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Verification and comparison of Sysmex KX-21N, Cell-Dyn 1800 And Horiba ABX Micros ES 60 automated hematology analyzers at Tikur Anbessa Specialized Hospital Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Henok Kebede, entitled: ***Verification and comparison of Sysmex KX-21N, Cell-Dyn 1800 And Horiba ABX Micros ES 60 automated hematology analyzers at Tikur Anbessa Specialized Hospital Addis Ababa, Ethiopia*** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology track) complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Abbreviations

AAU	Addis Ababa University
BD	Becton-Dickinson
CBC	Complete Blood Count
CLSI	Clinical Laboratory Standard Institute
CV	Coefficient of Variation
DC	Direct Current
DLC	Differential Leukocyte Count
EDTA	Ethylene Diamine Tetra Acetic Acid
FDA	Food and Drug Administration
HCT	Hematocrit
HGB	Hemoglobin
ICSH	International Council for Standardization in Hematology
LCD	Light Crystal Display
LED	Light Emitting Diode
LoA	Limits of Agreement
LYM %	Lymphocyte percentage
LYM #	Lymphocyte absolute number
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume

MPV	Mean Platelet Volume
MXD %	Mixed cells percentage
MXD #	Mixed cells absolute number
NCCLS	National Committee for Clinical Laboratory Standards
NEU %	Neutrophil percentage
NEU #	Neutrophil absolute number
PDW	Platelet Distribution Width
PI	Principal Investigator
PLCR	Platelet Large Cell Ratio
PLT	Platelet
QA	Quality Assurance
RBC	Red Blood Cell
RDW-CV	Red cell Distribution Width Coefficient of Variation
RDW-SD	Red cell Distribution Width Standard Deviation
RT	Room Temperature
SOP	Standard Operating Procedure
SPSS	Statistical Package for Social Sciences
TASH	Tikur Anbessa Specialized Hospital
WBC	White Blood Cell

Operational definitions

Carry over: Indicates, in percentage, how much a sample with high results may falsely raise results of a cytopenic sample subsequently analyzed.

Linearity: linear correlation between theoretical values and those observed in practice by means of the analysis of several dilutions of a certain sample.

Precision: closeness of agreement between measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions.

Agreement: Measurement of the extent to which the three methods give the same result.

Determination of agreement: If the two measurements lie mean$\pm$1.96 SD on Bland-Altman plot for 95% or above of cases we can say the two measurement agree with each other.

Limits of agreement: The mean difference $\pm$1.96 SD of differences.

Analyte: Parameter whose constituents are being identified and measured.

Flags: Written or displayed output intended to signal or attract attention. Flags are generated by the instrument to alert the operator to instrument malfunctions that occur during sample processing, or to data abnormalities detected during data analysis.

Correlation coefficient - A statistic that indicates the degree, to which two measurements are related, expressed as a value from -1.0 to +1.0, with +1.0 indicating that results are in total agreement, and -1.0 indicating that results are in opposites. A 0.0 value indicates that the two measurements are unrelated.

CBC includes:- WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, LYM%, MXD%, NEUT%, LYM#, MXD#, NEUT#, MPV, RDW, PDW, and PCT.

Major CBC parameters includes: - WBC, RBC, HGB, and PLT.

WBC category: Leukopenia (WBC<3.98x10^9/L), Normal, Leukocytosis (WBC>10.04 x10^9/L)

HGB category: anemia (HGB<12.2g/dl), Normal, High (HGB>17.5g/dl).

PLT category: Thrombocytopenia (PLT<150x10^9/L), Normal, Thrombocytosis (PLT>369x10^9/L)

Abstract

Background: Laboratory testing plays a crucial role in the detection, diagnosis and treatment of disease in patients. Analytical evaluation of a new analyzer or method should be done in order to confirm the declared specifications before its introduction into the routine use. Agreement of all automated hematology analyzers must be carried out in all laboratories. But, the practice of using automated analyzers interchangeably without evaluation is common in our country including Tikur Anbessa specialized hospital (TASH).

Objective: To verify performance and compare agreement of Cell-Dyn 1800, Sysmex KX-21N and Horiba ABX micros ES $_{60}$ hematology analyzers found in TASH.

Methods: A cross sectional study was conducted to verify performance and assess agreement between Cell-Dyn 1800, Sysmex KX-21N and Horiba ABX micros ES $_{60}$ hematology analyzers at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia. The study period was from February to May 2017. According to the CLSI guideline a total of 267 samples were analyzed in duplicates on the three analyzers to verify and compare them. Data cleaning and analysis were performed by Microsoft excel 2007 and SPSS version 20.

Result: We found that minimal carryover (< 0.5%) and excellent linearity for white blood cell, hemoglobin, red blood cell and platelet ($r > 0.999$). Precision was good for all major CBC parameters with % CV< 2.0. Agreement between most of the parameters of the analyzers lies in the limits of agreement for more than 95% of cases according to the Bland-Altman plot analysis. Pearson correlation and linear regression graph showed that the three analyzers were well correlated ($r > 0.900$) for major CBC parameters. The correlation of WBC differential-neutrophil and lymphocyte were excellent ($r > 0.920$). Poor correlation was observed in mixed cells ($r=0.005$) especially in the high levels between Horiba and Sysmex. There was also moderate correlation in mean platelet volume (MPV) between analyzers of Horiba and Sysmex ($r= 0.593$ and $r=0.572$) at normal and high levels respectively.

Conclusion: The overall performance of the three analyzers is excellent. They agree well with each other and can be used interchangeably with care in mixed cells and MPV.

Key words: Method comparison, performance evaluation, verification of hematology analyzers Sysmex KX-21, Horiba ABX micros ES 60, Cell-Dyn 1800, hematology.

1. Introduction

1.1 Background

Laboratory testing plays a crucial role in the detection, diagnosis and treatment of disease in patients. An estimated 70 % of all decisions regarding a patient's diagnosis and treatment, hospital admission and discharge are based on laboratory test results. As health care reform focuses on diagnosis and early intervention, timely and accurate laboratory results are necessary for the quality of patient care and for managing downstream costs. Laboratories practicing quality assurance program improperly and without a strong commitment cannot expect to maintain a satisfactory laboratory result. It is only with a complete quality assurance (QA) system you can know where you are and have the right to say so [1]. Automated hematology analyzers have taken a key place in hematology laboratories, because of their high analytical performances for blood cell count and their ability to flag abnormal cells [2].

Nowadays, the overwhelming majority of laboratory results in clinical laboratories are being generated by automated analyzers. Modern automated analyzers are highly sophisticated instruments which can produce a tremendous number of laboratory results in a very short time. As a result, the laboratory routine work has diminished significantly [3].

Even if these analyzers bring a significant change in different aspects of the laboratory, they could also produce inaccurate results. Thus, they should be evaluated against the specifications of the manufacturer claim to verify that whether the specific instrument meets the manufacturer's specifications at the working site [4].

Clinical Laboratory Standard Institute (CLSI) also recommends that the end-user laboratory should follow the principles and procedures of the manufacturer's validation protocol to verify that the manufacturer's stated performances are correct for the specific instrument at the working site [5].

After selection of new hematology analyzer, each laboratory must compare the new method with the one currently in use to see whether their measurements are indeed comparable. There are different statistical approaches to compare the new and the reference method. This analysis consists of paired measurements by the two methods and testing for the agreement of the two analytical methods [6].

The Cell-Dyn 1800 is a hematology analyzer with a throughput of 60 specimens per hour. It provides a basic blood count with a 3-part white blood cell differential. Due to its capacity, the Cell-Dyn 1800 is well suited in a small clinical laboratory as a primary analyzer or a backup analyzer in a medium-size clinical laboratory. Cell-Dyn 1800 uses two independent measurement methods; they are electrical impedance method for determining WBC, RBC and PLT and modified met hemoglobin method for determining HGB. During each instrument cycle, the sample is aspirated, diluted, and mixed before each parameter is measured. The sample used is a whole blood collected in an EDTA tube. The instrument aspirates 30 µl of patient sample [7].

The Sysmex KX-21 is an automatic multi-parameter blood cell counter for *in vitro* diagnostic use in clinical laboratories. The KX-21 processes approximately 60 samples per hour and displays on the LCD screen the particle distribution curves of WBC, RBC, and PLT along with data of 18 parameters including the 3-part differential as the analysis results. To assure easy sorting of abnormal samples in the laboratory, the instrument displays abnormal analysis data with abnormal marks attached on the LCD screen. Thus, displayed analysis data allows detecting those samples which are outside the tolerance and need further analysis and reconsideration [8].

The Horiba ABX micros ES 60 is a fully automated hematology analyzer used for *in-vitro* diagnostic testing of whole blood specimens. The rate of determination is approximately 55 samples per hour in the optimum configuration. The system is totally automated including the cap-piercing of the sample tube, an internal dilution system, and a graphic printer for recording all test results including flags and graphics. Well mixed whole blood specimens collected in EDTA anti-coagulant and run within eight hours after collection provide the most accurate results for all parameters. The RBCs, PLTs and WBCs are measured by an electronic impedance principle. The hemoglobin measurement is based on photometry [9].

