ORIGINAL ARTICLE

COMPARISON OF SYSMEX-KX-21 WITH MANUAL METHODS: HEMOCYTOMETERY, SAHLI-HELLIGE AND MICROHEMATOCRITE METHODS IN QUANTITATIVE HEMATOLOGY ANALYSIS

Baye Gelaw[1], Mulugeta Aemero[2], Salie Ayalew[3], Aschalew Gelaw[1]

ABSTRACT

Background: *The manual blood cell counting is currently replaced by automation hematology analyzers in many clinical laboratories. Sysmex is a discrete hematology analyzer designed for high-volume testing in clinical laboratories. Though automation is widely accepted and is on use in different health institutions in Ethiopia, it is not yet evaluated for precision and accuracy nor compared with manual methods. This study compared four manual hematology analysis methods with Sysmex kx-21.*
Objective: *To compare Sysmex-kx-21 hematology analyzer with manual hemocytometery (WBCs), Sahli-Hellige (hemoglobin concentration), microhematocrit centrifuge method (hematocrit or packed cell volume) and the differential white blood cell (WBC) count methods.*
Methods: *The white blood (WBC) cell enumeration, differential WBC count, hemoglobin and hematocrit results of three laboratory technicians (two manually and one by using Sysmex- kx-21) was compared. Blood samples from 130 patients and 30 students were collected between May and June, 2008. Each sample was investigated manually and by the SYSMEX-KX-21 automation. Data was registered and analyzed using Microsoft Excel 2003 and SPSS version 13 computer software programs.*
Results: *There was no significant difference in the total WBC count between the two manual readers and between the two manual readers and the automation. The results of the three readers strongly matched on the total WBC count and hemoglobin concentration. The result of the manual hematocrit readers was less correlated with the automation. The two manual readers were almost not correlated on the lymphocyte differential WBC count.*
Conclusions*: The overall correlation of the manual methods to the automation can be graded as good. Standardizing the automation, combined use of manual methods with automation at higher health institutions and the use of manual methods by the peripheral health unites are recommended.*
Key words: *White blood cell, Differential white blood cell, Hemoglobin, Automation, Hematocrit, Sysmex, manual methods.*

INTRODUCTION

Although highly automated modern hematology analyzers have adequate reproducibility, there are concerns over accuracy. For example, it is well known that erythrocyte fragments or microcytes cause a pseudo increase in platelet count (1). The major role of the hematology laboratory in the analysis of body fluids has been to provide accurate enumeration of blood cells (2). A variety of instruments and methods are available for the enumeration of hematological parameters. Among the standard parameters and methods used for evaluation, there exists a strong need for standardization, taking into account biological, analytical and pre-analytical variabilities all of which can significantly affect the data being obtained through hematological analysis(3). The visual counting of blood cells has been an acceptable alternative until recently (4). Manual blood cell counting is currently replaced by automation hematology analyzers in many clinical laboratories. One of the predominantly used automation is the Sysmex series. Sysmex is a discrete hematology analyzer designed for high volume testing in clinical laboratories (5).

The sysmex series is a cutting edge technology which considerably improves the quality of reports generated from the blood samples due to the innovative principle of fluorescence based flowcytometry (6). The Sysmex – kx-21 is an automatic multiparameter blood cell counter for invitro diagnostic use in clinical laboratories. It processes approximately 60 samples an hour and displays on the screen with the distribution curves for white blood cells, red blood cells, hemoglobin, hematocrit, and platelets

[1]*Department of Laboratory Technology, College of Medicine & Health Sciences, University of Gondar*
[2]*Department of Applied Biology, Faculty of Natural Science, University of Gondar*
[3]*Department of Statistics, Faculty of Natural Sciences, University of Gondar*
** Corresponding Author: Baye Gelaw, P.O.Box 196, Tele: 09 –18–70–37-23, e-mail- bayegel@yahoo.com*

along with data for 18 parameters as analysis results (operators manual, Sysmex-kx-21, Japan 2000).

