Comparing a compact hematology analyzer based on direct optical measurements using blue LED with the VCS reference technology for neutrophil differential count

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Abstract. Technologies for the automated blood cell count have been evolving over the last decades. Hematology analyzers enable identifying blood elements, including leukocytes differential. In the bloodstream, the most abundant leukocyte, the neutrophil, presents multi-lobed nuclei, requiring, therefore, combined measurement principles for their detection and classification. Due to the lack of white blood cells’ certified reference materials or reference measurement method, it has been customary to evaluate the performance of new hematology analyzers by comparing them with traditionally available and well-consolidated systems. In 2015, a technology based on axial light loss using a blue LED, the Beckman Coulter DxH500, was launched. This hematology analyzer presents reduced cost, fewer chemical reagents, and a compact design. This work compares the performance of the DxH500 with an analyzer frequently employed in large-scale laboratory routine, the Beckman Coulter LH750, based on the well-established combination of volume (impedance), conductivity and light scatter principles. The study examined 310 paired samples taken out randomly from the routine of a general hospital laboratory. The analyses evaluated the correlation coefficient, p-value, and the linear regression equation. The equivalence of results provided by both analyzers for the characterization of neutrophils in the leukocyte population points to the performance adequacy of the new low-cost and portable technology, suitable for small-sized clinical laboratory's routines.

1. Introduction
White blood cells (WBC) perform an essential role in immune systems, defending the body against foreign particles, infectious diseases, and cancerous cells. [1]. Bone marrow produces all blood cells, including the different types of WBC. There are mainly five major types of white blood cells circulating in the bloodstream: basophil, eosinophil, neutrophil, lymphocyte, and monocyte. The detection, classification, and determination of both the absolute value and the percentage of the overall WBC count consist of essential strategies for the diagnosis of a wide range of diseases.

Since 1870, with the use of microscopes and dyes in a blood smear, it is possible to differentiate the types of white blood cells and to quantify the number of cells by blood count [2]. Commonly based on a count of 100 cells, this method can introduce statistical errors, distribution errors caused by the unequal distribution of cells in the smear, and subjective interpretation errors [3]. The results of a WBC cell count and differentiation are interpreted by comparison. The comparator can be a population reference
interval, a decision point (from clinical studies), or a previous result on the same patient. For validating the comparison, the knowledge of methods’ limitations and errors is required to provide a reliable clinical diagnosis [4, 5].

By mid-XXth century, the emergence of automated blood cell counters significantly reduced the time spent on the procedure of blood analysis. Hematology analyzers evaluate thousands instead of 100 cells per sample, being, therefore, considered more reliable [6]. These methods contribute to avoiding the statistical and distributional errors associated with the white blood cell count in a blood smear [2].

Over the ensuing decades, flow-based cytometers using light, impedance, conductivity by radio frequency measurements, or the combination of these principles, were developed. These devices provided a more significant number of parameters enabling identifying blood elements, including leukocytes differential [2].

The first principle applied in hematology analyzers was the impedance, also called the Coulter Principle [7-9]. It is an electronic method for counting and sizing particles. Blood cells act as electrical insulators while suspended in a conductive liquid (dilution reagent), guided by a gentle vacuum; it passes through an aperture. Each blood cell, when crossing into the sensitive zone, increases the resistance for the electric current flow established between two submerged electrodes. The conductivity principle, in turn, is the measurement of intracellular content using a high-frequency electromagnetic probe. The cell’s constituents and their chemical composition result in measurable changes in conductivity [9, 10]. The third principle, light scattering, characterizes the cell’s surface, distinguishing cell structure, shape, and reflectivity [9, 11].

The combination of the three principles (impedance, conductivity by radio frequency and light scatter) brought, in 1970, a hematologic device that could differentiate WBC population in five parts: basophil, eosinophil, neutrophil, lymphocyte, and monocyte.

Currently, the well-consolidated combination of these three principles, called VCS technology (volume, conductivity and scatter), is the most frequently employed by automated hematology analyzers. An example of these VCS systems in use is the Beckman Coulter industry, LH750 model, launched in 2001, using a total of five types of reagents [12].

In 2011, an automated blood cell counter combining light scatter and fluorescence, the Sysmex XN, was launched. These instruments need fluorescence dyes polymethine based [13].

More recently, in 2015, the same manufacturer of LH750, Beckman Coulter, launched a more compact and less sophisticated hematology analyzer, DxH500, which combines the Coulter principle with the direct optical measurements of axial light loss (AxLL) with a blue led source incorporated [7]. This system uses three types of reagents [14]. Traditionally used in flow cytometry for abnormal cell's characterization, the axial light loss consists of the interruption of the optical path when a cell passes in front of the electromagnetic emission [15]. The amount of light falling on a sensor can be measured and varies depending on cell structure [7]. At the same aperture that measures impedance, an optical assembly projects the blue LED light onto an AxLL sensor [7].