1.2 Statement of the problem

The accurate automated enumeration and identification of WBC, HGB, PLT, and RBC is an important and often challenging aspect of the complete blood count (CBC). Confirmation of accuracy of any of these parameters is time consuming, costly and the delay in reporting may have a negative impact on patient management [10].

Currently the laboratories at the various hospitals in Ethiopia use hematology analyzer systems from different companies. Tikur Anbessa specialized hospital (TASH), for example, has different models of 3-part analyzers from three companies. Whenever a new analyzer is introduced, it was supposed to be evaluated for its performance against the company's requirements. Determining agreement with existing analyzers also ensures use of the analyzers for patient management interchangeably [11-13].

However, in TASH, where patients from all over the country are referred for the clinical management of hematological disorders, different analyzers are being used interchangeably without prior evaluation.

The hospital has 5 hematology analyzers. Among these, 4 of them are functional. Most of these analyzers are never been evaluated for their agreements with each other and against the manufacturers' specifications. As tertiary level referral and teaching hospital, all the machines must be evaluated for their performances and agreements. Tikur Anbessa specialized hospital hematology laboratory serves on average 350-450 patients daily [14]. From these patients, almost all patients are coming for hematology laboratory for CBC diagnosis and the analysis is done by different automated analyzers interchangeably. Sometimes there is a variation on CBC results from one analyzer to another. This may cause loss of confidence of the staffs on the analyzers. So, to overcome this variation and to give quality and reliable results to the patients confidently, these automated analyzers must be evaluated and compared for their agreements. This study, therefore, tried to confirm the performances of the three analyzers by the data they generate against the company's specification. Finally agreements of the three analyzers were also compared with each other.

1.3 Significance of the study

Considering that CBC is one of the most frequently ordered laboratory test in medical appointments and a fundamental examination to detect any pathology in the blood stream especially in cancer patients, the validation of results from the hematology analyzers becomes an item of special importance. The result of this research is important especially for reflecting the quality of laboratory work and directly influencing patients' clinical management in the hospital. It indicates the quality of CBC results provided by TASH laboratories and ensures that the obtained results are the most reliable for clinicians and patients. The findings will give confidence for laboratory personnel whether or not to use the three instruments interchangeably. The staffs will gain satisfaction by the result the analyzers generate. It also helps managers to take corrective actions if there is any discrepancy in using these machines. Finally the results will also be a base line for further researches.

2. Literature Review

Different hematological analyzers which utilize the pioneer Coulter principle (Electrical Impedance) and flow cytometry for cell sizing and counting exist globally [15]. Automated analyzers play an important role in the operation of medical laboratory especially in the disciplines of chemical pathology and hematology; and to a lesser extent, clinical microbiology and infection. Hence, laboratories are expected to carry out thorough verification of their auto analyzers as part of method validation before they are put into service [16]. Several studies tried to evaluate performance of the different analyzers as per the Clinical Laboratory Standard Institute (CLSI) guidelines [17]. Accordingly, a study conducted in China in 2001 has evaluated the performance of SE-9500 according to guidelines published by the International Committee for Standardization in Hematology (ICSH). The results demonstrated minimal carryover (< 0.01%) and excellent linearity for WBC, RBC, HGB and PLT ($r > 0.995$). Imprecision was generally acceptable for all CBC parameters (CV < 5%). Correlation between the SE-9500 and XN-1000 was excellent ($r > 0.970$) for all the major CBC parameters (WBC, RBC, HGB, and PLT). Their results indicate that the SE-9500 was an excellent tool for routine hematological examination [18].

A study conducted in USA at Saint Louis children's Hospital by Langford *et al* in 2003 on Sysmex XT-2000i automated hematology analyzer evaluated precision, linearity, carryover, method comparison and correlation. Linearity was up to 410 $\times 10^3/\mu l$ for WBC count and up to 6560 $\times 10^3/\mu l$ for PLT counts. The carryover was of minimal magnitude which is less than 1% for all determinations [19].

A study which was done by Fernandez *et al* in 2001 at Florida demonstrated an excellent performance of automated differential count as compared to the manual differential count method. In their study, Fernandez *et al* were evaluating Coulter LH-750 hematology analyzer against the Gen S system analyzer by regression analysis and the mean differences between methods. A total of 383 samples were analyzed. WBC, RBC, HGB, MCV, PLT, and RDW showed correlation of > 0.980 and MPV showed a correlation coefficient of > 0.960. The correlation coefficient for WBC was > 0.920 while for neutrophils, lymphocytes, and eosinophil the correlation coefficient values were > 0.990; for monocytes > 0.970 and for basophils > 0.810.

Coulter LH-750 hematology analyzer provides accurate and reliable results over a wide range of normal and abnormal sample types [20].

Veillon *et al* in 2000 at Louisiana State University Health Sciences Center compared three hematology analyzers: Coulter Gen S, Abbott Cell-Dyn 4000 and Bayer Advia 120. The correlation coefficient which is obtained by the study lied within the acceptable range [21].

A study was done in Peking University Hospital, Beijing, China in 2013 by Rui Q *et al* to verify the performance of Beckman Coulter LH-750, Mindray BC-5800 and Sysmex XE-2100 in clinical laboratories. They used a total of 15 fresh whole blood specimens. Except platelet inter day precision of BC-5800 analyzers, the rest complied with specification of each analyzer. Analytical measuring intervals of WBC and PLT of LH-750 and BC- 5800 were wider than those of XE-2100. The performance verification of XE-2100, LH-750 and BC-5800 showed excellent results [22].

The performance of Cell-Dyn 1800 hematology analyzer was evaluated by Ryhanen *et al* in Finland using commercial controls of different levels for precision. Whereas, high patient samples were used for carryover determination under routine conditions. The result showed that the precision of Cell-Dyn 1800 was very good in that the highest CV was 2.8% for low level control of platelet and the smallest CV was 0.3% for low level MCV and its carryover was 0.00% for WBC and 0.37% for platelet. The authors concluded that the Cell-Dyn 1800 fulfilled the specifications set by the manufacturer [23].

Ghys *et al* in Belgium evaluated the performance of Sysmex XS-1000i instrument in 2008 according to CLSI and ICSH guidelines. Precision, carry-over and linearity were determined using a total of 700 patient samples and results from the Sysmex XS-1000i were compared with those from Sysmex XE-2100, Abbott Cell Dyn 4000 and also with manual reference leukocyte differential. In all performance determinations and comparisons, the Sysmex XS-1000i demonstrated good analytical performance, is able to generate CBC with five part differential on low blood volumes and has considerable back-up capacity [24].

Another study was conducted by Lehto T and Hedberg P on the performance evaluation of Abbott Cell-Dyn Ruby in Finland. The analyzer was evaluated by comparing its results with the results obtained from Cell-Dyn sapphire. Precision, linearity, and carryover between patient samples were also assessed. Precision was good at all levels for CBC parameters. In the

comparative study, the Cell-Dyn Ruby results showed a good correlation ($R^2 > 0.98$) with Cell-Dyn sapphire for the CBC parameters. In the differential leucocyte count (DLC), the precision was good for all parameters except basophil count. The comparison of neutrophils, lymphocytes, and eosinophil showed excellent correlations ($R^2 > 0.97$). The authors concluded that the results of the evaluation showed good performance of Cell-Dyn Ruby and compared well with Cell-Dyn sapphire [25].

Performance evaluation of Abbott Cell-Dyn and Sysmex XT-2000i hematology analyzers was conducted in Germany in 2011 by Leers MPG and his colleagues. Inter-instrument agreement for most parameters was good, although small numbers of discrepancies were observed. Systematic biases were found for MCV, PLT, and MPV. WBC subpopulation counts were in good agreement with no major outliers. They finally concluded that the Sysmex XT-2000i and Abbott Cell-Dyn analyzers had broadly comparable analytical performance [26].

Another study conducted in London in 2011 evaluated performance of the Celltac F hematology analyzer and compares it with Sysmex XE-2100. They found precision, linearity, and carry-over met manufacturers claim. Comparisons were excellent for WBC, RBC, HGB and platelets ($r > 0.980$). All other red cell and platelet parameters showed good correlation with the XE-2100 ($r > 0.930$) except MCHC. The differential was comparable to the XE-2100 for neutrophils, lymphocytes, and eosinophil and acceptable for monocytes. Finally they concluded that the Celltac F showed comparable analytical performance to the XE-2100 for the parameters assessed [27].