Though automation is widely accepted and is on use in Ethiopia in general and the University of Gondar Hospital Laboratory in particular, it is not yet evaluated for precision and accuracy nor compared with the manual methods which is the objective of this study. The output of this study will help to reduce the uncertainty during the hematological parameter enumeration and investigation by selecting the appropriate test procedure for the intended hematological test.

METHODS

The subjects of this institution based cross-sectional study were patients seeking hematological investigation at the University of Gondar Hospital Laboratory from May to June, 2008. The control groups were students of the Department of Laboratory Technology.

Sample size was determined using a pilot sample since there was no similar study concerning this problem. We took 30 students as a pilot sample from third year laboratory technology students. From each of the 30 samples total WBC; differential WBC, hematocrit and hemoglobin values were determined.

We used hemoglobin values of the pilot sample to determine the minimum sample size that represent the study population. The hemoglobin mean and standard deviation were 12.48 g/dl and 1.41 g/dl respectively. The margin of error, d, for this pilot test was 0.5.

Therefore, we took 0.25 as a margin of error to calculate a rational representative sample size. Considering an assumption of a level of significance alpha 0.05, variance of 1.99 and the margin of error 0.25 the calculated sample size was 123.Because the target group, study subjects, were accessible during the sample collection time, we took 130 patients for this study.

Since the study variables had different units of measurement, it was necessary to evaluate the consistency of the variables using the coefficient of variation (CV).

Accordingly, the CV of the study variables among the pilot samples between the manual readers and the automation were 0.016, 0.027, 0.23, and 0.18 for lymphocyte, total WBC, hematocrit and hemoglobin, respectively. Because the CV of the pilot study seemed consistent, we used a simple random sampling method to select the study subjects on daily bases and involved 130 patients during the study period. The pilot sampled 30 students who were randomly selected served as the control group, to minimize dilution error effects that can particularly happen on patients with low WBC or low hemoglobin values, since they were apparently healthy, in a similar age group and similar living standard.

Three milliliters of venous blood was collected from each patient and student. Trisodium citrate solution with a concentration of 32 gram per liter was used as anticoagulant. Three hundred micro liter of the anticoagulant dried in vials and the three milliliter blood drawn was added to the vials. After a thorough mixing, each blood sample was investigated for total WBC, differential WBC, hemoglobin and hematocrit values.

The blood cell enumeration result of two laboratory technicians, (reader-1 (R_1) and reder-2 (R_2)), who used the manual methods like hemocytometery for counting white blood cells, Sahli-Hellige for hemoglobin determination, microhematocrit centrifuge method for hematocrit determination and stained blood films for differential white blood cell count was compared with the result of another laboratory technician, (reader-3 (R_3), who used Sysmex-kx-21 automation. The investigation was done blindly and all the three laboratory technicians were the same standard of qualification (B.Sc. in Medical Laboratory Technology).

Blood samples from the patients and students were investigated manually and by the automation. From each sample an aliquot (up to 0.5 marks) of blood was taken using standardized white blood cell pipette (Thomma white blood cell pipette) and diluted 1:20 with 2% acetic acid. An aliquot from the diluted blood was charged on a white blood cell (WBC) counting chamber (improved Neabauer counting chamber) and the four WBC areas were counted. The counted WBC was multiplied by a factor of fifty (dilution factor).

Thin blood film was prepared from each sample and stained with Wright's stain solution. Each stained blood film was examined microscopically and the different white blood cells (Neutrophils, Lymphocytes, eosinophils, basophils, monocyts and nucleated red blood cells) were counted in percentage.

The hematocrit (packed cell volume) of each sample was determined using microhematocrit capillary tubes and centrifuged at a speed of 10,000 revolution per minute (rpm) for five minutes and the hematocrit value quantified using hematocrit reader. The hemoglobin value of each sample was determined using Sahli-Hellige visual comparative method. A 0.1 N HCl solution was filled up to the "20" mark of Sahli-Hellige hemoglobinometer.

