Evaluation of the Differential Leukocyte Count and Screening Efficiency of the Sysmex SE-9000 Haematology Analyser

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The purpose of this study was to evaluate the white blood cell (WBC) differential count and screening efficiency of the Sysmex SE-9000 haematology analyser compared to the visual 400-cell WBC differential count according to the NCCLS H20-A protocol. Automated WBC differential counts were performed on 756 samples during the first 10 days of use of the analyser in the Department of Haematology at King Hussein Medical Centre.

Reference WBC differential counts were performed by microscopy examination of 400 WBC cell counts on May-Grünwald-Giemsa stained blood films.

Automated WBC differential counts were considered positive if they contained a WBC flag indicating the presence of abnormal cells. The automated WBC differential counts were then classified as true positive (TP), false positive (FP), true negative (TN), and false negative (FN).

Correlations were excellent with the exception of basophils: for neutrophils r = 0.99, for lymphocytes 0.98, monocytes 0.81, eosinophils 0.95, and basophils 0.74. Sensitivities for morphologic and distributional abnormalities were 68.4 % and 98 %, respectively.

Our evaluation demonstrated excellent performance of the SE-9000 with reference to the automated WBC differential count. The screening efficiency of the SE-9000 was very high, significantly reducing the manual workload. On the other hand, analysis of qualitative flags underscored the continuing importance of the blood film examination.

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Key Words Automated Hematology Analyzer, SE-9000, Differential, Accuracy, Sensitivity, Specificity, Efficiency

INTRODUCTION

Since 1992 the Sysmex K-1000 automated haematology analyser has been used in the department of haematology at King Hussein Medical as a tool for screening differential counts[1]. In common with others, increasing workload and changes in the laboratory environment[2] necessitated change and upgrading of instrumentation. In August 1998, the laboratory obtained the Sysmex SE-9000 fully automated haematology analyser which performs 23 parameters including the 5-part white blood cell (WBC) differential count. It was anticipated that the latter would reduce the time spent preparing blood films and undertaking visual differential counts[3].

Since the SE-9000 was the first fully automated haematology analyser in our department, we were motivated to evaluate its performance. The present study was designed to assess the ability of the automated WBC differential count to reduce the number of visual differential counts performed while maintaining high accuracy and efficiency and to evaluate the sensitivity and specificity of the WBC flags generated by the instrument.

MATERIALS AND METHODS

Instrument

The Sysmex SE-9000 uses a WBC channel for enumeration of total WBC count, by means of a strong lysing reagent and based on electrical impedance measurements at direct current (DC).

The WBC differential count, identification of granulocytes, lymphocytes and monocytes, is performed in a second WBC channel, the DIFF-channel. After a specific haemolyses of red blood cell (RBC) and a differential lyses of WBC, sub-populations of WBCs can be identified in a scattergram, based on electrical impedance measurements of both, DC which reflects cell size, and radio frequency (RF) which reflects the internal structure of cells[4].

Identification of eosinophils and basophils is accomplished in separate channels using DC but based on their sensitivity to special lysing reagents, while cells of the immature granulocyte series are identified using RF/DC measurements after chemical pretreatment in the Immature Myeloid Information (IMI) channel.

Thus two scattergrams are established, one for cluster analysis of WBC subpopulations and the other for the immature granulocytes, besides five histograms for RBCs, PLTs, WBCs, Eosinophils and Basophils.

WBC differential counts evaluation

In this evaluation of WBC differential counts, accuracy, clinical sensitivity and efficiency were studied as described previously[5-10]. Seven hundred and fifty six (756) blood samples received from patients from different wards and outpatient clinics with requests to perform differential counts were selected randomly during a 10 day period for inclusion in the study. These blood specimens were anticoagulated with K-EDTA in 4.5 mL tubes and analysed within two hours. The reference differential counts were performed by microscopy examination of 400 cell counts on May-Grünwald-Giemsa stained preparations by 2×200 cell counts on separate slides by qualified senior technologists.

Accuracy

WBC differential counts obtained by the SE-9000 were compared with the results of the 400 cell counts. Any constant bias was identified by calculating the percentage differences between SE-9000 and the visual method for each WBC sub-population. A regression analysis was constructed for each sub-population.

