Comparative Analysis of Hematological Parameters Determined By Cell-Dyn Ruby Automated Hematology Analyser and Manual Analysis

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Abstract:
Introduction: Automated laboratory hematology analysers (ALHA) have replaced the traditional technique of microscopic peripheral blood smear examination and manual methods of analysis in hematology laboratories. The change is due to advantages of ALHAs over manual methods – short turnaround time, easy reproducibility of results etc. But, manual methods help in providing essential information by which the ALHA results are validated.
Methods: A review of one hundred randomly selected samples from outpatient hematology laboratory at Prince Mutaib Bin Abdul Aziz Hospital, Sakaka, Al Jouf, Kingdom of Saudi Arabia (KSA) was carried out. Thorough analysis was done using ALHA (Cell-Dyn Ruby) and by manual methods.
Results: In an unbiased randomly selected sample of one hundred patient samples, all the samples showed complete leucocyte differential counts in manual methods and one showed incomplete leucocyte differential count. Blood film examination showed normocytic normochromic picture in 65%. Rest showed abnormal red blood cell pictures. All the parameters revealed statistically significant difference (p value<0.0001) and correlated positively when comparative analysis was done.
Conclusion: The present study revealed that ALHA reports are closely correlating with the manual methods of analysis. ALHA usage decreases the workload and preparation time of the laboratory personnel. For promising correlation of reports the ALHAs should always be under good maintenance with regular calibration and control checks.
Key words: ALHA (Automated laboratory hematology analyser), DLC (Differential leucocyte count), manual assay, PBS (Peripheral blood smear), RBC indices (Red Blood Cell indices)

I. Introduction

Automated laboratory hematology analysers (ALHA) have slowly replaced the traditional microscopic blood film examination and standard manual methods of analysis in the hematology laboratories. The individual manual assay methods are now a days occasionally used as he ALHAs are capable of carrying over differential leucocyte counts (DLC), morphological evaluation of red blood cells (RBC) and platelets with greater accuracy and precision than that of manual scanning by peripheral blood smear[1-3]. On the contrary there is a drastic difference in the turnaround time (TAT), which is very less in ALHA when compared to peripheral blood smear examination and other individual manual assays (WBC, RBC, Platelet counts). Thus, the manual procedures are time consuming and comparatively expensive when individual test expenditure is calculated[3,4]. Thus, the traditional review of hematology samples by preparation, staining, manual counting under chamber and microscopic examination of blood film is not being practiced as a routine in most of the hematology laboratories and institutions. The accuracy and clinical usefulness of automated DLC has been

validated in numerous studies[1,2,5,6]. Thus, performing DLC manually while ALHAs are yielding identical results may undermine efficiency and low productivity in a hematology laboratory[7]. In this recent transition there is a dramatic reduction in number of medical technologists and technicians in medical laboratories after the advent of ALHAs, which is simpler compared to manual and individual assay techniques – thus, demands less manpower[8]. Even though, the ALHAs are major breakthrough in the laboratory technology, the machines need continuous monitoring with controls, calibration, maintenance, and reagent replenishment which is a cumbersome process, which in turn has to be strategically managed in at regular intervals. Any derailment in the regular maintenance of the machine will result in erroneous results immediately. This will have a great effect over the accuracy and precision of the machine resulting in wear and tear of the instruments[9].

The manual methods of evaluation, if at all are time consuming are still at the upper hand over ALHAs as false results related to counts or morphological evaluation were observed in several instances. False low white blood cell (WBC) counts are often seen because of agglutination in presence of ethylene diamine tetra acetic acid (EDTA)[10]. Red blood cell counts and Hemoglobin determination may be erroneous in case of hemolysed samples. Some of the abnormalities of RBC, WBC and platelets may not be apparent from instrument results alone. In these instances, a meticulous review by manual methods, individual assays and peripheral blood smear examination is warranted, which in turn has to be correlated with clinical findings in co-ordination with the physician. Thus, appropriate training programs for the technical staff in automation and manual methods is to be ensured and it is to be taken into consideration that, manual analysis and evaluation always helps in cases of erroneous results with flags and proper care has to be taken, so that, all the analysis is not solely left to the machine[9].