Blood sample was drawn up to the 0.02 mark of the Sahli-Hellige pipette and blown into the graduated tube of acid solution. After the graduated tube was placed in the hemoglobin meter and the sample was diluted with distilled water and stirred by a glass rod, the color of the sample tube matched with the standard color on the hemoglobin meter. The result was the concentration of hemoglobin in gram per deciliter.

Each blood sample was again analyzed for total WBC count, differential WBC count, hematocrite and hemoglobin concentration values by Sysmex-kx-21 automated hematology analyzer on the same day.
The total WBC enumeration quality was controlled by each manual reader by considering the accepted sources of error and differences which must be less than 11 WBCs among the four WBC areas in the counting chamber.

Moreover, the proper dilution of blood in the thomma white cell pipette was performed avoiding air bubbles while charging the counting chamber. The counting chamber was surveyed with low power objective of the microscope by the laboratory technologist intended to do the particular test to ascertain whether cells were evenly distributed.

Effort was also made to maintain the quality of the differential WBC count by beginning enumeration in the thin area of the slide and following a pathway for differential WBC count until 100 WBCs were counted by which the even distribution of WBCs was also evaluated (the exaggerated Battlement Method).
The visual comparative method (Sahli-Hellige) is not recommended because of its unacceptable impression and inaccuracy although it is still used in many health centers and hospitals in Ethiopia. This is the limitation of this study. All sources of error in hematocrite determination including incomplete packing, incorrect reading, hemolysis and others were considered.

Quality control of the Sysmex-kx-21 needs to be ensured by running control blood samples (operators manual Sysmex-kx-21, Japan 2000). However, because there were no control blood samples for the automation, the quality was not fully assessed.
Pair wise student t test (t test) and normal standard test (Z test) considering a=0.05 was used for the data analysis since the experimental units used by the three readers (R_1, R2, and R_3) were the same.

Data was analyzed using Microsoft excel 2003 and SPSS version 13 computer soft ware programs.

Ethical approval was obtained from the University of Gondar Research and Publications Office. Informed consent was obtained from each student (control groups) and confidentiality was maintained.

RESULTS

A total of 130 patients and 30 students (normal population or controls) were included in the study. The socio-demographic characteristics of patients show that 69 were males and 61females. Of the students 15 were males and the other 15 females. The highest age distribution category among patients lies between 20-30 years of age (30.8%) followed by 30-40 (22.3%). The mean age of the students was 20 years and the maximum age distribution was between 11-

Table 1: *Socio-demographic characteristics of subjects included in the study at UOG Hospital Laboratory, 2008.*

Patients				Students			
Age Category	**Male**	**Female**	**Total**	**Age category**	**Male**	**Female**	**Total**
0-10	9	6	15	0-10	-	-	-
11-20	6	9	15	11-20	12	15	27
21-30	25	15	40	21-30	3	-	3
31-40	9	20	29	31-40	-	-	-
41-50	11	7	18	-	-	-	-
51-60	3	3	6	-	-	-	-
61-70	4	1	5	-	-	-	-
71-80	2	0	2	-	-	-	-
Total	**69**	**61**	**130**	**Total**	**15**	**15**	**30**

The total white blood cell count agreement between the two readers on patients using a manual white blood cell count method has no significant difference on average at 0.05 level of significance (Z= average/ standard error=0.21).

Moreover, there was no significant difference between the two manual readers and the Sysmex-kx-21 automation for WBC count on patients (Z_1 = 1.08 and Z_2 = 0.32, respectively).

However, in patients with lower total WBC count, there was a relative cell enumeration difference between the two readers and even a significant difference with Sysmex-kx-21 (Table 2).