Clinical sensitivity, specificity and efficiency

The presence of flags generated by the SE-9000 indicated abnormal (positive) samples. "Not reported" results were considered abnormal when one or more morphologic abnormalities were detected by the visual reference method and these samples were considered as positive. Truth tables were constructed so test results were classified as true positive (TP), true negative (TN), false positive (FP) and false negative (FN).
From the truth tables the following equations were used to calculate FP ratio, FN ratio, clinical sensitivity, specificity and efficiency.

$$\text{FP Ratio} = \frac{FP}{FP + TN} \times 100\ \%$$

$$\text{FN Ratio} = \frac{FN}{FN + TP} \times 100\ \%$$

$$\text{Sensitivity} = \frac{TP}{TP + FN} \times 100\ \%$$

$$\text{Specificity} = \frac{TN}{TN + FP} \times 100\ \%$$

$$\text{Efficiency} = \frac{TP + TN}{(TP+FP+TN+FN)} \times 100\ \%$$

RESULTS

Accuracy

The WBC counts for the study population ranged from $0.3 - 162.5 \times 10^9$/L.
Fig. 1 show the results of comparison of the WBC differential count with the visual differential count.
The SE-9000 did not report 24 (3.2 %) cases for neutrophils, 11 (1.5 %) cases for lymphocytes and 23 (3.0%) cases for monocytes. The main causes for not reporting results were the presence of high or low WBCs associated with leukemic patients with high percentages of blast cells, or patients on chemotherapy with WBC counts less than 0.5×10^9/L. Thirteen cases were neonatal samples who suffered from septicaemia, and 6 cases were acute myeloid leukemia with blasts in the range of 70-85 %.
Four cases of eosinophilia were not reported, and in 65 (8.6%) cases the SE-9000 did not report the basophils. The latter had normal, low and high WBC counts and the only common factor was the presence of immature granulocytes.

Clinical sensitivity specificity and efficiency

Table 1 shows the truth table data for classification of differential counts as normal, quantitative normal and qualitative normal or abnormal by the SE-9000 using the 400-cell differential as the reference method.

Table 2 shows the results of calculations from the truth table, which include sensitivity, specificity and agreement in addition to FP and FN. For each classification higher sensitivity, specificity and efficiency were obtained for distributional classification rather than the morphologic classification.

Tables 3 - 5 show features of FN results, features of the quantitative abnormal results, and features of the qualitative abnormal results, respectively.

DISCUSSION

An excellent correlation was established between the SE-9000 differential counts and the visual differential counts, except for basophils.
Evaluation of results concerning monocytes and eosinophils was of particular interest, as several investigators found the monocyte and eosinophil counts to correlate poorly with the visual differential count[5, 11, 12] while others found good correlation[13, 14].
Some investigators have reported poorer agreement of instrument basophil counts with visual results[14]. Our results indicated a more acceptable agreement, and this may have been due to the large number of samples analysed possessing a wide range of abnormalities and therefore a better analysis resulted.
Evaluation of results concerning the quantitative and the qualitative flags showed high sensitivity, specificity and efficiency. This was equivalent to, or higher than that found in other investigations[14-16]. Furthermore the SE-