Hence, the present study was carried out to determine the relationship and to compare the results between ALHA (Cell-Dyn Ruby, Abbott Hematology) and manual counts using randomly selected subjects' blood samples attending outpatient department at Prince Mutaib Bin Abdul Aziz Hospital, Sakaka, Al Jouf, KSA.

II. Material and Methods

Blood samples were collected from one hundred (N=100) unbiased randomly selected patients attending various outpatient departments on a single week day. Intravenous blood drawn from left antecubital vein. The patient population comprised of normal healthy individuals and individuals with blood disorders.

An informed consent was obtained from each subject during the collection of blood sample. Under strict aseptic precautions 4 ml of intravenous blood sample was collected from each subject into tri potassium EDTA (anticoagulant) sample tube. The sample tubes were gently mixed by inversion and were subjected for mixing on roller for 5-10 minutes. Then, blood sample was divided into two equal parts, 2 ml of was taken for manual method and 2 ml for automated analysis with Cell-Dyn Ruby AHLA. CELL-DYN Ruby system is a multiparameter automatic hematology analyser for invitro diagnostic use in clinical laboratories. This instrument utilizes MAPSS (Multi Angle Polarized Scatter Separation) technology, laser flowcytometry – coupled with state of art software from Abbott Hematology[11].

The automated analysis as carried out according to the instruction manual provided by the manufacturer within 30 minutes of collection. Manual analysis was performed for Hemoglobin estimation with Cyanmeth hemoglobin method. Hematocrit was estimated by the microhematocrit method. Peripheral smear examination was carried out to evaluate and analyse findings regarding morphology of RBC, WBC (DLC and morphology) and platelets. All individual manual assays of RBC, WBC and platelet counts were carried out by trained technical staff with the help of appropriate dilution fluids for RBC (hayem's), WBC (2% acetic acid) and platelets (1% w/v ammonium oxalate) in respective pipettes. RBC indices were calculated with the above data from hemoglobin, RBC count and hematocrit using respective formulae for Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC).

All the parameters analysed were checked for correlation between ALHA analysis and manual analysis and statistical evaluation was carried out using SPSS *ver.*16 software.

III. Results

One hundred randomly selected unbiased sample of patients samples were analysed both in automated analyser and by manual methods. All the samples showed complete differential count in manual method. Only one sample showed incomplete DLC in automated method. There are equivalent reports showing neutrophilia in 12% in automated method and 15% in manual method. Band form neutrophils were observed in 8% in automated method and in 12% in manual method. Accordingly, the RBC picture was observed in the blood film which revealed various pictures of RBCs. Both normal and abnormal, which has been depicted in Table No: 1 with mixed population of RBCs revealing anisocytosis and poikilocytosis (elliptocytes, pencil shaped cells, ring shaped cells etc). The platelet related abnormalities were also observed and there was quite good correlation in between automated method and manual methods for the above parameters.

The mean values and standard deviation (S.D) of the all the parameters analysed by automated and manual methods of analysis are tabulated in Table No: 2. All the parameters studied show statistically significant differences (p value < 0.0001). The correlation between automated and manual methods for all the parameters is graphically represented as a scatter plot in Figure No: 1 & 2.

Table 1: Findings of Manual Peripheral Smear examination in all the subjects studied (N=100)

Parameter	Number (n) Percentage (%)
RBC findings:-	
Normocytic - normochromic	65 (65%)
Normocytic – hypochromic	5 (5%)
Microcytic – hypochromic	23 (23%)
Macrocytic – hypochromic	7 (7%)
Specific findings:-	
Anisocytosis&Poikilocytosis	4 (4%)
Band forms (Neutrophil)	3 (3%)
Atypical lymphocytes	1 (1%)
Thrombocytosis	2 (2%)
Thrombocytopenia	12 (12%)

