Verification of Analytical Performance of LH750 and HmX Hematology Analyzers

Thesis

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List of Abbreviations

Abb.	Mean
AHAs	Automated Hematology Analyzers
AMR	Analytical Measurement Range
CAP	College of American Pathologists
CBC	Complete blood count
CD61	Cluster of differentiation 61
CLIA	Clinical laboratory improvement amendment
CLSI	Clinical Laboratory Standards Institute
CPA	Clinical Pathology Accreditation
CRR	Clinical Reporteable Range
CV	Coeffecient of variation
DLC	Differential leukocyte count
EQA	External quality assessment
EQC	Exernal quality control
fL	femto liter
GLP	Good Laboratory Practices
Hb	Hemoglobin
Hct	Hematocrit
ICSH	International Committee for Standardization in Hematology
IQC	Internal quality control
ISO	International standardization organization
K_2 EDTA	di-potassium ethylenediamine tetraacetic acid
MCH	Mean corpuscular hemoglobin

Abb.	Mean
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean cell volume
MPV	Mean platelet volume
MSR	Manual slide review
NCCLS	Clinical and Laboratory Standards Institute
NE%	Neutrophil percent
NRBC	Nucleated red blood cell
pg	Pico gram
PLT	Platelet count
QC	Quality control
RBC	Red blood cell count
RDW	Red cell distribution width
RET	Reticulocyte
RET%	Reticulocyte percent
RF/DC	Radio frequency/ direct current method
RNA	RNA ribonucleic acid
SD	Standard deviation
SDI	Standard deviation index
TEa	Total Allowable Error
VCS	Volume, conductivity, scatter
WBC	White blood cell count

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INTRODUCTION

Human blood cell analysis dates back 330 years to Leeuwenhoek, when he provided the first description of red blood cells using his simple microscope consisting of a minute biconvex lens. Wallace Coulter developed the first automated analyzer for counting and sizing cells and presented it in 1956 **(Chapman, 2000).**

The first hematology analyzers were based on the principle of impedance (resistance to current flow). The analyzer prepares one lysed sample to measure white blood cells (WBCs) and hemoglobin (Hb) values and unlysed sample for the red blood cells (RBCs), hematocrit (Hct), mean corpuscular volume (MCV) and platelet count **(Zandecki et al., 2007).**

The automated counting of RBC have been based on electrical impedance or later on light scattering technique, the hemoglobin is measured optically. The MCV is measured by the electronic cell counter while Hct, MCH and MCHC are calculated **(Chapman, 2000).**

The platelet count is often measured by impedance, both RBCs and platelets are discriminated by their volume and volume histogram is generated later. Flags are triggered for cases corresponding to inability to differentiate platelets from RBCs. Manual evaluation is required if platelet count is very low, clumping was observed or large platelets were present **(Zandecki et al., 2007).**

The automated differential leukocyte count is a more complicated process; at first the analyzer used volume to provide three part differential analysis of neutrophils, lymphocytes and monocyte. By the use of this reliable inexpensive method the identification of monocytes, immature myeloid cells and nucleated RBCs is difficult and peripheral smear review is necessary **(Gopal et al., 2005)**.

Current hematology instruments combine laser technology, impedance, radiofrequency, direct current, optimized temperature and volume to maximize the sensitivity and specificity of WBCs **(Kanzowaska and Bystryk, 2011).** The newer instruments provide five WBCs parameters in relative and absolute count but don't report the band numbers which is detected by manual review **(Senzel et al., 2010).**

The fully automated Coulter instruments Coulter HmX and LH750 produce a five-part differential white cell count, which is based on various physical characteristics of white cells, following partial stripping of cytoplasm. Three simultaneous measurements are made on each cell; impedance measurements, conductivity measurements and forward light scattering **(Bain, 2006).**

With the latest Beckman–Coulter instrument, the LH750, precision is improved by counting white cells, red cells and platelets in triplicate and by extending the counting time if the WBC or platelet count is low. The instrument is able to count NRBCs and corrects the WBCs for NRBCs interference **(Bain, 2006).**

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).**

Quality control (QC) can be separated into 2 major categories: internal QC and external QC. Most of the quality control activities take place internally to evaluate the data, and takes rapid corrective action. However, the external QC results are useful as an objective test of the internal QC procedures and may identify errors (i.e., biased or contaminated standards. The validation of automated hematology analyzer results by manual slide review (MSR) is currently an inevitable work process in clinical hematology laboratories **(Hur et al., 2011).**

ISO 15189:2012 specifies requirements for quality and competence in medical laboratories. It can be used by medical laboratories in developing their quality management systems and assessing their own competence and also for confirming or recognizing the competence of medical laboratories by laboratory customers, regulating authorities and accreditation bodies. Verification of automated cell counter specification is a requirement of ISO 15189: 2012 **(ISO15189, 2012).**

AIM OF THE WORK

To verify the analytical performance of LH750 and HmX hematology analyzers as regards the complete blood picture parameters as a preparatory step for laboratory accreditation according to the ISO standard ISO 15189: 2012.

AUTOMATED CELL COUNT

The most commonly performed test in a clinical hematology laboratory is a complete blood count, generally referred to as CBC. The second most commonly performed hematologic test is what is traditionally called differential leukocyte count (DLC) **(Carr, 2012).**

During the first half of the 20th century, the complete blood count (CBC) was performed using exclusively manual techniques: **(George, 2014).**

- Blood cell counts (red cells, white cells, platelets) were performed using appropriately diluted blood samples and a hemocytometer.
- Hemoglobin concentration was analyzed by the cyanomethemoglobin method.
- The hematocrit (Hct), was measured by high speed centrifugation of a column of blood, either in the Wintrobe tube or in sealed microcapillary tubes.
- The white blood cell differential was obtained by examining a suitably stained blood smear **(George, 2014).**

In 1932, Wintrobe developed a set of calculated indices that estimated erythrocyte size and hemoglobin