A study in a university hospital in Belgium carried out by Guerti *et al* in 2009 evaluated Pentra $60C^+$ hematology analyzer and assessed its agreement with the Advia 2120. First they assessed reproducibility and carry-over to evaluate the analytical performance. They used Pearson correlation and regression analysis to evaluate agreement with the Advia 2120. Reproducibility was excellent for the majority of CBC and 5 differential count parameters. Their data showed very good correlation for most CBC parameters. Lower correlation coefficients were observed for RDW, MCV and MPV. As compared to the Advia 2120, the 5-differential count performed very well. Agreement was poor for low level eosinophil and basophil counts [28].

Another study was carried out by Shu G *et al* in 2013 in a university hospital in China to evaluate BC-3600 auto hematology analyzer and compare it with Mindray BC-3200 3-part

differential and Sysmex XE-2100 5-part differential hematology analyzer in the hematology laboratory. The BC-3600 was evaluated according to guidelines published by CLSI, ICSH, and Department of Food and Drug Administration (FDA). They found that there were no background, minimal carryover (< 0.5%) and excellent linearity for white blood cell, hemoglobin, red blood cell, and platelet counts ($r > 0.999$). Precision was good at all levels for the routine CBC parameters: CV % being ≤ 2.0 except for platelet count at the low level with CV % of ≤ 5.0 % and WBC at the low level with CV % of < 3.0%. Correlation between the BC-3600 and BC-3200, XE-2100 were excellent ($r > 0.990$) for all major CBC parameters. Finally, they concluded that the overall performance of the BC-3600 was excellent and compared well with that of BC-3200 and XE-2100 [29].

An earlier study conducted in Belgium in 1991 compared the automated hematology analyzer Sysmex NE-8000 with the Technicon H. One hundred seventy samples from the daily routine workload comprising specimens from healthy adults and patients with various ailments were analyzed on the Sysmex NE-8000 and the Technicon H. Comparison of the results from the two blood cell counters showed good correlation ($r > 0.900$) for the white blood cell count, hemoglobin, hematocrit and platelet. For the red blood cell count and mean cellular volume, the correlation coefficients were greater than 0.8. In the differential leukocyte count, Sysmex NE-8000 and Technicon H showed good correlations for the neutrophil ($r = 0.953$), lymphocyte ($r = 0.763$), and eosinophil ($r = 0.904$). Correlation coefficients were very low for monocyte ($r = 0.130$) and basophil ($r = 0.006$). They concluded, the Sysmex NE-8000 was well suited for routine blood cell analysis and was a valuable tool for the diagnosis and screening of various hematological abnormalities [30].

The Carle Clinic Laboratory, Urbana, USA evaluated the performance of the XE-2100 and compared it to the SE-9500TM and R-3000TM in 1999. Carryover, reproducibility, Linearity, and correlation were performed. Carryover and reproducibility were well within the manufacturer specifications and the linearity met the specified range for all parameters studied. The XE-2100 showed excellent correlation with the results from the SE-9500 for WBC, Neutrophil %, Lymphocyte %, Monocyte %, eosinophil %, RBC, HGB, HCT, MCV, MCH, MCHC, and PLT. The overall performance of the XE-2100 was very good and compared well to the SE-9500 and R-3000TM [31].

A study by Meintker and his colleagues in USA in 2013 compared 4 modern hematology analyzers namely -Abbott Sapphire, Siemens Advia 120, Sysmex XE-2100, and Beckman Coulter DxH 800. The comparison includes CBC and differential leukocyte count in a total of 202 samples from hematology patients and normal controls. The analyzers exhibited very good correlation for CBC parameters. Neutrophils and eosinophils also showed very good correlations, whereas lymphocytes and monocytes correlated fairly. The Advia 120 displayed notably lower measurements for both parameters, which is attributable to classification of some events as large unstained cells. Basophil counts were unreliable with all analyzers [32].

The performance of Mindray BC-6800 was evaluated and compared with Advia 2120 and LH-750 in 2013 in Anyang, Korea. Specimens from 100 healthy controls and 95 patients were used. They performed precision and correlation studies of CBC and differentials leucocyte count. The coefficients of variations (CVs) of precision were < 2 % for most CBC parameters and < 5 % for neutrophil and eosinophil counts. The results obtained using the BC-6800 were well correlated with those of the Advia 2120 (Siemens, USA) and LH-750 (Beckman Coulter Corporation, USA). The correlation coefficients (r) were > 0.980 for CBC except erythrocyte indices and > 0.950 for differentials leucocyte count except monocyte and basophil. They concluded that the BC-6800 showed good precision and correlation with pre-existing hematology analyzers. Their study demonstrated that the BC-6800 hematology analyzer exhibited suitable performance and is helpful in routine laboratories [33].

A similar study was conducted by Young L *et al* in Korea in 2015 on the evaluation of Sysmex KN-2000. They evaluated the analytical performance and low white blood cell mode of the KN-2000. Precision, linearity, and carryover were evaluated for the analyzer. CBC and DLC parameters were compared between KN-2000 and XE-2100 (Sysmex) using 200 samples from normal controls and patients. For 80 samples with a WBC count < 1.5×10^9 cells/L, the low WBC mode was evaluated by comparing the automated count. The coefficients of variation of precision were < 5 % for most CBC parameters and < 10 % for DLC. All results obtained with the KN-2000 showed good correlation with those obtained from the XE-2100. The correlation coefficients (r) were >0.980 for all CBC parameters except MCHC, MPV, and PDW, and > 0.990 for DLC except monocytes and basophils. The low WBC mode provided accurate counts for neutrophils and lymphocytes with r > 0.930 for samples with a WBC count of 0.1-1.5×10^9 cells/L. They concluded KN-2000 showed good analytical performance and correlated with the

existing model, the XE-2100. The KN-2000 provided accurate results for DLC in samples with a WBC count of 0.1-1.5×10^9 cells/L [34].

Another study was conducted in Royal Bolton, UK hospital in 2013 on the evaluation of the QBC Star centrifugal three-part differential hematology system. The CBC performance was evaluated according to the National Committee for Clinical Laboratory Standards (NCCLS) document H20-A. Imprecision, correlation and linearity studies all showed excellent results. Overall, the hemoglobin, hematocrit, white cell count and platelet count parameters showed excellent correlation. MCHC results showed poor comparability. The DLC parameters showed good correlation within certain clinically significant limits. Imprecision for hemoglobin, hematocrit, WBC, MCHC and platelet count was considered acceptable [35].

Taken together, the literatures reviewed above indicate that different versions of hematology analyzers perform well against the manufacturers' specifications as well as agreed well against each other for the major CBC parameters. However, deviations are noted for differential counts and other RBC and platelet parameters. As to my knowledge, there are no published researches done on analyzers verification and comparison in Africa particularly in Ethiopia. Two unpublished studies comparing Cell-Dyn 1800 (3 part differential) versus Sysmex XT-2000i (5 part differential) by Lulit Hailu at TASH [36] and Sysmex KX-21(3 part differential) versus Cell-Dyn 1800 (3 part differential) by Tibebe Adinew [37] at St Paul's hospital revealed similar results. Verification study of Sysmex KX-21 versus Cell-Dyn 1800 by Tibebe Adinew gave acceptable results. As shown in the above literatures, the verification and comparison studies done on different models of hematological analyzers in different countries gave varying conclusions in terms of recommending a certain machine. Thus, generating more evidence in our set up is of importance.

3. Objectives

3.1 General objective

To verify performance and compare agreement between Cell-Dyn 1800, Sysmex KX-21N and Horiba ABX micros ES $_{60}$ hematology analyzers at Tikur Anbessa specialized hospital Addis Ababa, Ethiopia.

3.2 Specific objectives

- To assess linearity of Cell-Dyn 1800, Sysmex KX-21N and Horiba ABX micros ES $_{60}$
- To determine carry-over of Cell-Dyn 1800, Sysmex KX-21N and Horiba ABX micros ES$_{60}$
- To evaluate precision of Cell-Dyn 1800, Sysmex KX-21N and Horiba ABX micros ES $_{60}$
- To compare Cell-Dyn 1800, Sysmex KX-21N and Horiba ABX micros ES $_{60}$ hematology analyzers by WBC, HGB, and PLT categories (Low, Normal, High)

4. Hypothesis

The three instruments agree with each other and comply with the manufacturers' specifications with no difference amongst them.

5. Materials and Methods

5.1 Study area

This study was conducted at Tikur Anbessa specialized hospital which is located in Lideta sub city. The emergency department of the hospital serves around 80,000 patients per year. It is the largest specialized referral hospital in Ethiopia, with over 700 beds and serves as the training center for undergraduate and postgraduate medical students, dentists, nurses, midwives, pharmacists, medical laboratory technology, radiology technology, and others who shoulder responsibilities to alleviate the health problems of the community and the country at large [14]. The hospital had about 201 staff physicians, >10 pathologists, 5 hematologists, 627 nurses, and >115 other health professionals dedicated to provide the health care services [38].

5.2 Study design and period

A hospital based cross sectional study was conducted at Tikur Anbessa specialized hospital from February to May 2017.

