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ORIGINAL ARTICLE

Correlation of Complete Blood Count Performed by Hematology Analyzer with Manual Methods at District Bannu, Khyber Pakhtunkhwa, Pakistan

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Abstract

Objective: To measure the correlation between the results of complete blood count performed by automated Hematology Analyzer Sysmex XP-100 with the manual methods at District Bannu, Khyber Pakhtunkhwa, Pakistan.

Methods: This Comparative Cross-sectional descriptive study was conducted in Samad Clinical Laboratory, District Bannu, Khyber Pakhtunkhwa, Pakistan, from October 2020 to October 2021. The Selection of 50 patients was made from all those who came to Samad clinical Laboratory for Blood complete determination from District Bannu. Using Standard Operating Procedure for Phlebotomy, 3ml of venous blood sample was obtained into tri-potassium Ethylenediamine tetra-acetic acid (EDTA K3) bottle for the determination of Blood Complete using the automated and the manual methods. Patients of any Age, Sex & Area having Advised Blood Complete tests were included in this study. Those patients which blood samples were hemolyzed during phlebotomy, during sample processing, improper mixing, poorly prepared and poorly stained slides of blood samples were excluded from this study

Results: Total of 50 samples were processed. Statistically significant differences ($p < 0.05$) were seen between the two methods (Manual & Automated). Similarly, the mean values of platelets, Hemoglobin (HB), Total Leucocyte (TLC), White Blood Cell (WBC), and Red Blood Cells (RBC) showed a statistically significant difference ($p < 0.001$) and correlated positively when both methods were compared.

Conclusion: It can be concluded from the present study that the results obtained from the hematology analyzer correlated well with those by the standard manual method, although the latter method provided additional diagnostic information on the blood pictures.

Keywords: Blood Manual CBC, Blood automated CBC

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Introduction

A complete blood count (CBC) is performed as an important screening test to diagnose, differentiate, and prognosis the patients. Despite advances in hematology automation and the application of molecular techniques, the blood complete smear has still an important diagnostic test for a variety of clinical diseases.

There are approximately 5-6 liters of blood in each human adult. Blood conveys oxygen and nutrients to living cells and waste products were taken by it. Immune cells are also delivered by it to combat infections and consist of platelets that have the function to make a plugin a damaged blood vessel to stop bleeding.[1] The methods that are used for the determination of white blood cells are important because the accurate results of white blood cells count have a great effect on the diagnosis and treatment of patients. Therefore, accurate and reliable results are necessary. Moreover, White blood cells values give valuable information about the blood and the bone marrow, in which blood cells are formed.[2] The White blood cells count can be used for the following purposes: to investigate those patients who may have inflammation, especially an infection, acute and chronic illness, blood

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disorders for example white blood cell disorders such as leukemia, to monitor treatment and prognosis particularly to find the effects of chemotherapy and radiotherapy on blood cells.[1,3] The count of blood cells can be made by Hematology analyzer which is an automatic machine working on the aspiration of a blood sample flow through an electric field. This method has Tayseer Ibrahim Elsidigge *et al.,* proved its value when used clinically in hospitals instead of the traditional manual method that depends on the visual counts of the blood cells, which takes time and effort.[4] For the determination of blood complete by the automated method is commonly in use in routine practice laboratory. However, due to poor automated results, most labs still use manual procedures, and also laboratory workers in the laboratory standardized their results by doing tests on the manual microscopic procedure as quality control for automated methods.[5] In modern hospitals and health institutions the old manual method has been substituted by an automated hematology analyzer with Blood complete (CBC) results.[6] Manual differentiation for the presence of old results for leukocytes is superior to the automated cell counter.[7.] The immune system consists of white blood cells (WBCs), also termed leukocytes that have the function to protect the body from pathogens.[8]

The handling of clinical specimen preservation and storage transportation identification and processing of data (including results reporting and recording interpretation of results and feedback), all of these elements should be included in Quality assurance (QA). Internal quality control (IQC) and external quality assessment (EQA) are also included in Quality assurance.[9] With each batch of the test procedure, one or more control materials should be included to assure Internal Quality Control. The control materials are treated in the same way as the test material by the analytical procedure.[10] The number of counted cells and a reasonable level of effort determines the precision and accuracy.[11.]The quality of the test procedure is checked by an external quality source outside the laboratory.[12] For the determination of blood complete most medical laboratories trust automated hematologic analyzers[13] there is still a need to review the Peripheral blood smear to confirm cell morphology or cell numbers which cannot be classified by devices' algorithms when the algorithms show abnormalities.[14] Slide review is very useful for test accuracy and also for patients. According to the type of hematology analyzer and need for slide review, and also patient characteristics which are age, disease, and disease condition, the rate of slide review is different. In general, an analyzer flag is the bases for manual review.[15]

