cells (RBC), WBC, and Platelets are measured by electronic impedance.

Cells passing through an aperture create resistance (impedance) when they are suspended in an electrolytic diluent between two electrodes and a voltage potential.

The voltage change measured as a cell passes through the aperture is proportional to the size of the cell. Since the current is constant and remains unchanged, the larger the cell, the more resistance it creates.

The individual voltage pulses generated by cells passing through the aperture are amplified, channeled according to size and threshold, and then mathematically integrated to give final numeric values for the RBC, WBC, and Platelet concentrations in the diluted sample. (Figure 1)

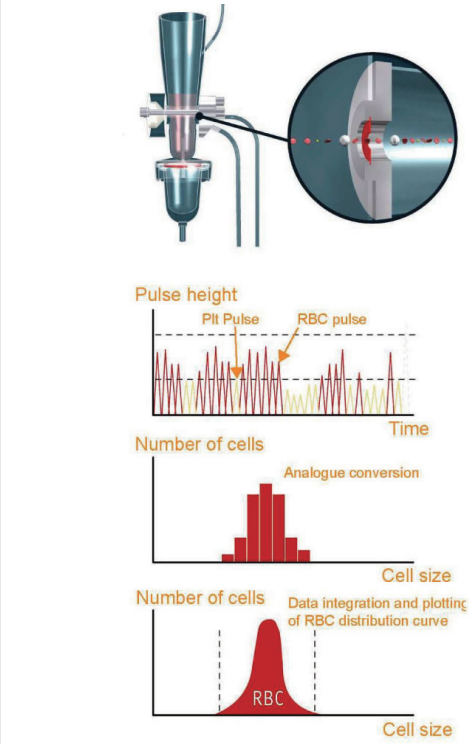

Figure 1

HEMOGLOBIN by ABSORBANCE SPECTROPHOTOMETRY

Traditional methods for measuring RBC hemoglobin used potassium cyanide (KCN) to form a cyanmethemoglobin compound. Absorbance was then measured by spectrophotometry at a wave length of 550 nm. The more ecology friendly Lysebio hemoglobin reagent formulation used on the HORIBA Medical ABX Pentra DX120 relies on an oxy-hemoglobin method eliminating the use of KCN. The absorbance value obtained is then multiplied by a coefficient in order to obtain the hemoglobin value.

WBC DIFFERENTIAL MEASUREMENT

In addition to impedance measurement, white blood cells in the sample are also evaluated by flow cytometry using a double hydrodynamic flow cytometer. Cell complexity is measured using cytochemical staining with chlorazol black and light absorbance from a polychromatic light source. Cell volume determination is made by measuring impedance changes. (Figure 2)

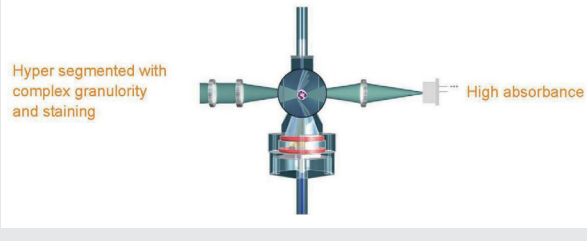

Figure 2

Identification and quantification of mature and immature WBC cell lines is accomplished by graphic representation in a double differential matrix. Lymphocytes, Monocytes, Neutrophils, and Eosinophils (LMNE) white cell types are distinguished by their size and complexity. Immature cell lines are further identified in an extended area of the matrix. (Figure 3)

RETICULOCYTE MEASURING PRINCIPLES

Reticulocytes are immature red blood cells characterized by remnant nuclear material within the cells. The Retic module of the HORIBA Medical ABX Pentra DX120 quantitates reticulocytes fluorimetrically with thiazole orange stain and an argon ion laser (488 nm). Thiazole orange is a fluorescent stain which is specific for nucleic acids.

A red cell passing through the Retic flowcell gives three

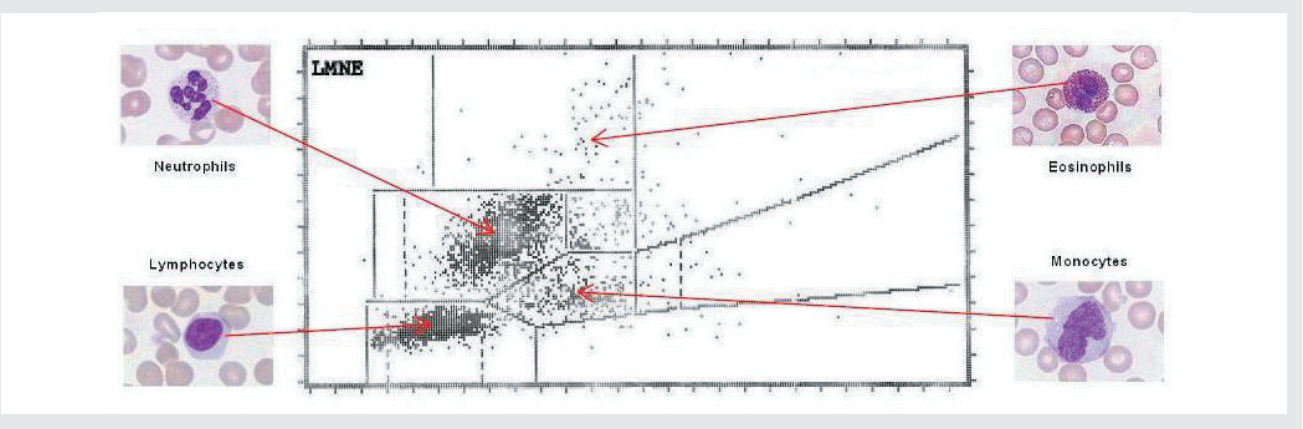

Figure 3

types of information: (1) The size of the cell is measured by resistivity (impedance); (2) Forward Scattered Light (FSL) is measured 200 microseconds after the aperture measurement; and (3) Orthogonal Fluorescence Light (OFL) is measured simultaneously with the FSL. (Figure 4)