***Table 2**: Frequency distribution of white blood cell count by the three readers (R_1, R_2, R_3) at UOG hospital laboratory, 2008.*

WBC	Patient			Student		
	R_1	**R_2**	**R_3**	**R_1**	**R_2**	**R_3**
< 5000	38(29.2%)	41(31.5%)	47(36.2%)	11(36.7%)	10(33.3%)	12(40%)
5000-10,000	85(65.4)	82(63.1%)	75(57.7%)	18(60%)	19(63.3%)	17(56.7%)
10,000	7(5.4%)	7(5.4%)	8(6.2%)	1(3.3%)	1(3.3%)	1(3.3%)
Total	**130**	**130**	**130**	**30**	**30**	**30**

The trends of the white blood cell count of the three readers in both patients and students showed similar patterns even though there was significant difference in the result of some of the test parameters (Table 2).

Nevertheless, there was a significant difference between the two manual total WBC readers and the Sysmex-kx-21 among the control groups (Students) (Z R_1 = - 9.71 and Z R_2 = -19.81).

On the differential white blood cell count of patients, there was no significant difference between reader one (R_1) and reader two (R_2) on neutrophil and lymphocyte counts (ZR_1= -0.21 and ZR_2 = -0.075). Eosinophil, Basophil and Monocyt count results showed that there also was no significant difference between the two manual readers, but the three cells were enumerated as mixed by the Sysmex-kx-21 automation.

On the control groups, a significant difference between the two manual readers and the Sysmex-kx-21 was observed in the neutrophile count (ZR_1 = -4.13 and ZR_2 = -3.9).

The hematocrit and hemoglobin reading showed a significant difference between the two manual readers in the patients (Z = 0.12 and Z = -0.14 respectively).

There also was a significant difference between the two manual readers and the automation values for the hematocrit and hemoglobin ($ZHctR_1$=-5.37, $ZHctR_2$ =-5.19; $ZHgbR_1$ = -3.31, ZHgb R_2 = -4.04).

There was no significant difference between the two manual readers on hematocrit and hemoglobin values on the control groups. However, the hematocrit reading between one reader (R_1) and Sysmex-kx-21 showed a significant difference (Z=3.89) and the manual readers had the same conclusion on hemoglobin determination.

The four investigated variables with the corresponding result of investigators were assessed for correlation. Accordingly, the results of the three readers (R_1, R_2 and R_3) were strongly consistent on white blood cell count (r>0.87) and hemoglobin concentration (r>0.86) on both the patients and the control groups.

The hematocrit reading values among the three readers on the control group was strongly correlated, while the results of the manual hematocrit readers were less consistent with the results of Sysmex-kx-21 (r_1 = 0.19 and r_2 = 0.2 respectively).

The association of readers on lymphocyte differential WBC count on patients showed that the two manual readers R_1 versus R_2 andR_2 versus R_3 were almost not correlated (r_{12}= 0.016 and r_{23}=0.019 respectively).

On the other hand, the three readers were moderately correlated on the neutrophile differential WBC count on the same study subjects. On the control groups, the two manual readers strongly matched on their differential lymphocyte and neutrophile counts (r=0.97), but the differential lymphocyte and neutrophile counts of the two manual readers were less correlated with the automation (Table-3).

Table 3: *Correlation (r) among readers on the quantitative hematological analysis of patients and control groups, UOG, 2008.*

	Total white blood cell count		Lymphocyte		Neutrophil		Hematocrit		Hemoglobin	
	patient	**student**	**patient**	**student**	**patient**	**student**	**patient**	**student**	**patient**	**student**
R_1Vs R_2	0.92	0.94	0.016	0.97	0.60	0.97	0.96	0.94	0.92	0.94
R_1Vs R_3	0.97	0.93	0.63	0.36	0.42	0.37	0.19	0.94	0.86	0.96
R_2Vs R_3	0.94	0.87	0.0194	0.35	0.43	0.40	0.20	0.92	0.89	0.88

