Comparison of Leukocyte Differential Count by Beckman Coulter Unicel DxH800, Beckman Coulter LH780 and Sysmex XE-5000

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ABSTRACT

Objective: Leukocyte differential count (LDC), a component of complete blood count (CBC), is important in the diagnosis and follow-up of various diseases. Inter-instrument comparison of LDC is important in the laboratory using multiple analyzers to ensure the precision and reliability of test results.
Methods: One hundred and twenty EDTA blood samples were collected and analyzed using the DxH, LH, and XE. Regression and correlation analysis, intraclass correlation coefficients (ICC), and difference plots between the LDC findings from each analyzer and the means of all methods were analyzed.
Results: Neutrophil, lymphocyte, monocyte, and eosinophil counts from 3 automated hematology analyzers were strongly correlated ($r^2 > 0.97$, p-value < 0.001, and intraclass correlation coefficient (ICC) 0.89-0.99). However, a negative bias was observed when comparing the monocyte count from the Sysmex analyzer and the mean of all 3 analyzers.
Conclusion: Leukocyte differential counts between or among different models and/or brands of hematology analyzers may be different. From this study, the correlations of leukocyte differential counts between analyzers were excellent, except for basophil count. Monocyte counts from the Sysmex analyzer showed a negative trend. Laboratories should evaluate this significance, in terms of both quality management and clinical aspects.

Keywords: Leukocyte differential count, automated hematology analyzer, Beckman Coulter DxH 800, Beckman Coulter LH 780, Sysmex XE-5000

INTRODUCTION

The leukocyte differential count (LDC), a component of complete blood count (CBC), is important in the diagnosis and follow-up of various diseases.[1,2] LDC can generally be measured by manual method under light microscopy or by automated hematology analyzer (HA). Due to the laborious process associated with the manual method, the current and most popular method involves the use of HA. Automated LDC technology is currently based on 4 different principles: electric impedance, radiofrequency conductivity, light scattering, and cytochemistry.[3] Although previous studies have demonstrated LDC comparability among these methods, some studies have reported that LDC differences may occur, especially in monocyte and basophil counts.[4,5] At our laboratory, we use Beckman Coulter Unicel DxH800, Beckman Coulter LH780, and Sysmex

XE-5000 for routine analysis. In our review of internal quality control data, we too have encountered LDC differences among these instruments, although the cause and extent of the differences was never fully explored or explained. As such, the objective of this study was to compare and analyze the LDCs from our routine HAs for purposes of understanding and verifying the comparability of LDC among methods.

MATERIALS AND METHODS

Specimens

This study was conducted at the central laboratory of the Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. One hundred and twenty venous blood samples in dipotassium ethylenediaminetetraacetic acid tubes obtained from the out-patient and in-patient departments during the month of September 2012 were used. The samples with blood volumes of less than 3 ml were excluded from the study.

Instruments

The Beckman Coulter Unicel DxH800 (Beckman Coulter, Miami, FL, USA) (hereafter, "DxH") is a fully automated hematology analyzer that uses a red laser with 7-angle scattered light, volume, and conductivity for the measurement of LDC. Five light scatters (2 upper median angle light scatters, 2 lower median angle light scatters, and a median angle light scatter), as well as volume and conductivity are used to determine and evaluate granularity and membrane surface of leukocytes. Axial light loss measurement and low angle light scatter are used for cellular transparency and complexity index analysis.[4,6,7] Differing from the DxH, LDC analyzed by the Beckman Coulter LH780 (Beckman Coulter Miami, FL, USA) (hereafter, "LH") uses only volume, conductivity, and one light scatter analysis.[4,6]

The Sysmex XE-5000 (Sysmex Corporation, Kobe, Japan) (hereafter "XE") uses a semiconductor laser to analyze LDC by detecting forward-scattered light and side-scattered light for the evaluation of internal cell structure. The XE also uses side fluorescence to determine DNA and RNA contents in cells and analyzes them according to their side-scattered light and fluorescence intensity characteristics.[8]

Each instrument was calibrated and all interval maintenance activities were performed according to the manufacturer's recommendations. The internal quality control values for all units being evaluated were acceptable before testing.

Manual differential counts were performed on Giemsa-stained slides by a Sysmex SP-1000i (Sysmex Corporation, Kobe, Japan) automated hematology slide maker. Each blood film was examined independently by 2 experienced clinical pathologists who were blinded to the automated analysis results.