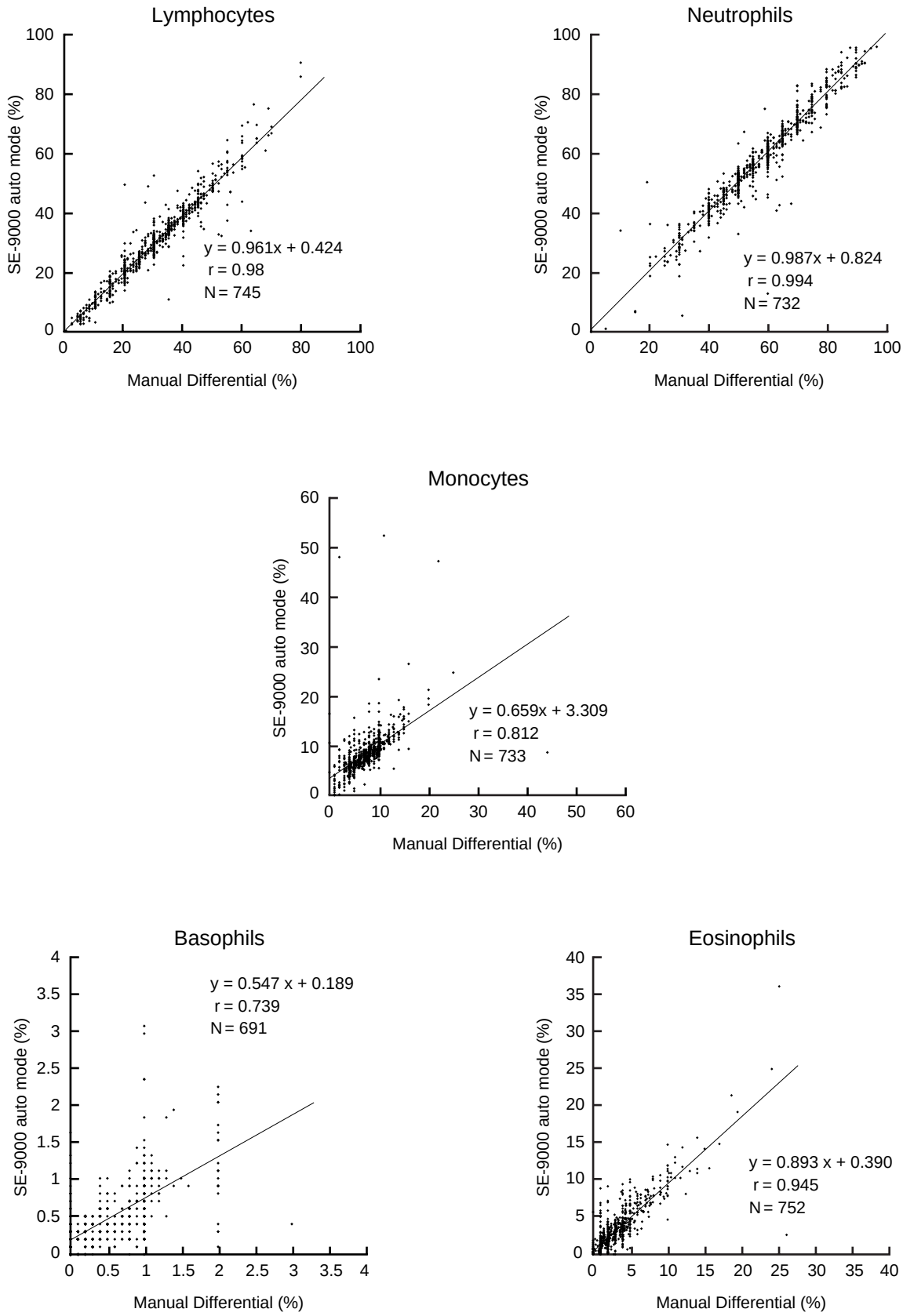

Fig. 1 *Comparison of the WBC differential count with the manual method*

Table 1 *Truth table data for classification of 756 differential counts as Normal, Quantitative, Qualitative Normal or Abnormal by SE-9000 and the manual method*

	TP	FP	TN	FN
Total Normal or Abnormal	331	90	307	28
Quantitative Normal or Abnormal	258	6	142	2
Qualitative Normal or Abnormal	73	84	165	26

Table 2 *Calculations from Truth Table*

	FP Ratio (%)	FN Ratio (%)	Sensitivity (%)	Specificity (%)	Efficiency (%)
Total Normal or Abnormal	22.8	7.8	92.2	77.3	84.4
Quantitative Normal or Abnormal	4.0	0.8	99.2	95.9	98.0
Qualitative Normal or Abnormal	33.7	26.2	73.7	66.2	68.4

Table 3 *Features of FN results*

	No. of Cases
Myelocytes + Metamyelocytes (1-3 %)	10
Hypersegmented Neutrophils	8
Blasts<1%	2
Atypical Lymphocytes (5-10 %)	6
Eosinophilia	2
Total	28

Table 4 *Features of the Quantitative Abnormal Results*

	TP	FP
Monocytosis	90	4
Neutrophilia	90	3
Eosinophilia	44	2
Lymphocytopenia	156	4
No. of Cases	258	6

Table 5 *Features of the Qualitative Abnormal Results*

	TP	FP
Blasts	23	57
Imm. Granulocytes	61	16
Left Shift	65	17
NRBCs	9	39
NRBCs/PLTCL	2	2
No. of Cases	73	84

9000 has the ability to detect blasts and NRBCs although the false positive rates are high for both flags (***Table 5***). However, we considered this as an advantage for the analyser, since the appearance of NRBCs flags in FP samples was related to the presence of giant platelets or platelets clumps. The appearance of FP blast flags on the other hand was affected by the presence of immature granulocytes or left shift flags. This may be resolved by carefully consulting the Q-flag displays.