5.3 Sample

5.3.1 Source Sample

All blood samples that were collected for CBC analysis using 5 ml EDTA Tubes.

5.3.2 Sample acceptance criteria

All blood samples that meet the requirements for specimen acceptance criteria according to the laboratory SOP.

5.3.3 Sample rejection criteria

- Clotted sample
- Hemolyzed sample
- Inadequate sample
- Overfilled sample
- Old sample

5.4 Study variables

5.4.1 Dependent variables

Precision, carry-over, linearity of the three analyzers and agreement between them.

5.4.2 Independent variables

Hematological parameters at various levels (Low, Normal, High): WBC, RBC, PLT, HGB, Granulocyte % & #, Lymphocyte % & #, Mixed cells % & # and analyzer types.

5.5 Measurement and data collection

5.5.1 Sample size determination

Sample size was determined according to the Clinical and Laboratory Standard Institute (CLSI) guideline. The CLSI guideline recommends to follow the manufacturer's principles and procedures for method evaluations. For the agreement study of the three analyzers, the CLSI 2012 guideline was followed. The guideline recommends using a minimum of 40 samples; accordingly for this study a total of 240 samples were analyzed in duplicates on each analyzer.

5.5.2 Sampling method

Convenient sampling method was used to collect data from the study site.

5.5.3 Data collection procedure

Blood samples were collected using 5ml EDTA tubes and transported to the hematology laboratory as soon as possible. Laboratory technologists and phlebotomists at Tikur Anbessa specialized hospital collected the blood according to the blood collection SOP of the laboratory under the supervision of the principal investigator (PI).

5.5.4 Laboratory analysis

The collected samples were analyzed for all the hematological parameters aimed to be assessed in this study as per the analyzers' manuals and procedures. Accordingly for reproducibility determination, one sample with all parameters within the normal range and with no flag was utilized. For linearity verification, samples whose platelet, RBC, hemoglobin and WBC values can cover the high range of the analytical measuring interval were used. High value samples for hemoglobin, RBC, WBC and Platelet parameters were used for carryover verification. For

agreement study CBC was performed in duplicates on each analyzer by the PI. The samples were selected conveniently from leftover samples that fulfill the required sample quality criteria.

5.5.4.1 Principle of Cell-Dyn 1800 hematology analyzer

The Cell-Dyn 1800 Hematology analyzer uses two independent measurement methods; they are:

- Electrical impedance method is used for determining WBC, RBC, and PLT count. As the cells pass through the aperture of the van Behrens transducer, a change in electrical resistance occurs generating an equivalent voltage pulse. The number of pulses sensed during each cycle corresponds to the number of WBC, RBC and PLT counted. The amplitude of each pulse is essentially proportional to the cell volume.
- Modified met hemoglobin method for determining HGB through photometric measurement.

After the WBCs have been counted and sized, the remainder of the lysed dilutions transferred to the hemoglobin (HGB) flow cell assembly. In the flow cell, the Cell-Dyn 1800 measures the ability of the dilution to absorb light at a wavelength of 540nm. The absorbed light is proportional to the concentration of hemoglobin.

Cell-Dyn 1800 (Abbott, USA)

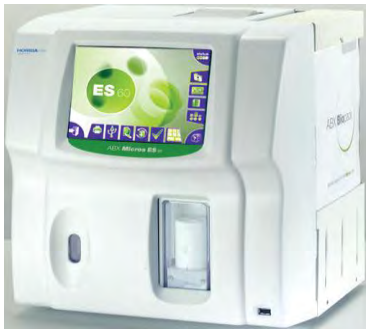
5.5.4.2 Principle of Sysmex KX-21N hematology analyzer

The KX-21 performs speedy and accurate analysis of 18 parameters including a 3-part WBC differential plus histograms for RBC, PLT and WBC in blood (WBC, LYM%, MXD%, NEUT%, LYM#, MXD#, NEUT#, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, PDW, MPV, and P-LCR). The KX-21 employs three detector blocks and two kinds of reagents for blood analysis. The WBC count is measured by the WBC detector block using the Direct current (DC) detection method. The RBC count and platelets are taken by the RBC detector block, also using the DC detection method. The HGB detector block measures the hemoglobin concentration using the non-cyanide hemoglobin method.

Sysmex KX-21N™ (Sysmex Corporation, Kobe, Japan)

5.5.4.3 Principle of Horiba ABX micros ES $_{60}$ hematology analyzer

The RBCs, PLTs and WBCs are measured by an electronic impedance principle. This means that an electronic field is generated around the micro-aperture in which the blood cells pass through. The cells create a resistance in the electronic field as they pass through the calibrated micro-aperture. This in turn causes an electronic pulse to be generated which is amplified, measured, and then mathematically calculated to create a numerical value. The hemoglobin measurement is based on a start-up cycle. This cycle includes a hemoglobin blank test sequence which includes hemoglobin blank measurements. Each analysis cycle run after start-up also has a HGB blank measurement which is compared to the initial start-up HGB blank. Each analysis cycle run thereafter compares the HGB blank reading to the previous cycle HGB blank reading.



Horiba ABX micros ES60 (Horiba international corporation, France)

5.5.4.4. Determination of Precision, Carryover and Linearity

Precision was determined by using one sample with all parameters within the normal range and with no flag and analyzed 20 times. Then mean, standard deviation (SD) and coefficient of variation (CV) were calculated.

Carryover was determined by analyzing a high patient sample followed by 3 background counts. Then by subtracting background run 3 from background run 1 and dividing this to the difference of high sample run 3 to background run 3 and multiplying the whole result by 100 and the result is expressed as percentage.

Linearity was determined by taking samples whose platelet, RBC, hemoglobin and WBC values can cover the high range of the analytical measuring interval. The means of the measured results were plotted on the x-axis and the expected results were plotted on the y-axis. Then the reportable range was assessed by linear regression statistics.

5.6 Data Quality Assurance

5.6.1 Pre-analytical

Patient identification and labeling was made with great care and samples were properly collected and transported without delay. Clotted samples, wrong samples (e.g. CBC in coagulation profile vial) and others that were mentioned in the sample rejection criteria list were also controlled in this stage of quality assurance.

5.6.2 Analytical

Three levels of commercially prepared hematology cell controls (Normal, Low and High) for the respective analyzers were run. Analysis was performed by following standard operating procedure (SOP) after running and passing of these levels of controls. The principal investigator performed all the analysis with close supervision from the advisors.

5.6.3 Post-analytical

For avoiding any clerical error, printout results that were generated by the analyzers were used. No results from the screen of the analyzers were recorded by hand. Then the results were documented and recorded using SPSS version 20 with great care.

5.7 Data analysis and interpretation

Data was collected through hard copy (print out) from all the analyzers, entered and analyzed using both excel 2007 (Microsoft Corporation, USA) and SPSS version 20 (SPSS INC, Chicago, IL, USA). Standard deviation, coefficient of variation and correlation coefficient were used to compare continuous variables. T-test was used to see difference between measurements in the analyzers. Bland-Altman plot was used to assess agreements of the three analyzers. If the coefficient of variation for precision and carry over tests fulfills the manufacturer's specification for each analyzer, we could say that the precision and carry over met the company's specification and if a straight line obtained between expected and observed values after serial dilution of the selected samples in case of linearity, we could say that the linearity test met the manufacturer's specifications. Linear regression graph was also used to see the correlation between the two measurements. In the linear regression graph, if most of the points lie on straight line and r values close to or exactly 1 we could say that the two measurements had excellent correlation and if the graph was not straight line and r value became zero, the two measurements were not correlated well and if r value far from 1 and close to zero we could say there was poor

correlation between the analyzers. Figures and tables were also used for the description of the different data. In the Bland-Altman plot, the differences in hematological parameters between two methods were plotted against mean values. Agreement was considered acceptable when the difference is lying between mean $\pm$ two standard deviation (Mean$\pm$1.96SD) for 95 % and above of cases. P-value < 0.05 was considered statistically significant.

5.8 Ethical considerations

Before the research work, ethical clearance was obtained from research ethical committee of Addis Ababa University, Department of Medical Laboratory Sciences to use left-over blood samples collected for routine examination. Then the study was commenced after getting approval from the ethical committees.

5.9 Dissemination of the result

The result of this study will be communicated to the hospital for appropriate action. The thesis will be submitted to the Department of Medical Laboratory Sciences. Information will also be presented to the medical and scientific community at different conferences. Manuscript will be submitted to peer reviewed journals for possible publication.

6. Result

Total samples used in this study were two hundred sixty seven (267); from these, 139 (52.1%) were from females and 128 (47.9%) were from males. Majority of the study participants were within the age range of 25-44 (Table 1). From the total samples, two hundred forty (240) were used for agreement tests, three (3) samples (at normal level for each parameter) was used for precision (within sample), twelve (12) samples (high levels for each parameter) were used for linearity study and twelve (12) samples (high levels for each parameter) were used for carryover study. For precision study, samples were selected based on the recommendation of the manufacturer, which is a sample where all its parameters lay in the normal range. For linearity and carryover verifications, samples were manipulated in order to get the best matrix which is needed for the studies.