IV. Discussion

After the advent of ALHAs the conventional manual techniques of hematology have slowly waned off. In some laboratories still, as a routine practice, manual methods are being carried out. But in some laboratories the manual methods are being practiced only in case of abnormal automated results or instrument "flags" before any manual triage step[3]. In present study all the samples showed complete WBC count in DLC manual method. But, automated method showed complete DLC in 99% only, which reflects the ability of the instrument to perceived differential morphology of WBCs. The immature neutrophils were marked as "BAND" and lymphocytes with abnormalities were reported as "ATYPICAL LYMPHOCYTES". In a similar study carried out by Samuel et al.,[9] using SYSMEX KX-21 ALHA, 6.6% of cases showed incomplete differential count. Another study done by Lewis et al.,[12] also revealed similar results. Thus, all the laboratories should establish its own policy for taking further action in case of samples with instrument flagged results. Most important step in this instance is to cross check the result with manual method of analysis. The advantage of manual analysis is the ability to identify specific clinically important cell types like blasts, hyper granular cells, immature granulocytes, nucleated RBCs, large platelets, hemoparasites etc., that are not quantifiable by instruments, but to some extent the flags will be displayed in these abnormal cases by ALHA[12-15]. These alarming results demands manual scanning of peripheral smear for the

Table 2: Comparison statistics of the hematological analysis of automated and manual methods with correlation coefficient of respective parameters

PARAMETER	Automated (mean±S.D)	Manual (mean ±S.D)	Correlation coefficient (r)
Hemaglobin (g/dl)	12.3 ± 3.02	12.17 ± 3.09	0.9827
Hematocrit (%)	35.33 ± 7.99	35.70 ± 8.82	0.9580
RBC (millions/cu.mm)	4.26 ± 1.08	4.28 ± 1.13	0.9584
MCV (fl)	83.6 ± 6.86	84.33 ± 8.66	0.9412
MCH (pg)	29.03 ± 2.56	28.54 ± 2.75	0.9328
MCHC (g/dl)	34.78 ± 2.35	33.57 ± 1.65	0.8914
Total WBC (10^3 /cu.mm)	8.53 ± 3.63	8.74 ± 4.03	0.9878
Neutrophils (%)	62.56 ± 14.94	62.53 ± 14.25	0.9919
Lymphocytes (%)	23.84 ± 11.65	25.37 ± 10.90	0.9179
Platelets (10^3 / cu.mm)	244.84 ± 100.67	237.96 ± 101.93	0.9870

validation of ALHA reports. At the same time, if a manual scan shows normal cells as quantified by the ALHA, but no abnormal cells, then also there is no need to invalidate the ALHA reports[3]. In contrast to the above statement it has been proposed by Tabuko and Tatsuni[16] that, there are discrepancies in the quality control survey in a manual DLC which was due to poor differentiation of segmented neutrophils and band neutrophils.

In spite of wide acceptance of ALHA reports, manual analysis and peripheral blood smear (PBS) examination reveals and confirms many findings which are given more weightage than ALHA reports. In present study PBS revealed 65% of cases with normocytic normochromic picture and rest 35% samples showed abnormal pictures which aided in the diagnosis of other blood disorders. Similar reports were revealed in a

study done by Samuel et al.,[9]. Thus, it has been stated by Rock et al.,[8] that, manual method even though is slow and needs meticulous efforts, still has advantages over the automated methods.

In present study the accuracy of ALHA and correlation of the same with manual methods of analysis has been promising and all the parameters were observed to be correlating positively. It has been noticed that, though there was positive correlation for the studied parameters, there were some statistical variations observed and were tabulated in Table No: 2. In a similar study carried out by Pierre[1] and Novis et al.,[7] it has been stated that, ALHA results are more accurate in detection of specimens with distributional or morphologic abnormalities than by traditional eye count method.

Another interesting finding in present study was, majority of cases were flagged for RBC or platelet abnormalities and in these cases WBC count and morphology was almost normal. These flagged reports comprising of Microcytic cells (23%); Macrocytic cells (7%); Anisocytosis and Poikilocytosis (4%); Thrombocytosis (12%); Thrombocytopenia (2%). There were minimal number of cases with band forms (3%) and atypical lymphocytes (1%). Identical findings were observed in a study carried over by Lantis et al.,[3].