R_1= Reader 1, **R_2**= Reader 2**, R_3**= Reader 3, **r** = Sample correlation ($-1 \leq r \leq 1$)

The precision of each reader was also assessed by a coefficient of variation (CV). Both the two manual readers and the Sysmex-kx-21 (R_1, R_2, R_3) quantification on the total white blood cell were not standardized in their precision (CV_1, CV_2 and CV_3 = 0.4, 0.4 and 0.30, respectively) in the patients and the controls (CV_1, CV_2 and CV_3=0.30, 0.30, and 0.30, respectively).

The Sysmex-kx-21 was less precise than the two manual readers on the total WBC count, differential neutrophils count and hematocrit value on patients. The CV was similar on WBC count, hematocrit and hemoglobin values on the control groups even if the CV in hematocrit and hemoglobin values were precise enough (Table 4).

Table 4: *Coefficient of variation (CV) among the three readers for each hematological variable, UOG, 2008*

	White blood cell		Lymphocyte		Neutrophil		Hematocrit		Hemoglobin	
	patient	**student**	**patient**	**student**	**patient**	**student**	**patient**	**student**	**patient**	**student**
R1	0.40	0.30	0.53	0.19	0.31	0.46	0.20	0.10	0.21	0.11
R2	0.40	0.30	3.64	0.19	0.25	0.44	0.20	0.11	0.19	0.10
R3	0.43	0.30	0.45	0.27	0.34	0.60	0.52	0.10	0.21	0.11

The trend of precision was higher in the two manual differential white blood cell readers (less CV) than the Sysmex-kx-21 automation except for lymphocyte differential counts (CVR_1,CVR_2, CVR_3 =0.53, 3.64 and 0.45, respectively). Very low precision of lymphocyte differential count was observed by the second manual reader (R_2) (CV=3.64).

DISCUSSION

The determination of the number of leukocytes (white blood cells, WBC) in blood is the most important tool (together with the percentage of polymorphonuclear cells) to discriminate between inflammatory and non-inflammatory diseases (7).

In this study, there was no significant difference in the total WBC count between the two manual readers (R_1 Versus R_2) and that of the manual readers with the Sysmex-kx-21 (R_1, R_2 versus R_3) on patients. Surprisingly, there was a significant difference between the two manual total WBC readers and the Sysmex-kx-21 among students (control groups, normal population) (ZR_1, = -9.71 and ZR_2 = -19.81).
A significant difference between the two manual readers and Sysmex-kx-21 was also seen in patients with lower total WBC counts. The possible reason might be the dilution effects which should have been corrected by increasing the volume of blood.

Whenever the white blood cell count dropped below 3000 per cubic milliliters of blood, the blood sample should be drawn up to the 1.0 mark, rather than up to the 0.5 mark of the white cell pipette and diluted to the 11 mark with the WBC diluting fluid to make the dilution 1:10, rather than 1:20 (9).

The two manual readers agreed on differential WBC count. However, the three cell counts (Eosinophils, basophils and monocyts) were counted and merged by the automation.

An increase of the three different cells that could have resulted due to specific diseases (basophilic leukocytosis, chronic myelogenous leukemia, polycythemia, chronic sinusitis, foreign protein injection, ionization, etc; eosinophilia, lymphoma, multiple myloma, aplastic anemia, parasite or bacterial infec-

tions, etc and monocytosis, monocytic leukemia, postsplenoctomy, chronic ulcerative colitis, rheumatoid arthritis etc) (10) was not encountered by the machine.

The occurrence of a significant differential WBC difference between the two manual readers and the automation is supported by other studies in which the analysis of the automated blood cell count is an essential tool in hematology. However, incase of the white blood cell differential the microscopy method often serves as reference (11).