The preparation of blood smear and staining procedure and then a microscopic examination of that smear are not used in most of medical institutions for review of all automated hematology analyzer results.[16] In most medical laboratories, the number of medical technologists and technicians is low. Blood Complete and differential counts by automation are useful to reduce the number of technologists required to perform these tests.[17.]Moreover, hematology analyzers give quick and accurate results in most circumstances. But inaccurate results regarding platelet or other tests from complete blood count may be seen in several circumstances, false decrease in the numbers of total leukocytes is due to aggregation which may be because of the presence of ethylenediamine tetra-acetic acid (EDTA).[18]

Therefore, the aim of this present study was made and preceded to find the relationship between Sysmex XP-100 automated hematology analyzer blood counts and manual counts by usage of blood samples of patients.

Materials and Methods

This cross-sectional comparative study was done on patients referred to and attending the Samad Clinical Laboratory near women and Children Teaching Hospital, District Bannu, Khyber Pakhtunkhwa, Pakistan (District Bannu and its adjacent areas) from October 2020 to October 2021. The sample size was calculated by using the WHO sample size calculator considering a confidence level of 95% and a level of significance of 5%. Non-probability sampling is defined as a sampling technique

Inclusion criteria included all patients of any age, sex, and area referred to Samad Clinical Laboratory were included in this study. Exclusion criteria included those Patients whose blood samples were hemolyzed during phlebotomy, during sample processing, improper mixing, poorly prepared, and poorly stained slides of blood samples were excluded from this study.

Sample collection is carried out by a person attending the Samad clinical laboratory district Bannu, after informed consent, a brief clinical record including age, sex, name, place of residence, and referring paper were recorded.

Using standard operating procedures (SOPs) for phlebotomy (according to WHO guidelines) from the vein of selected patient 3 ml of blood sample was obtained in a tube which contain Tri-potassium ethylene di amine tetra acetic acid (EDTA K3, Shanghai Orsin Medical Technology) as an anticoagulant. By gentle inversion, this was well mixed for blood complete analysis. EDTA blood for blood complete tests was placed at room temperature till the test was performed. All samples were processed by an automated cell counter using the Brand: Sysmex, Model: XP-100 (Kobe, Japan), following the manufacturer's operational guidelines. Also, by using the standard hematological method. The analysis of all specimens was made by manual method. Within half an hour of collection, the analysis of all samples was done.

Hb was determined by the cyan-met hemoglobin method also called Dropkin Method (Biozyme Diagnostic Division, Lahore, Pakistan). The percentage of PCV (hematocrit) was measured manually by filling plain capillary tube and sealing with Modeling clay and centrifuging (800 centrifuge machine, China) at 3,000 g for 5 minutes, tabulating the result using HCT reader. Also, WBCs were counted manually using improved Neubar counting chamber (Paul Marienfeld GmbH & Co. KG, Germany). The blood was diluted with WBC diluting fluid (Biozyme Diagnostic Division, Lahore, Pakistan) by using WBC pipette(1:20 ratio). WBC fluid contains glacial acetic acid which ruptureRBC and methylene which stain WBC..WBC s were counted in the four large square of the Neubar counting chamber and multiplied by 50.Similarly for RBC counting, one in two hundred dilution (1: 200) was made with RBC diluting fluid (Biozyme Diagnostic Division, Lahore, Pakistan) using RBC pipette. RBCs were counted in five small squares of the Neubar counting chamber and multiplied by 10000. Mean cell Hemoglobin (MCH), Mean cell volume (MCV) Mean Cell Hemoglobin Concentration (MCHC) was calculated from Hb and RBCs count, HCT and RBC count, and Hb and HCT count respectively. PLT was estimated by the thin blood film, stained with Giemsa stains (Haq Chemicals, Peshawar, Pakistan). The quality control was maintained by running three levels of controls daily and Levy-Jennings Control Chart. This study utilized both internal and external quality control procedures and obtained consistently satisfactory results.

All the data obtained were entered in Microsoft excel 2016, Data processing was performed by the Statistical Package for the Social Sciences (IBM SPSS Statistics, Version 21.0) statistical programmed, A p-value $\leq$ 0.05 was taken as statistically significant.