Table 1: Demographic characteristic of study participants in verification and agreement studies.

Variable	Number	Percent
Sex		
Male	128	47.9
Female	139	52.1
Age group		
16-24	40	14.9
25-44	139	52.2
>44	88	32.9

6.1 Precision (reproducibility) verification

The objective of this test was to determine the analyzer capacity to reproduce the results for a certain parameter in a given sample. Analysis was carried out in normal samples by the same operator, within a short period of time, same location and with the same reagents. In order to fulfill the manufacturer's recommendation, sample to the analytical ranges of normal values and

no flags was used. The prepared sample was analyzed for 20 consecutive times in Cell-Dyn 1800, Sysmex KX-21 and Horiba ABX micros hematology analyzers. The mean, standard deviation (SD) and coefficient of variations (CVs) were calculated for each parameter. Before samples were analyzed, controls were run and checked for their passing. Prior to the analysis, samples were homogenized and inverted 20 times. Table 2 summarizes the calculated coefficient of variation values of Cell-Dyn 1800 for the directly measured parameters. As shown in the table, the precision (reproducibility) values of the directly measured parameters lie within the specification of the manufacturer.

Table 2: Within sample precision of Cell-Dyn 1800 hematology analyzer for directly measured parameters at TASH; Addis Ababa, May 2017.

Parameter*	Mean	Standard deviation(SD)	Coefficient of variation (%CV) (actual)	Manufacturer's Specifications(CV%,95% confident limit)
WBC	5.10	0.11	2.2	≤2.5%
RBC	4.44	0.04	0.8	≤1.7%
HGB	13.80	0.11	0.9	≤1.2%
MCV	93.40	0.50	0.6	≤1.5%
PLT	298.20	8.50	2.9	≤6.0%
MPV	9.40	0.22	2.8	≤6.0%

***WBC $x10^9$/L; RBC $x10^{12}$/L; PLT $x10^9$/L; HGB gm/dl; MCV fl, MPV fl**

On the other hand, Cell-Dyn 1800 Precision specifications for the WBC differential parameters are given as a range of tolerance for each of the WBC subpopulations. The specification is based on running within-sample precision runs of N=20 fresh whole blood. The result tolerances for the WBC differential parameters were determined by obtaining the difference of individual results in N=20 determinations of the same sample from the mean of the determinations. Thus, based on the results and range of tolerances given by the manufacturer, the three part differential values LYM%, MXD%, and GRAN% for all runs were within the tolerance limit of the manufacturer.

Precision values of Sysmex KX-21 analyzer for WBC, RBC, PLT, HGB, MCV, MPV and WBC subpopulations as measured by the coefficient of variation are shown in Table 3. From the table we can easily understand that all the parameters of fresh whole blood that were found within the reference range and tested for reproducibility study had coefficient of variations (% CV) which lay within the manufacturer specifications.

Table 3: Within sample precision of Sysmex KX-21 hematology analyzer for directly measured parameters and WBC subpopulations at TASH, Addis Ababa, May 2017.

Parameters*	Means	Standard deviation (SD)	Coefficient of Variation (% CV) (actual)	Manufacturers Specifications (% CV)
WBC	5.36	0.09	1.7	≤3.5
RBC	4.37	0.05	1.1	≤2.0
HGB	13.81	0.13	1.0	≤1.5
PLT	336.70	9.77	2.9	≤6.0
MCV	89.90	0.45	0.5	≤2.0
MPV	7.22	0.09	1.2	≤5.0
LYM%	36.38	1.83	5.0	≤15.0
MXD%	16.40	2.16	13.3	≤30.0
GRAN%	47.27	1.11	2.4	≤15.0

***WBC x10^9/L; RBC x10^{12}/L; PLT x10^9/L; HGB gm/dl; MCV fl, MCV fl**

On the other hand, precision values of Horiba analyzer for WBC, RBC, PLT, HGB, and HCT as measured by the coefficient of variation are shown in Table 4. From the table, all the parameters of fresh whole blood that were found within the reference range and tested for reproducibility study had coefficient of variations (% CV) which lay within the manufacturer specifications.

Table 4: Within sample precision of Horiba ES60 hematology analyzer for directly measured parameters and WBC subpopulations at TASH, Addis Ababa, May 2017.

Parameters*	Means	Standard deviation (SD)	Coefficientof variation(%CV) (actual)	Manufacturers specifications (% CV)
WBC	4.90	0.14	2.9	<3.0%
RBC	4.40	0.12	2.8	<3.0%
HGB	13.97	0.22	1.6	<2%
HCT	36.70	0.67	1.8	<3.0%
MCV	86.25	0.44	0.5	<2%
PLT	250.00	12.24	4.9	<5%
LYM%	34.73	0.97	2.8	<5%
MXD%	9.12	0.51	5.6	<10%
GRAN%	56.14	1.02	1.8	<5%

***WBC x10^9/L; RBC x10^{12}/L; PLT x10^9/L; HGB gm/dl; HCT%**

6.2 Carryover verification

Table 5 summarizes carryover result of Cell-Dyn 1800. It indicates, in percentage, how much a sample with high results may falsely raise results of a cytopenic sample that is analyzed subsequently. This evaluation was performed in triplicate of a sample with high concentration of the analyte (H1, H2 and H3); with a subsequent analysis in triplicate of a background count (B1, B2, B3). The percentage of carryover for each parameter was calculated with the following formula: carryover (%) = |B1 - B3|/ (H3-B3) × 100. Where B1 and B3 were the results of the first and the third measurement of background counts respectively and H3 was the third measurement of the sample with high concentration. For determination of carryover using the Cell-Dyn 1800, the samples used were based on the manufacturer's specifications of high target value and the result showed that all the parameters for carryover study were within the acceptable range of the manufacturer's specification.

Table 5: Carryover of Cell-Dyn 1800 hematology analyzer for directly measured parameters at TASH, Addis Ababa, May 2017.

Parameters	H3*	B1	B3	% Carry-over (actual)	% Carry-over (claim)
WBC	98,500/mm^3	0.1	0.1	0%	<1.0%
RBC	6.78M/mm^3	0	0	0%	<0.5%
HGB	22.1g/dl	0.1	0	0.45%	<0.8%
PLT	970,000/mm^3	0	0	0%	<1.0%

***M=10^6**

For carryover determination of Sysmex KX-21, all the samples were taken based on the manufacturer specification of high target value and the result revealed that all the parameters tested for carryover study lay within the acceptable range of the manufacturer specification, (Table 6).

Table 6: Carryover of Sysmex KX-21 hematology analyzer for directly measured parameters at TASH, Addis Ababa, May 2017.

Parameters	H3*	B1	B3	%Carry-over (actual)	% Carry-over (claim)
WBC	96,200/mm^3	0	0	0%	<3%
RBC	6.14M/mm^3	0.02	0.01	0.16%	<1.5%
HGB	22.7g/dl	0	0	0%	<1.5%
PLT	985,000/mm^3	0	0	0%	<5%

***M=10^6**

On the other hand, for carryover determination of Horiba ES60, all the samples were taken based on the manufacturer specification of high target value and the result revealed that all the parameters tested for carryover study lay within the acceptable range of the manufacturer specification, (Table 7).

Table 7: Carryover of Horiba ES60 hematology analyzer for directly measured parameters at TASH, Addis Ababa, May 2017

Parameters	H3*	B1	B3	% Carry-over (actual)	%Carry-over(claim)
WBC	98,900/mm^3	0.1	0.1	0%	<0.5%
RBC	6.69M/mm^3	0.01	0	0.14%	<0.5%
HGB	22.8g/dl	0	0	0%	<0.5%
PLT	1,804,000/mm^3	0	0	0%	<2%

***M=10^6**

6.3 Linearity Verification

A quantitative analytical method is said to be linear when measured results from a series of sample solutions are directly proportional to the concentration in the test specimens and a straight line can be used to characterize the relationship between measured results and the concentrations of the analytes. In this study WBC, RBC, HGB, and PLT were tested for linearity on each analyzer. If the difference between the predicted and measured values is less than the allowable error for each specimen, then the method is said to be linear.

One high target value sample that covers most of the reportable ranges was used to test for linearity verification. This high target value sample was diluted by the analyzers own diluent solution into five dilutions that are equidistant to each other i.e. 20%, 40%, 60%, 80% and 100% of the undiluted sample and measured twice on each analyzer. The average of the two was taken as mean measured value.

6.3.1 Linearity verification of cell-Dyn 1800 hematology analyzer

Linearity range verified for high target value of WBC count of 98,900/µl presented with an excellent correlation coefficient of 1.000 between expected and observed values with a regression equation of y=0.903+0.991x. All the measured values lie within the manufacturer specification of ±0.3 difference between the measured and the predicted values, figure 1 (A).