Study protocol

All specimens were analyzed within 4 hours of blood collection and run on all instruments within a 2-hour window. All specimens were tested using the XE as the primary routine method and were then tested using the DxH and the LH.

Data analysis

Statistical analysis was performed using PASW Statistics for Windows, Version 18.0 (SPSS Inc., Chicago, IL, USA) and Excel (Microsoft Corporation, Redmond, WA, USA). Linear regression studies and coefficient of determination (r^2) of parameters for each analyzer against the mean values of all analyzers were evaluated. Difference plots between LDC for each analyzer against the means of all and intraclass correlation coefficients (ICC) were used to determine agreement between methods. For the interpretation of ICC, the Landis and Koch criterion was applied: ICC of 0.00 to 0.20, 0.21 to 0.40, 0.41 to 0.60, 0.61 to 0.80, and more than 0.80 for slight agreement, fair agreement, moderate agreement, substantial agreement, and almost perfect agreement, respectively.[9] A p-value of less than 0.05 was regarded as statistically significant. Microscopic counts were performed for control purposes only, so the data from this method was not included in the calculation of mean values of the LDC.

TABLE 1. Interinstrument comparison of leukocyte differential counts. Individual measurement was compared with the mean of all methods.

Parameters	DxH			LH			XE			Intraclass correlation coefficient (95 % CI, p value)
	Regression equation	r^2	Mean/median ± SD (range)	Regression equation	r^2	Mean/median ± SD (range)	Regression equation	r^2	Mean/median ± SD (range)	
Neutrophils (%)	y = 0.994x + 0.945	0.99	61.05 ± 14.22 (25.50-96.90)	y = 0.982x + 1.015	0.99	61.76 ± 14.40 (25.10-99.00)	y = 1.013x - 1.299	0.99	62.15 ± 13.95 (29.10-97.30)	0.998 (0.997-0.999, p < 0.001)
Lymphocytes (%)	y = 0.998x + 0.438	0.99	27.29 ± 12.65 (1.20-65.70)	y = 0.987x + 0.666	0.99	27.35 ± 12.78 (0.20-66.10)	y = 1.002x - 0.753	0.99	28.37 ± 12.60 (1.30-63.20)	0.998 (0.997-0.998, p < 0.001)
Monocytes (%)	y = 0.909x - 0.138	0.99	7.98 ± 3.31 (0.40-18.20)	y = 0.923x + 0.210	0.98	7.48 ± 3.24 (0.00-18.20)	y = 1.115x + 0.574	0.97	5.89 ± 2.64 (0.00-14.10)	0.984 (0.978-0.988, p < 0.001)
Eosinophils (%)	y = 0.984x + 0.001	0.99	3.10 ± 2.30 (0.00-13.50)	y = 0.990x + 0.140	0.99	2.00 ± 2.86 (0.00-13.30)	y = 1.001x - 0.066	0.99	2.4 ± 2.82 (0.00-13.30)	0.996 (0.994-0.997, p < 0.001)
Basophils (%)	y = 0.832x + 0.024	0.83	0.50 ± 0.30 (0.00-1.50)	y = 0.919x + 0.068	0.79	0.50 ± 0.27 (0.00-1.50)	y = 0.769x + 0.141	0.88	0.40 ± 0.33 (0.00-1.60)	0.897 (0.860-0.925, P < 0.001)

Ethical Consideration

This study was reviewed and categorized as a "Research with Exemption" study by the Siriraj Institutional Review Board (SIRB Protocol No. 488/2555).

RESULTS

The regression equation, coefficient of determination (r^2) and intraclass correlation coefficient (ICC) of the LDC from each analyzer and a comparison of the means of all methods, along with mean/median and standard deviation (SD), have been described in Table 1.

The LDC of DxH, LH, and XE showed very strong correlations with the means of all methods ($r^2 > 0.97$) for neutrophil, lymphocyte, monocyte, and eosinophil counts. For the basophil counts, the correlations were fair for all HAs, with the lowest correlation coefficient of 0.79 for LH. The ICC for neutrophil, lymphocyte, monocyte, and eosinophil for the 3 automated HAs were 0.99, 0.99, 0.98, and 0.99, respectively (p < 0.001 for all).