The discrepancy in hematocrit and hemoglobin values between the two manual readers compared to the automation ($ZHctR_1$ = -5.37, $ZHctR_2$ =-5.19 and $ZHgbR_1$ = -3.31, $ZHgbR_2$ = - 4.0) in patients could be due to decreased number of red blood cells and hemoglobin concentration. In some previous studies, as high as 5-6 percent decrease in hematocrite or hemoglobin values was documented in abnormal sampling and up to 20 percent in very ill individuals (12) as compared to the normal ones. In this study there was no significant difference between the two manual readers on hematocrite and hemoglobin concentration of the students (control groups).

Correlation results show that the results of the three readers (R_1, R_2 and R_3) on total WBC is strongly correlated ($r<0.87$) which is in agreement with the study on white blood cell counts done on pleural fluids in Saint Thomas Hospital and Vender bit University, Nashville, TN. The total pleural fluid WBC counts obtained with manual and automated counting methods on the EDTA- treated fluid samples were nearly identical ($r = 0.92$; $P<0.01$) (13). On the other hand, there was no significant relation between the two manual readers with that of Sysmex- kx-21 (r_1 = 0.19 and r_2 = 0.2 respectively) concerning hematocrit.

This information is supported by another study where variability increased in hematocrit measurement suggesting that hematocrit is not the best parameter for red cell quantitative assessments (14). In addition, it is documented in many books that hematocerit value variation results due to speed (rpm), time of centrifugation, volume of blood taken in the capillary tube and readers' individual difference.

In terms of consistency, the two manual readers and the Sysmex-kx-21 were not sufficiently precise on the total WBC count (cv_1, cv_2 and cv_3 = 0.4, 0.4 and 0.30 respectively) on patients which was in disagreement with a study done to evaluate the Sysmex UF – 100 automated urinalysis analyzer on which the within run imprison cell counts expressed as coefficient of variation (CV) for leukocytes was 0.18 (15).

It is well documented in literature that the manual hematological methods are laborious and time consuming. The increasing workload and the necessity to decrease the turn around time (TAT) impose the need for automation. In this regard, it is documented that automated hematology system saves 222 minutes of manual activities arising in a large routine hematology laboratory with a mean throughout of 612 samples per day (16).

However, the cost effectiveness of automation in countries like Ethiopia still raises questions. The cost of a Sysmex-kx-21 hematology analyzer is greater than Birr 100,000.00 which is by far costly than the collective cost expense (about Birr 12,600) of hemocytometer, Sahli-Hellige and microhematocrite centrifuge. The reagent costs of the automation are even damn expensive as about Birr 4520.00 is expected to run an average of 400 samples (operator's manual, Sysmex-kx-21, Japan, 2000).

In the case of manual methods, only diluted (1-2%) hydrochloric acid or acetic acid, Wright's stain solution and hematocrit tube are required. On the other hand, in the result of the automation, immature WBC's and nucleated red blood cells (NRBCs) are not selectively enumerated. It is true that finding even 1NRBC per 100 WBC in a blood smear from an adult is abnormal by itself, but the significance of such a finding from the clinical stand point remains debatable (17).

In a study designed to evaluate Sysmex XE-2100 in the enumeration of NRBCs compared to the manual method, the overall correlation was excellent (18). Previous documents (19, 20) indicated that both the supper vital stain and the Miller eye Disc methods (both manual methods) were good enough to selectively enumerate NRBCs particularly reticulocytes based on the comparison of NRBCs per erythrocytes.

The overall correlation of the manual methods to the automation can be graded as good without forgetting the discrepancies on hematocrit and differential white blood cell count. The automated hematocrit results and WBC count are prone to certain errors which are no more a problem with manual methods. Although the manual methods are slow, they are preferable to the automated methods (21).

The exaggerated cost of automation and reagents for

the hematology analyzer which is unaffordable to the peripheral health units, still dictates the uses of the manual methods. Thus automation is recommended for higher health institutions and manual methods for peripheral health units.

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