Results

The blood film reported by manual method showed that 56% of the subjects were normocytic normochromic, while the other 44% revealed different abnormal blood pictures like 2 (4%) normocytic-hypochromic, 1 (2%) microcytic-normochromic, 1 (2%) increased platelet distribution, 3 (6%) decreased platelet distribution, 1 (2%) target cells, 1 (2%) Polychromasia, 3 (6%) rouleaux formation, 1(2%) myelocytes and Blast cells and 9 (18 %) showed microcytic and hypochromic blood pictures. No reactive lymphocytes were found.

Moreover, when the mean and Standard Error values of the two procedures (automated and manual) were compared. There is statistically significant differences noted ($p < 0.0001$) among Hb%, Total Leucocyte Count (TLC), White Blood Cell Count (WBC), Red Blood Cell Count (RBC) and platelet count and correlated positively ($r = 0.974, 0.890, 0.761, 0.855$ and 0.910 respectively).

The test results also showed that Hb%, Total Leucocyte Count (TLC), White Blood Cells (WBC), Red Blood Cells (RBC), and PLT counts are positively correlated along the regression line between Automated hematology analyzer (Sysmex) and manual methods (figures 1-5) All the data points are homogeneously distributed along the regression line in all five test parameters. The slop and intercepts of regression lines are variable depending upon the nature of test parameters, but regression patterns remains identical in all cases.

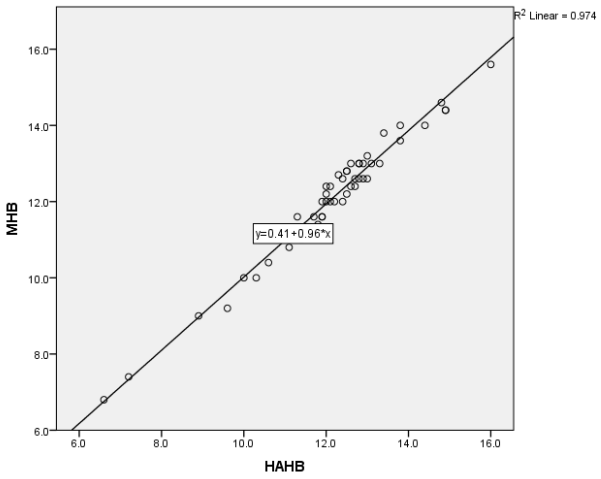

Figure I. Correlations of Hemoglobin (HB) results by Hematology analyzer and by manual methods.

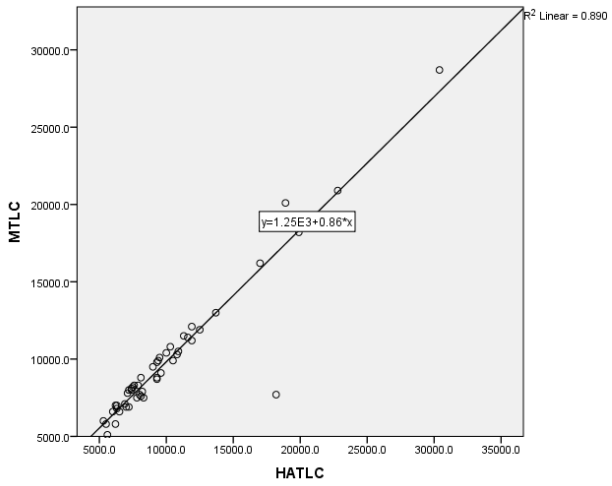

Figure II: Correlations of Total leucocytes count (TLC) results by Hematology analyzer and by manuals methods.

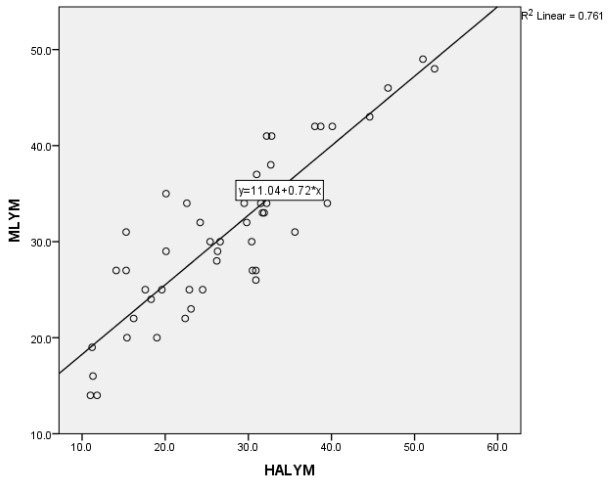

Figure III. Correlations of White Blood Cells (WBC) results by Hematology analyzer and by manuals methods.