Linearity range verified for high target value of RBC count of 6.8M/µl presented with an excellent correlation coefficient of 1.000 between expected and observed values with a regression equation of y=0.02+1.000x. All the measured values lie within the manufacturer specification of ±0.1 difference between the measured and the predicted values, figure 1 (B).

Linearity verification of Cell-Dyn 1800 for HGB count of 23 gm/dl presented an excellent correlation coefficient of 0.995 between theoretical and measured values with a regression equation of y=0.987x-0.1. All the measured values also fall within the acceptable range of the manufacturer specification of ±0.2 difference between the measured and the predicted values , figure 1 (C).

Linearity range verified for high target value of PLT count of 990,000/µl presented with an excellent correlation coefficient of 1.000 between expected and observed values with a

regression equation of y=1.021x-22.6. All the measured values lie within the manufacturer specification of ±12 difference between the measured and the predicted values, figure 1 (D).

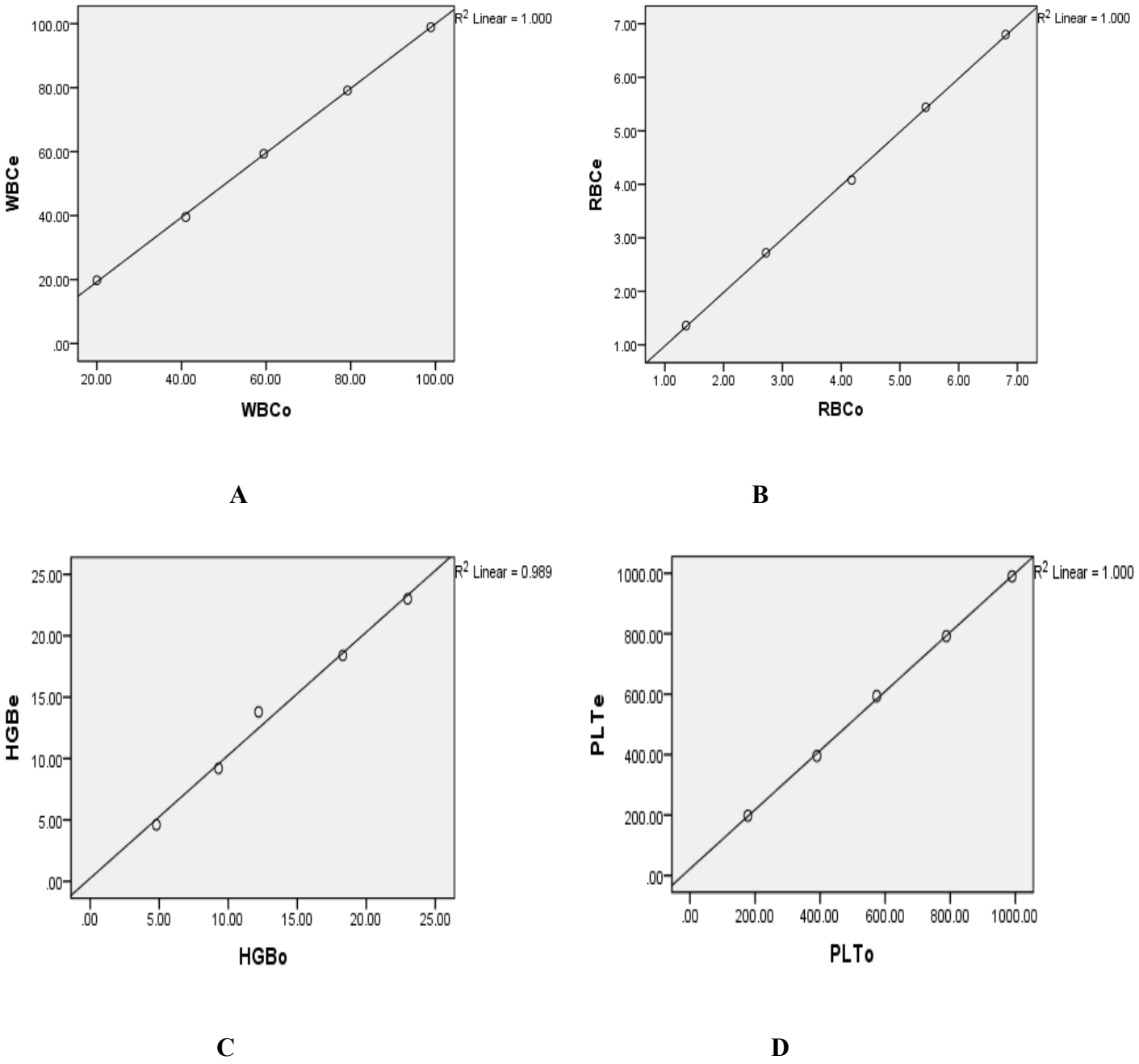


Figure 1: Linearity of A) WBC B) RBC C) HGB D) PLT of Cell-Dyn 1800. Where o & e on underscript means observed and expected value respectively on Cell-Dyn 1800 analyzer for each parameters.

6.3.2 Linearity of Horiba ES60 hematology analyzer

Linearity verification of Horiba ES 60 for a value of 99,900/µl WBC count presented an excellent correlation coefficient of 0.998 between theoretical and measured values with a regression equation of y=1.024x-1.79. All the measured values lie within the acceptable limit of the manufacturer specification of ±0.3, figure 2 (A).

Linearity verification of Horiba ES 60 for a value of 7.5M/µl RBC count presented an excellent correlation coefficient of 0.999 between theoretical and measured values with a regression equation of y=1.019x-0.025. All the measured values lie within the acceptable limit of the manufacturer specification of ±0.1 figure 2 (B).

Linearity verification of Horiba ES 60 for a value of 23.8g/dl HGB count presented an excellent correlation coefficient of 0.986 between theoretical and measured values with a regression equation of y=1.134x-1.91. All the measured values lie within the acceptable limit of the manufacturer specification of ±0.3 figure 2 (C).

Linearity verification of Horiba ES 60 for a value of PLT 1,900,000/ µl count presented an excellent correlation coefficient of 1.000 between theoretical and measured values with a regression equation of y=1.011x-22.8. All the measured values lie within the acceptable limit of the manufacturer specification of ±12, figure 2 (D).