Neutrophil, lymphocyte, eosinophil, and basophil showed no systematic bias and demonstrated little scatter among the 3 hematology analyzers in the difference plot. However, a negative bias was observed in the plot when comparing monocyte counts from the Sysmex analyzer (Fig 1). The microscopic count for monocytes was equivalent to the Sysmex analyzer data.

DISCUSSION

Inter-instrument comparison of hematology analyzers is very important in the laboratory using multiple analyzers. This process helps to ensure the reliability of hematologic analysis, a key component affecting patient care. Previous studies that compared the performance of various HAs (including LDC) found that leukocyte differential counts were most often closely aligned, but not always so,[4,6,10,11] with some studies reporting that differences in LDC may occur, especially in monocyte and basophil counts.[5,6,11,12]

However, there is no data specific to LDC comparison between DxH, LH, and XE. This
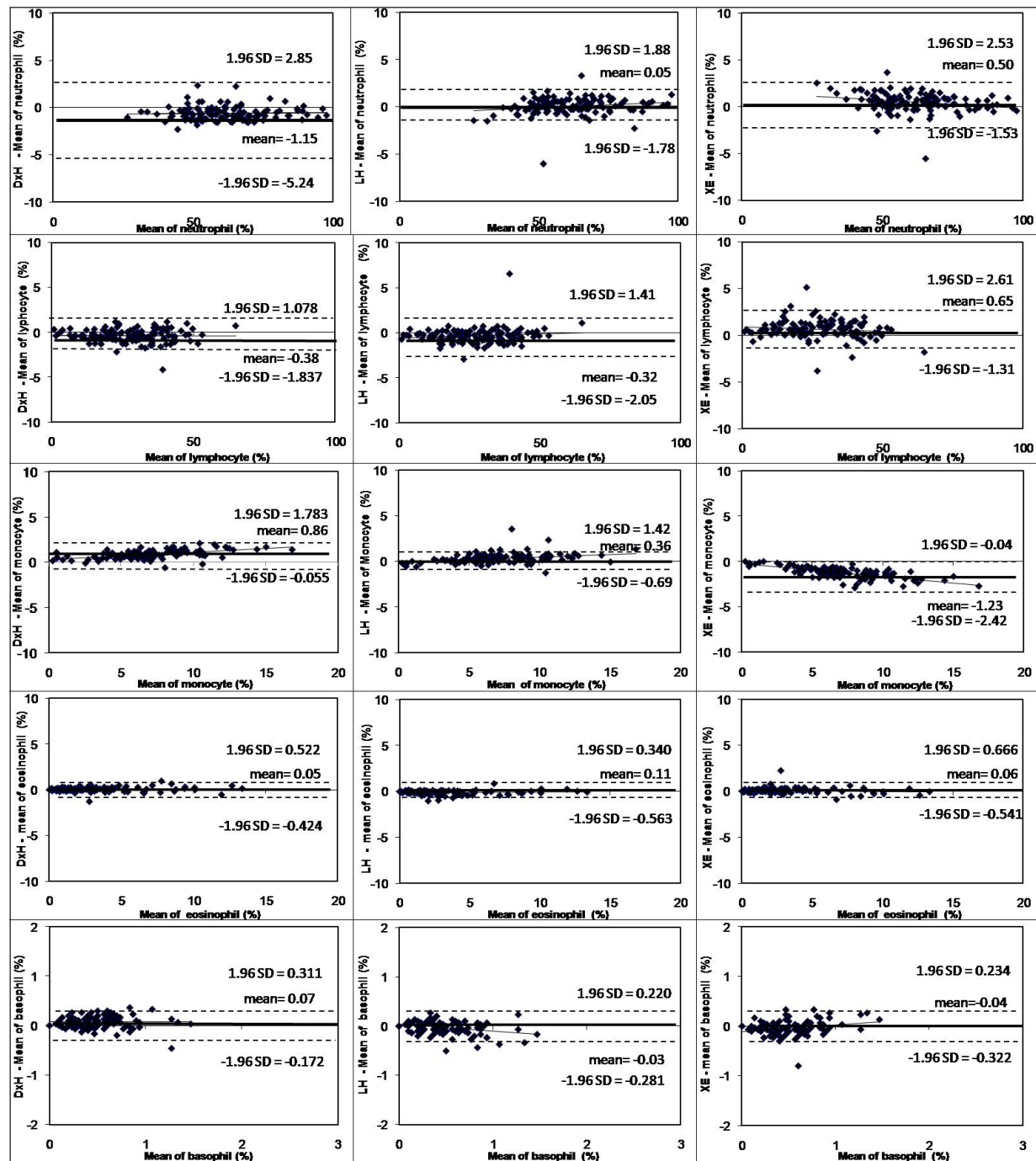
Fig 1. Difference plots of neutrophil, lymphocyte, monocyte, eosinophil and basophil.