In conclusion, the SE-9000 has a very high screening efficiency. This significantly reduces the manual workload and enables us to identify pathologic samples using the qualitative and quantitative flags and scattergram inspection. The last has been reported recently to aid in the differentiation of acute and chronic lymphocytic and myeloid leukemias[17].

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References

1) *Fares AK : A study of one thousand cases using the Sysmex K-1000™ Haematology Analyzer. Sysmex J Int, 6 : 155-159, 1996.*

2) *Macdonald AJ : The evaluation of an automated laboratory. Sysmex J Int, 7 : 31-36, 1997.*

3) *Fodinger M, et al. : Evaluation of a total haematology analysis system Sysmex HS-430™ - Benefits of large laboratories by reducing manual workload and optimizing screening efficiency for pathologic samples. Sysmex J Int, 6 : 123-128, 1996.*

4) *TOA MEDICAL ELECTRONICS : Sysmex SE-9000, Operator's Manual. TOA Medical Electronics Co., Ltd., KOBE, JAPAN, 1996.*

5) *Weber N, Mast BJ, Houwen B : Performance evaluation of haematology analyzers : An outline for clinical laboratory testing. Sysmex J Int, 5 : 103-113, 1995.*

6) *Shinton NK, England JM, Kennedy DA : Guidelines for the evaluation of instrument used in haematology laboratories. J Clin Pathol, 35 : 1095-1102, 1982.*

7) *International Committee for Standardization in haematology : protocol for type testing equipment and apparatus used for haematology analysis. J Clin Pathol, 31 : 275-279, 1992.*

8) *National Committe for Clinical Laboratory Standards (NCCLS) : Reference leukocyte differential count (proportional) and evaluation of instrumental methods. NCCLS Approved Standard NCCLS Document H20-A, NCCLS, Villanova PA, 1992.*

9) *Percy-Robb IW, et al. : A recommended scheme for the evaluation of kits in clinical chemistry laboratory. Ann Clin Biochem, 17 : 217-266, 1980.*

10) *Rosner B : Fundamentals of biostatistics, 3rd edition, 11 : 398-472, PWS-KENT Publishing Company, Boston, Massachusetts, 1990.*

11) *Hubl W, et al. : Precision and accuracy of monocyte counting. Amer J Clin Pathol, 103 : 167-170, 1995.*

12) *Bentley SA, Johnson A, Bishop CA : A parallel evaluation of four automated haematology analyzers. Am J Clin Pathol, 100 : 632-636, 1993.*

13) *Bartels PCM, Schoorl M : Influence of storage conditions on results of monocyte counting on Sysmex NE-8000™ and SE-9000™ automated haematology analyzers. Sysmex J Int, 6 : 143-146, 1996.*

14) *Hubl W, Andert S, Bayer PM : Evaluation of the Sysmex SE-9000™ haematology analyzer. Sysmex J Int, 5 : 85-95, 1995.*

15) *Buttarello M, Bulian P, Temporin V, Rizzotti P : Sysmex SE-9000™ haematology analyzer : Performance evaluation on leukocyte differential counts using an NCCLS H20-A Protocol. National Committee for Clinical Laboratory Standards. AM J Clin Pathol, 108 : 674-686, 1997.*

16) *Thalhammer Scherrer R, Knobl P, Korninger L, Schwarzinger I : Automated five-part white blood cell differential counts. Efficiency of software-generated white blood cell suspect flags of haematology analyzers Sysmex SE-9000™, Sysmex NE-8000™ and Coulter STKS. Arch Pathol Lab Med, 121 : 573-577, 1997.*

17) *Van Dor Mer W, Swinkels DW, Willems HL : The characterization of leukemias with the Sysmex NE-8000™. Acta Haematol, 98 : 195-198, 1997.*