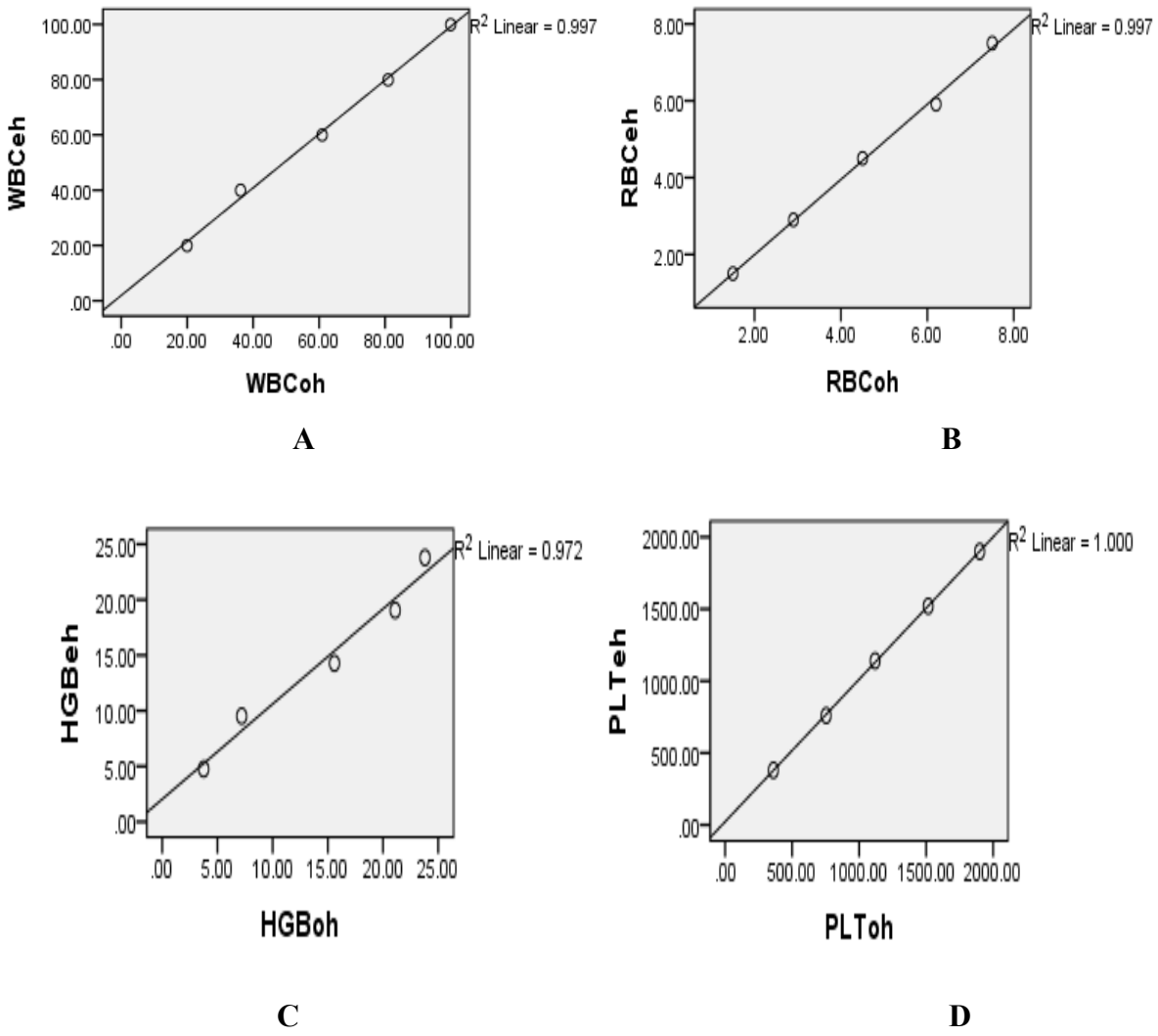


Figure 2: Linearity of A) WBC B) RBC C) HGB D) PLT of Horiba ES60. Where oh & eh on underscript means observed and expected value respectively on Horiba analyzer for each parameters.

6.3.3 Linearity of Sysmex KX-21 hematology analyzer

Linearity verification of Sysmex KX-21 for a value of WBC 98,300/ µl count presented an excellent correlation coefficient of 0.999 between theoretical and measured values with a regression equation of y=1.023x-1.679. All the measured values lie within the acceptable limit of the manufacturer specification of ±0.3, figure 3 (A).

Linearity verification of Sysmex KX-21 for a value of RBC 6.9 M/µl count presented an excellent correlation coefficient of 0.970 between theoretical and measured values with a regression equation of y=1.102x-0.105. All the measured values lie within the acceptable limit of the manufacturer specification of ±0.03, figure 3 (B).

Linearity verification of Sysmex KX-21 for a value of HGB 23.5gm/dl count presented an excellent correlation coefficient of 0.991 between theoretical and measured values with a regression equation of y=1.104x-1.85. All the measured values lie within the acceptable limit of the manufacturer specification of ±0.02, figure 3 (C).

Linearity verification of Sysmex KX-21 for a value of PLT 995,000/ µl count presented an excellent correlation coefficient of 0.999 between theoretical and measured values with a regression equation of y=1.021x-24.9. All the measured values lie within the acceptable limit of the manufacturer specification of ±10, figure 3 (D).

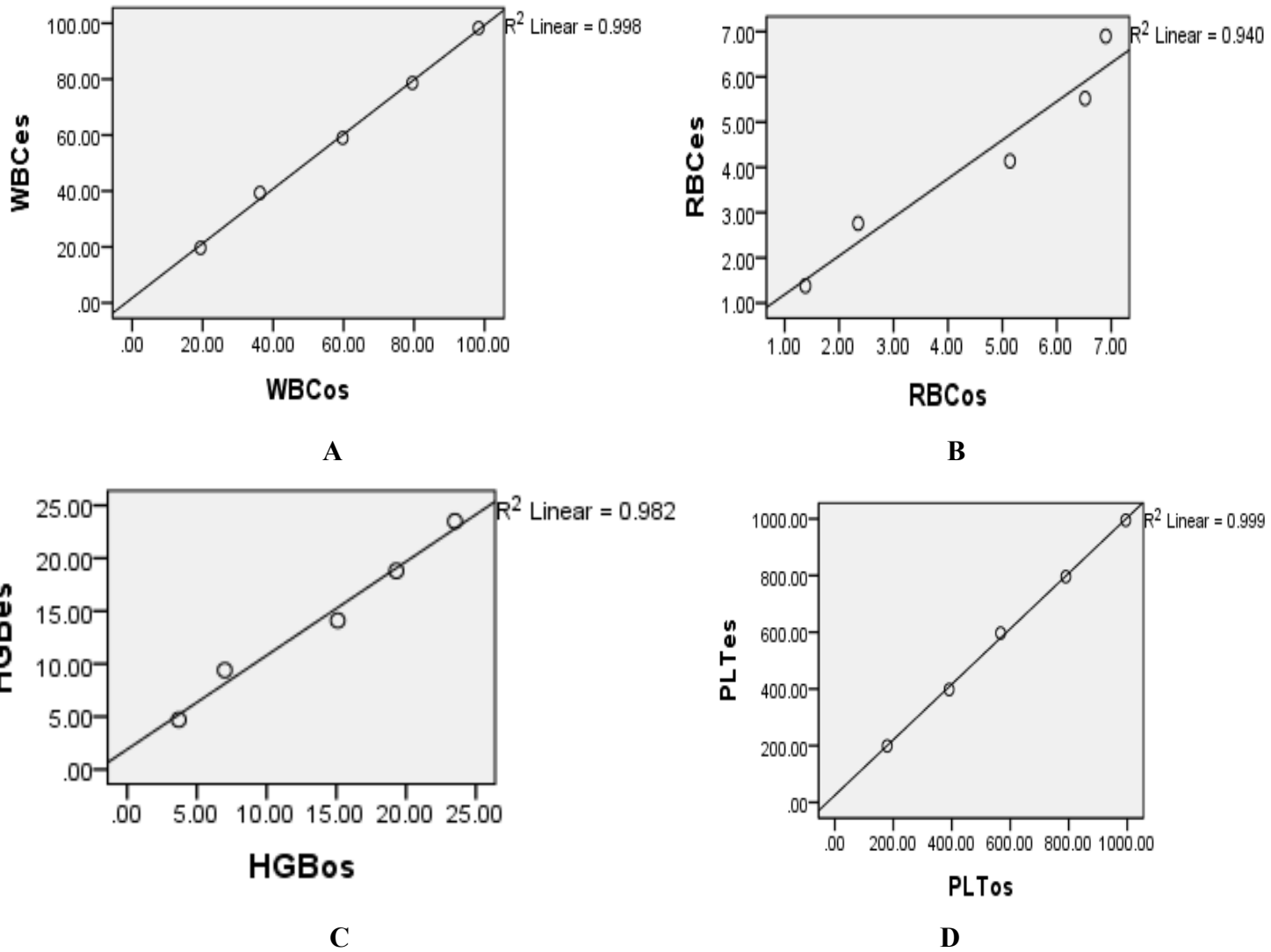


Figure 3: Linearity of A) WBC B) RBC C) HGB D) PLT of Sysmex KX-21. Where os & es on underscript means observed and expected value respectively on Sysmex analyzer for each parameters.

Table 8: Linearity specification of Cell-Dyn 1800, Horiba ES60, and Sysmex KX-21 hematology analyzers

Analyzers	Parameters*	Analytical measurement range
Cell-Dyn 1800	WBC (K/mm^3)	0.5-99.9
	RBC (M/ mm^3)	1.0-7.00
	HGB (g/dl)	2.5-24
	PLT (K/mm^3)	10-999
Horiba ES60	WBC (K/mm^3)	0-100
	RBC (M/ mm^3)	0-8
	HGB (g/dl)	0-26
	PLT (K/mm^3)	0-2200
Sysmex KX-21	WBC (K/mm^3)	10.0-99.9
	RBC (M/ mm^3)	1.00-7.00
	HGB (g/dl)	10.0-25.0
	PLT (K/mm^3)	10-999

***K= 10^3, M=10^6**

6.4 Agreement studies of major CBC parameters and WBC differential on different levels

6.4.1 Agreement test of WBC in the low level

The agreement of Cell- Dyn and Horiba in low level is acceptable because in the Bland-Altman plot, the difference is lying between Limits of agreement (LoA) for above 95% of cases and from the linear regression graph, we can see an excellent correlation of r=0.933 with the equation of y= 0.314-0.165x, figure 4 (A).

The agreement of Cell-Dyn and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between the LoA for at least 95% of cases and from the linear regression graph, we can see an excellent correlation of r=0.944 with the equation of y= 0.240+0.96x, figure 4 (B).