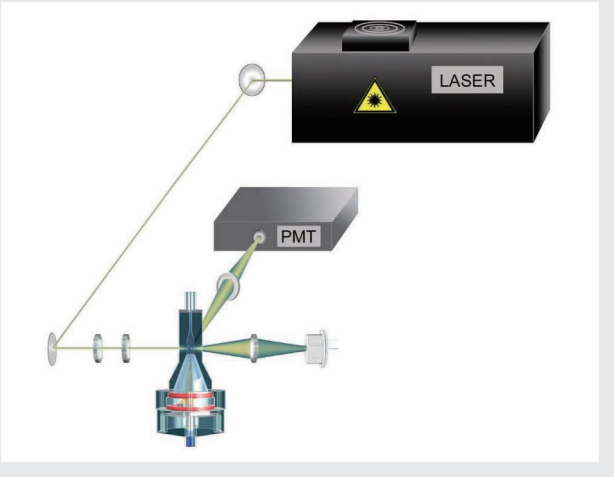

Figure 4

The fluorescence (OFL) is collected using a lens focused on the optical flowcell and located at 90° from the light beam. An interference filter specific to thiazole orange stain selects only fluorescent light (530 nm) which is then detected by a photomultiplier tube.

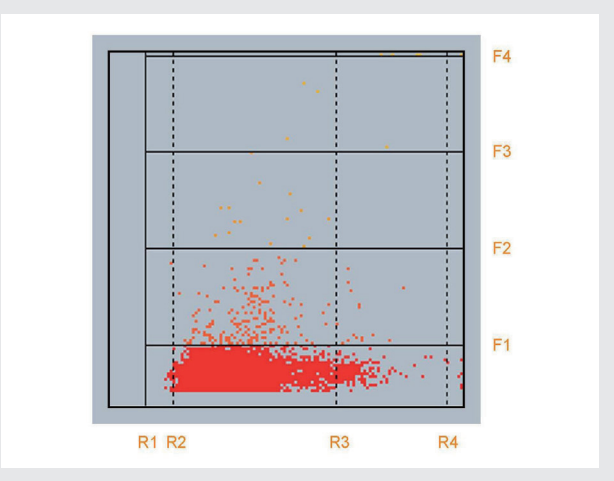

Figure 5

A reticulocyte matrix is then generated from two measurements: (1) Cell volume as determined by resistivity (impedance); and (2) Orthogonal Fluorescence. Mature red blood cells without nuclear material (RNA) show little or no fluorescent signal. They are located at the bottom of the matrix below F1 and horizontally distributed according to their mean corpuscular volume (MCV) and red cell distribution width (RDW). (Figure 5)

Reticulocytes are located in the upper portion of the matrix, and they are separated from the red blood cells by their fluorescence which is proportional to their RNA content and their maturity.

NRBC enumeration is accomplished in a manner similar to the enumeration of Reticulocytes.

CLINICAL USE of the HORIBA Medical ABX Pentra DX120

The CBC or Complete Blood Count is a routine panel of tests performed in many clinical laboratories. CBC test parameters include the enumeration of the Red Blood Cells, White Blood Cells with differential, Hemoglobin concentration, Platelet concentration and other test parameters.

The CBC is a valuable tool for the Physician to diagnose hematological disorders. A high White Cell count along with increased neutrophils in the WBC differential can indicate a bacterial infection somewhere in the body. Chronic bleeding and anemias can be diagnosed with decreased RBC counts and decreased hemoglobin concentrations. Oncology patients may have decreased platelets and other abnormalities in the CBC. Pediatric as well as adult and geriatric samples can be assayed on the HORIBA Medical ABX Pentra DX120.

In addition to CBC parameters, the HORIBA Medical ABX Pentra DX120 also can test for Reticulocytes and NRBC. These are immature forms of Red Cells which can be associated with blood loss or other disorders. Identifying an increase in the Reticulocyte percent can be a good sign indicating response by the body to blood loss with increased production of RBC. Manual enumeration of reticulocytes by hemocytometer is a tedious process requiring that the technologist count 1,000 red cells under a microscope. Automation of reticulocyte enumeration by the HORIBA Medical ABX Pentra DX120 is a valuable time saver for the clinical laboratory.

While valuable to the General Practitioner, the HORIBA Medical ABX Pentra DX120 is also valuable to specialty areas of medicine such as the Oncology practice. The extended differential of the HORIBA Medical ABX Pentra DX120 is valuable for identifying immature WBC

as found in certain types of leukemia patients.

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