Sensors development is continually evolving for improving detection sensitivity, signal to noise ratio and interferences, without the need for sophisticated and expensive instruments for the blood cell’s count [16-18]. In the clinical laboratory practice, however, for quality control, background counts (limit of blank) need to be performed daily for identifying and avoiding any possible interference [19].

Combinations of principles introduced by manufacturers may result in distinct performances and interferences. Each type of instrument has warnings signalizing interference effects and emits suspect messages in the presence of abnormal cells, identified as the flagging system. The literature shows that automated hematology analyzers are superior for white blood cell (WBC) differential counts when evaluating well characterized (mature) cell types, whereas optical microscopy is better for differentiating cells based on nuances of cytological features, especially for immature cells [20]. The flags, or a combination of them, show the difficulty of the automated method in characterizing the cell, indicating the need for a smear review [16]. Strategies for dealing with flags are available in the International Consensus Group for Hematology Review (ICGHR) published in 2005 [20, 21].
The appropriateness of performance evaluation of the various technologies coming from the WBC faces, however, a challenging metrological demand [5]. Despite the advances on the worldwide equivalence of measurement results in clinical laboratories with the creation, in 2002, of the Joint Committee for Traceability in Laboratory Medicine (JCTLM) [4], up to the moment, there are no certified reference material or reference methods for WBC analysis available. With the lack of WBC reference materials or reference measurement procedure, while commutability of reference material is not established [8], it has been customary to evaluate the performance of new hematology analyzers by comparing them with traditionally available systems [22].

The literature shows comparisons between the compact hematology analyzer recently launched (DxH 500), which uses AxLL and incorporates a blue LED source, with the Sysmex XN-3000, a technology based on Light scatter and Fluorescence [23]. Another comparison available is performed between DxH500 and Beckman Coulter HmX, which is one of the first VCS systems launched and still in use [24], presenting a more limited flagging system than LH750. All comparisons were performed with selected samples without morphological WBC flags or suspect messages.

Among the leukocytes in the bloodstream, neutrophils are usually the most abundant and have multi-lobed nuclei, requiring the use of combined principles for their detection and classification [1]. Therefore, neutrophils without morphological changes are useful parameters for comparing automated techniques for differential WBC.

This work aims at comparing the most recently launched Beckman Coulter DxH500 hematology analyzer, a compact technology, with reduced operational complexity, based on AxLL with a blue LED source, with the Beckman Coulter LH 750, which is a VCS system that presents a more robust flagging system than the HmX. This preliminary study focuses on the neutrophils’ differential, whose intracellular characteristics require combinations of measurement principles for automated detection and quantification.

2. Materials and Methods
Comparisons were performed during a one-week period in which the hematology analyzer DxH 500 (Beckman Coulter, Miami, USA) was placed in a hospital laboratory employing the LH750 (Beckman Coulter, Miami, USA). The laboratory is located at a general hospital that offers a diversity of medical specialties, such as surgery (including neurosurgery and transplants), oncology, orthopedics, pediatrics, and geriatrics.

Specimens were obtained from anonymized whole blood samples and submitted for routine blood count testing. Samples were stored at room temperature, and all analyses were completed within eight hours after sample entry into the laboratory. A total of 310 unselected samples were taken randomly from routine analyses. The average interval between the paired exams was one hour, with 46 min of standard deviation.

Background counts performed daily presented results within the acceptable values for both measuring instruments. The samples with positive flagging for the WBC count, or any suspect message that implies in the necessity of a smear review, were excluded from the analyses. One hundred six samples were removed from the comparison, representing 34.19 % of the total of 310 samples. As a result, 204 samples were used for paired comparison.

For data analysis and statistics, a two-tailed t-test was used on the paired results of neutrophils’ percentage ($NE_{\%}$) in leucocytes differential to evaluate the $p$-value to ascertain whether or not there was any significant discrepancy between the $NE_{\%}$ mean values obtained by each one of the hematology analyzers. The correlation coefficient and linear regression equation, with the plot of the linear regression, were evaluated for the $NE_{\%}$ provided by DxH500 and LH750 for each of the 204 samples.

3. Comparative Analysis
The automated evaluation of the 310 whole blood samples, covering a wide variety of underlying medical conditions, using both DxH500 and LH750 hematology analyzers, resulted in a total number of flag signals that required the removal of 106 samples (34.19 % of entire WBS evaluated).
Both hematology analyzers presented indications for smear review by flagging of abnormal white blood cells, but not always for the same set of samples (table 1). Forty-two samples were flagged exclusively by the DxH500 analyzer (13.55 % of the 310 WBS), and 43 were flagged only by LH750 VCS technology (13.87 %). Twenty-one samples were flagged by both analyzers (6.77 % of the total WBS).