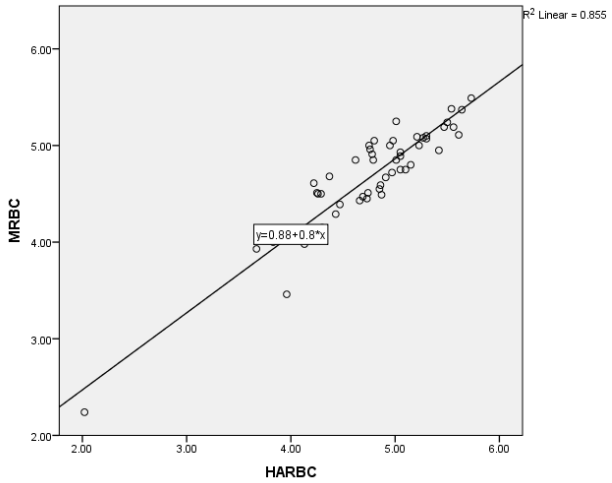

Figure IV. Correlations of Red Blood Cells (RBC) results by Hematology analyzer and by manuals methods.

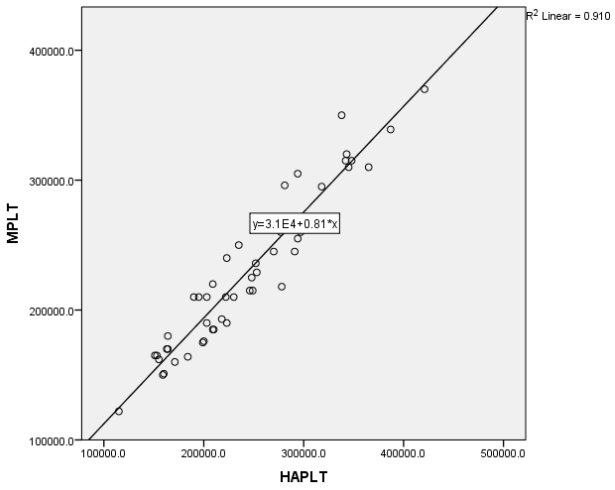

Figure V. Correlations of Platelet Count (PLT) results by Hematology analyzer and by manuals methods.

Discussion

The present study was aimed to conduct and correlate manual and automated hematology analyzer results of complete blood count. Automated blood counts are broadly accepted in routine clinical laboratories. The benefits of manual examination are the ability to identify significant cell types like teardrops, burr cells, schistocytes, target cells, sickle cells, and blast cells, etc. that are not assessable by hematology analyzer, but that produce flags on the automated results.[19]

In the present study, differential leucocyte count by automated method demonstrated “unflagged” samples with incomplete white cell count (<100 cells). This might probably be due to the inability of the automated analyzer to identify/differentiate the leucocytes, more particularly the immature cells.[7, 20] Also, this is in agreement with an earlier report by Takubo and Tatsuni [8] whose result demonstrated discrepancies in quality control (QC) survey in a manual leucocyte differential count which was attributed to poor differentiation of segmented neutrophils and band neutrophils.

The direct microscopic examination of stained blood film reported among the 50 subjects showed that 28 subjects (56%) were normocytic-normochromic, while the other 22 subjects (44%) showed different abnormal blood pictures which are very important in the diagnosis of different blood disorders. The imprecision in measurement of hemoglobin (Hb) and Hematocrit (HCT) by the manual method may produce variations in the Red blood cell (RBC) indices. This is best seen with mean cell hemoglobin concentration (MCHC) which may result in

misclassification of values for diagnosis of the anemia. This showed that the manual method still has some advantages over the automated methods, although slow and at times cumbersome.[11]

In addition, the mean and Standard Error values of the two procedures (automated and manual) were compared. There are statistically significant differences noted ($p < 0.0001$) among Hb%, Total Leucocyte Count (TLC), White Blood Cell Count (WBC), Red Blood Cell Count (RBC), and platelet count and correlated positively. This indicates that the automated hematology analyzer (Sysmex Xp-100) readings correlated well with the manual methods, this is in agreement with an earlier report by Atilola and McCarthy. [21, 22]

A good quality smear, thorough examination and proper interpretation in line with patient's clinical state should be ensured by the haemato-pathologist. Clinicians should be abreast with its clinical utility and proper application of the reports in the management of patients.

Conclusion

It is concluded from our study that the results of an automated hematology analyzer are more reliable than standard manual methods, even though the manual examination of blood smear provides extra diagnostic information. The findings of this study show that the automated hematology analyzer is suitable for screening purposes because it increases the turnaround time and reduces the labor cost. But to identify and differentiate different types of anemia and other blood diseases standard manual microscopic examination of the blood smear is still the gold standard, therefore standard manual method of blood should always be used to validate the automated methods.

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