finding of this study demonstrates that neutrophil, lymphocyte, and eosinophil counts from DxH, LH, and XE were not only very strongly correlated, but were also strongly in agreement with the means of all. The systematic deviation of each HA for LDC demonstrated by bias of method was very low. The values of biases were not considered to be of clinical significance. This indicated that, even when applying the different principles of testing, the LDC were considered comparable among the three HAs; although, a negative trend in monocyte count for XE was observed.

Studies undertaken by Park BG, et al.[5], Meintker L, et al.[12], and Grimaldi, et al.[13], report high monocyte counts with the Beckman Coulter hematology analyzer. In an earlier study, the Beckman Coulter CytoDiff (Beckman Coulter Miami, FL, USA) was compared with the Beck-

man Coulter DxH 800 and the Sysmex XE-2100. Monocyte counts from the CytoDiff were more strongly correlated with the XE-2100 than the DxH 800. Subsequently, Meintker L, et al, compared monocyte counts among 4 different analyzers, as follows: Abbott Sapphire, Siemens Advia 120, Beckman Coulter DxH 800, and Sysmex XE-2100. The DxH showed monocyte counts slightly above average, whereas, the Advia 120 showed significantly lower than average monocyte counts. Grimaldi, et al., also compared monocyte counts by Beckman Coulter LH 750 and Advia 120 with flow cytometry of immunostained cells. Beckman Coulter LH 750 demonstrated slightly higher monocyte counts, and the Advia 120 considerably lower monocyte counts than the reference method.

The XE series differentiates the white blood cell count into two distinct channels: WBC/BASO and DIFF-channel. In the WBC/BASO channel, the XE uses forward and side-scattered light signals to quantify total WBC and basophil. In the DIFF-channel, the XE can categorize four sub-populations: neutrophils, eosinophils, lymphocytes, and monocytes. There are 2 steps in this channel. First, all blood cells are pretreated with Stromatolyser-4DL, which is a surfactant reagent that is specific to the XE family. This reagent induces not only complete hemolysis and shrinkage of the red cell and platelet membrane, but also the formation of ultramicroscopic pores in the white blood cell membrane. This outcome facilitates the entry of Stromatolyser-4DS, a fluorescent dye with a high affinity for RNA and DNA, to enter the cells. Second, the leukocytes that have been sensitized to the two special reagents are detected by the flow cytometry principle. The signals from the cells relating to side scatter (complexity inside the cells) and side fluorescence (intensities of the cells) are depicted on a scattergram.[15] In addition, the number of differentiated cells also plays a role in the accuracy of statistical prediction in cell counting. With regard to microscopic count in this study, we found monocyte results to be equivalent to the results from XE.

This indicated that at a higher value of monocyte (e.g., at the value of more than 15%), the value from XE may be lower, as compared to values from DxH and LH. This data will also be helpful in patient case management. For example, patient samples showing a high monocyte count should be followed-up using the same HA in order to reduce potential variability in result interpretation. This study also confirms that various levels of quality control should be evaluated, especially when a laboratory uses more than one HA.

Although this study included various numbers of neutrophils, lymphocytes, and monocytes, the number of eosinophils and basophils were limited to the lower range, resulting in an unbalanced distribution of these two groups. Also as a result, the ICC for eosinophil and basophil might be open to only limited interpretation. Further investigation of higher ranges of these cells is required to ascertain comparability among HAs for eosinophil and basophil.

CONCLUSION

Inter-instrument comparison of hematology analyzers is very important in the laboratory using multiple analyzers. In this study, neutrophil, lymphocyte, monocyte, eosinophil, and basophil counts from three hematology analyzers (Beckman Coulter Unicel DxH800, Beckman Coulter LH780, and Sysmex XE-5000) were found to be comparable and in agreement with the means of all methods. Only the monocyte count reported by XE showed a negative trend. Patient samples with high monocyte counts should be followed-up using the same HA to reduce the possible variability of result interpretation.

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