Table 1. Number of samples flagged by each automated hematology analyzer (DxH500 and LH750) and their percentage regarding the total number of analyses (310 samples).

| Flagged Samples       | Percentage of the 310 samples |
|-----------------------|------------------------------|
| DxH500                | 42                           | 13.55 %                       |
| LH750                 | 43                           | 13.87 %                       |
| DxH500 and LH750      | 21                           | 6.77 %                        |
| Total                 | 106                          | 34.19 %                       |

After excluding the 106 flagged samples, the mean values for the percentage of neutrophils in leucocyte differential of the remaining 204 analysis using DxH500 and LH750 were, respectively, 61.07 % of leucocytes and 61.93 %.

The difference between the mean values for the percentage of neutrophils obtained by the two technologies was -0.86 ± 2.80 %, for a 95 % confidence interval. A paired, two-tailed t-test showed that there was no significant difference (p-value = 0.4148) between the NE% means obtained from LH750 and DxH500 measurements. Figure 1 shows the plot of the NE% values provided by both hematology analyzers for each of the 204 samples.

Figure 1. Linear regression, correlation coefficient R and linear regression equation y for neutrophils’ percentage (NE%) in leucocytes differential for the paired test between DxH500 and LH750.

The linear regression analysis demonstrates a high correlation (R = 0.99), with a slope of 0.95, between both results of neutrophils' percentage measured by DxH500 and LH750, as the reference VCS technology.
4. Discussion
Since its launch, in 2015, the literature presents two other comparisons for the results of neutrophils’ percentage in leucocyte differential provided by the new DxH500 technology [23, 24]. One of these comparisons was performed with one of the first VCS technologies launched and still in use [24], model HMX, from the same industry LH750, Beckman Coulter, but with a reduced flagging system. The other study compared DxH500 with another recently launched hematology analyzer, Sysmex XN-3000, which combines Light scatter and fluorescence. All these studies removed flags from the set of whole blood samples’ results being compared, and do not report the correspondence of signalized flagged samples between the compared technologies. Table 2 presents the correlation coefficients obtained by each study comparing DxH500 results for neutrophils’ percentage in leucocyte differential obtained with each one of the three alternative technologies: HMX [24], XN-3000 [23], and, in the present study, LH750.

Table 2. Correlation coefficients between DxH500 and each of three alternative hematology analyzers, for neutrophils’ percentage in WBC differential (NE\%).

| Hematology Analyzer (Physical Principle) | HMX (VCS) [24] | XN-3000 (light scatter)(fluorescence) [23] | LH750 (VCS) [present study] |
|-----------------------------------------|----------------|----------------------------------------|-----------------------------|
| Correlation coefficient comparing with DxH500 (Coulter)(AxLL with blue LED) | 0.990 | 0.9894 | 0.9917 |

The high correlation coefficient evidenced in all studies shown in table 2 points toward the appropriateness of results for leucocyte differential using the compact and simple technology based on the impedance and AxLL with Blue LED, recently launched.

5. Conclusions
While certified reference materials or reference methods are not available in the JCTLM database to enable metrological traceability for leucocyte differential, the comparison of measurement methods combining diversified chemical and physical principles remains essential for in vitro diagnostics (IVD) assessment [25].

The present study compares the performance of a new technique, DxH500, designed for small routines, needing fewer chemical reagents, which combines a simple principle by impedance measurements with axial light loss using blue led, with one of the conventionally employed technologies, the LH750. This analyzer applies the well-consolidated measurement principles, the VCS (impedance, conductivity, and light scatter).

Although using different measurement principles, the present study showed a high correlation between the results of DxH500 and LH750 for the detection and quantification of neutrophils’ percentage in leucocytes’ differential. These results confirm the outcome equivalence for neutrophils’ characterization, described in the literature, between the new hematology analyzer, DxH500, and the HxM, which is the most ancient VCS device still in use [24]; as well as with the also recently emerged device, XN-3000, based on the light scattering and fluorescence principles [23].

The present comparison completes the evaluation of the performance equivalence of results for neutrophil characterization provided by the compact new technique, with low-cost and few chemicals consume, appropriate for small clinical laboratory routines.

As future work, it is worth mentioning the extending of these studies for encompassing the whole set of white blood cells’ analyses. The discrepancies observed between the two hematology analyzers...
regarding their review warning flags associated with abnormal cells consist of additional demand for investigations.

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