The ALHA (Cell-Dyn Ruby) used in present study revealed acceptable and positive correlation for all the parameters studied with manual methods of analysis and has showed very good performance characteristics in all the parameters measured in both normal and abnormal cases. Thus, ALHA (Cell-Dyn Ruby) readings were correlating with manual methods of analysis and show only minor, negligible and acceptable errors. These errors can be corrected with regular maintenance, calibration and control checking of the instrument. Many other authors have studied the behavior of various ALHAs and correlated their findings with manual methods. Almost all of them reported the similar findings[17,18].

V. Conclusion

The present study suggests that, there is gradual disappearance of manual analysis after the advent of ALHAs. There are quite a good number of advantages like less turnaround time and less effort compared to manual methods. There is also gradual improvement in the efficacy of the latest ALHAs. The quality, performance and precision of these instruments can be safeguarded by setting the instrument threshold triggers and developing the strict internal laboratory policies regarding flagged reports and need for manual analysis. Though, standard manual methods give more additional information, these methods are to be warranted only to validate the automated methods by which the laboratories can optimize patient care. It is not to be practiced as a replacement of ALHAs. Thus, the manual methods are to be advocated as a special practice where there is definite need of morphological and quantitative evaluation of RBC, WBC, platelets.

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Figure 1: Correlation of Hemoglobin, Hematocrit, RBC and WBC results between ALHA and manual methods of analysis
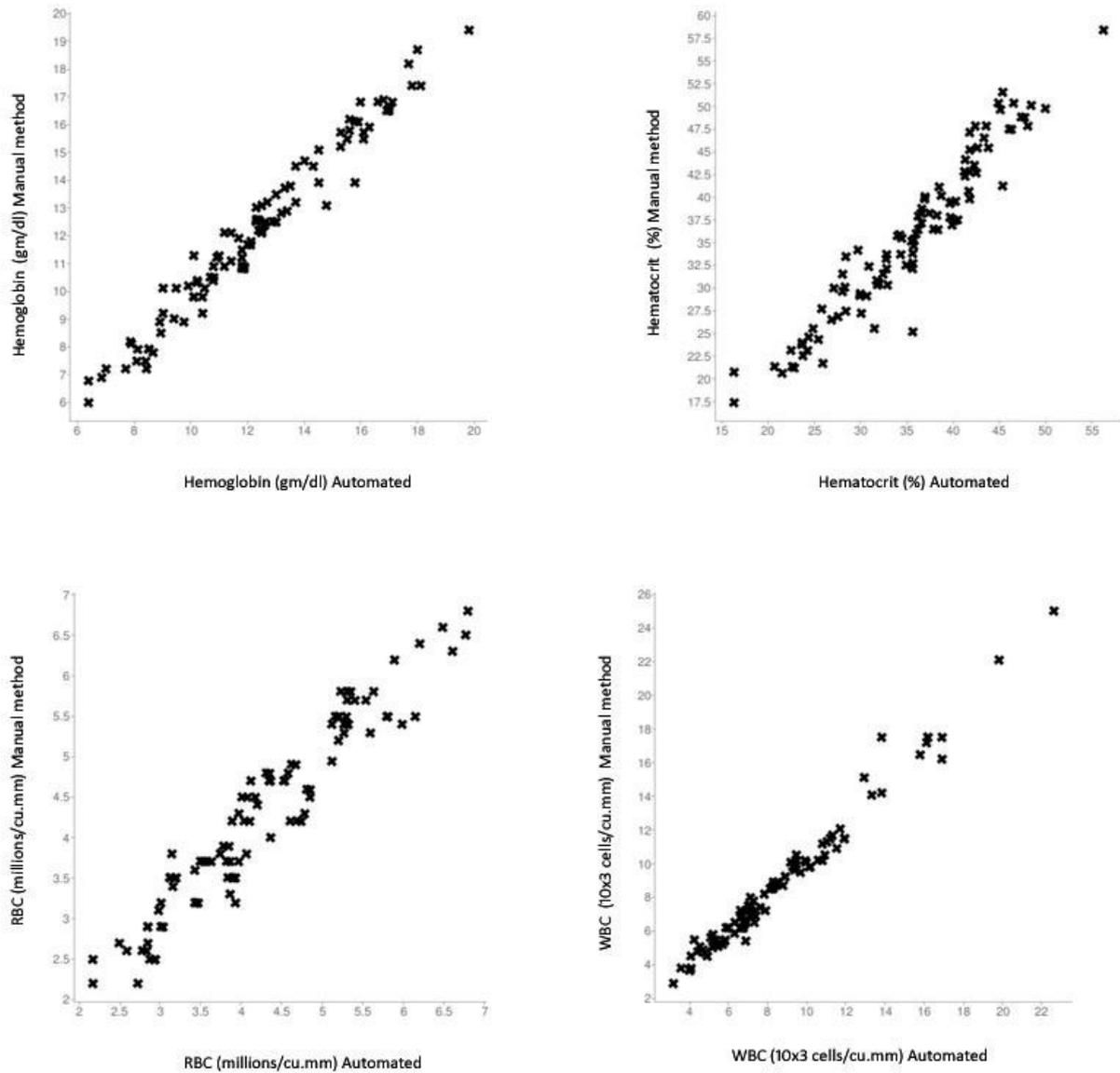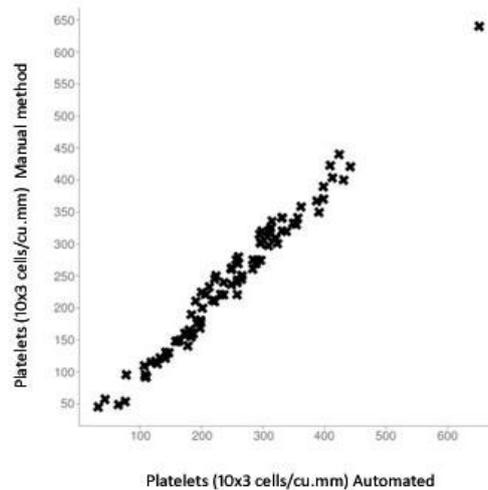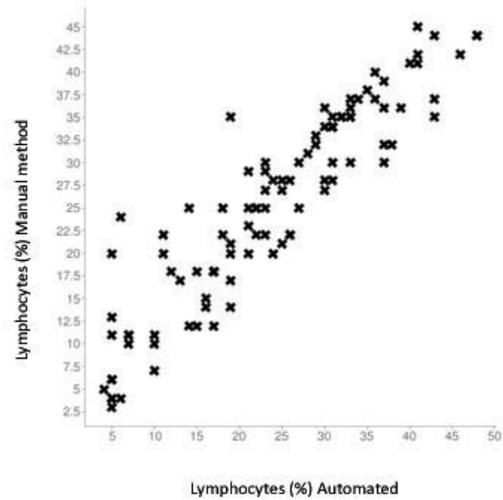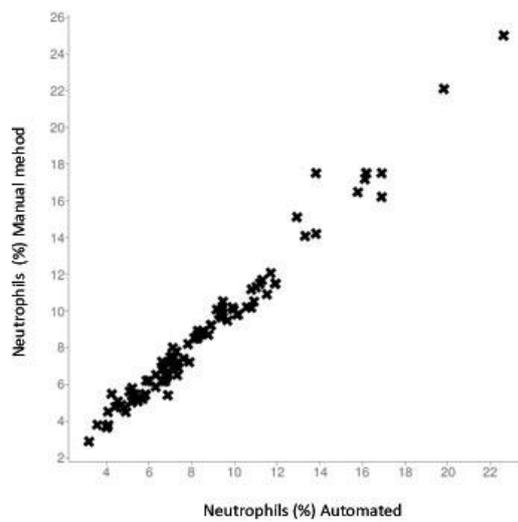

Figure 2: Correlation of Neutrophils, Lymphocytes and Platelets counts between ALHA and manual methods of analysis