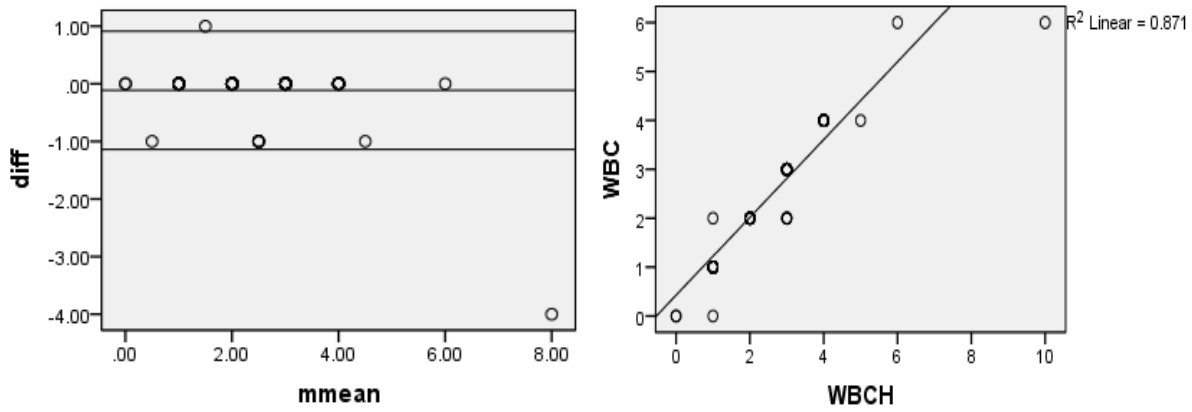
The agreement of Horiba and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between the LoA for 98.75% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.890 with the equation of y=0.63+0.771x, figure 4 (C).

6.4.2 Agreement test of HGB in the low level

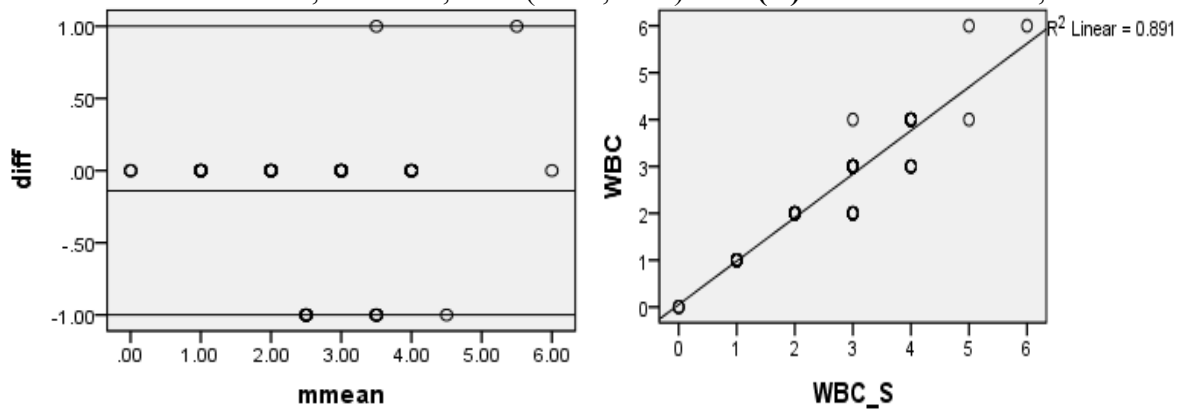
The agreement of Cell-Dyn and Horiba is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 95% of cases and from the linear regression graph, we can see an excellent correlation of r=0.990 with the equation of y= 0.072+0.983x, figure 5 (A).

The agreement of Cell-Dyn and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 96.25% of cases and from the linear regression graph, we can see an excellent correlation of r=0.989 with the equation of y=0.086+1.005x, figure 5 (B).

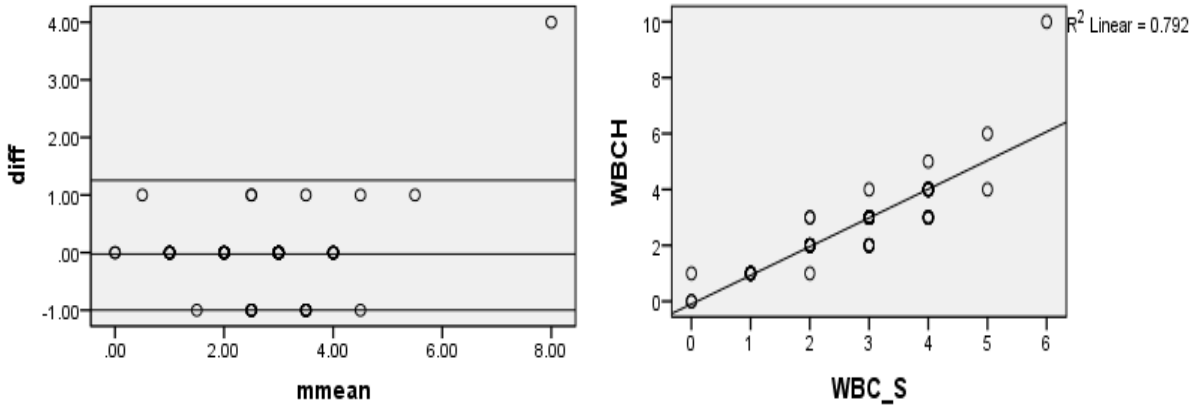
The agreement of Horiba and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 98.75% of cases and from the linear regression graph, we can see an excellent correlation of r=0.988 with the equation of y= 0.138+1.01x, figure 5 (C).



Mean=-0.11, SD=0.53, LoA (-1.14, 0.91) **(A)** Y=0.314-0.165x, r=0.933

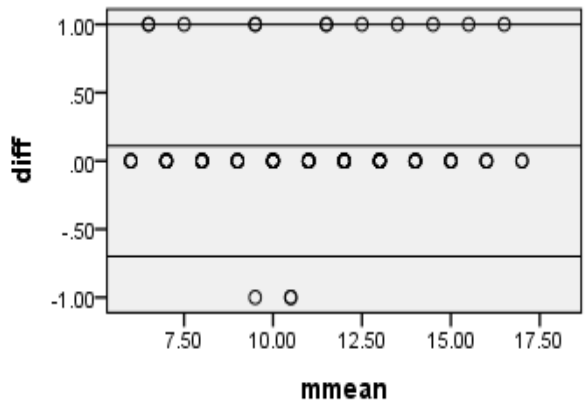


Mean=-0.14, SD=0.41, LoA (-0.95, 0.67) **(B)** Y=0.240+0.96x, r=0.944

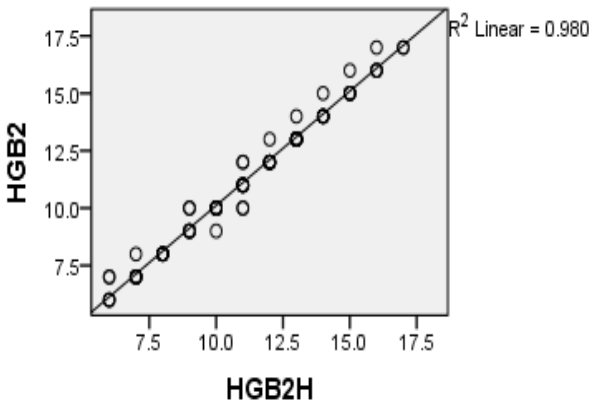


Mean=-0.03, SD=0.66, LoA (-1.31, 1.26) **(C)** Y=0.63+0.771x, r=0.890

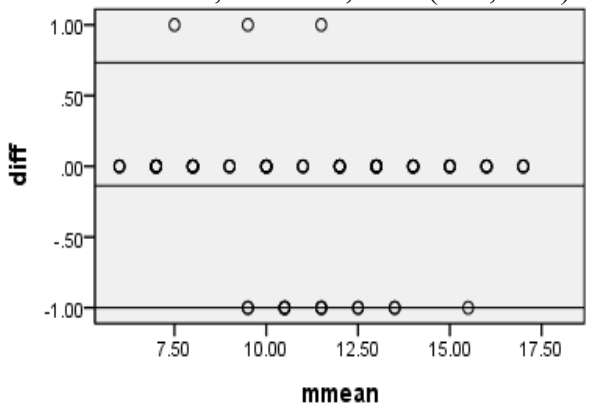
Figure 4: Bland-Altman and linear regression graph of WBC in low level on (A) Cell-Dyn and Horiba (B) Cell-Dyn and Sysmex (C) Horiba and Sysmex where diff and mmean, WBC, WBCH and WBC-S are difference and mean of the two measurement, WBC measured in Cell-Dyn, Horiba and Sysmex respectively.



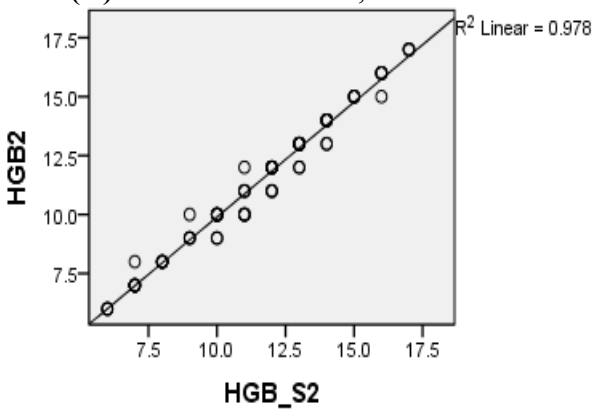
Mean=0.11, SD=0.42, LoA (-0.7, 0.93)



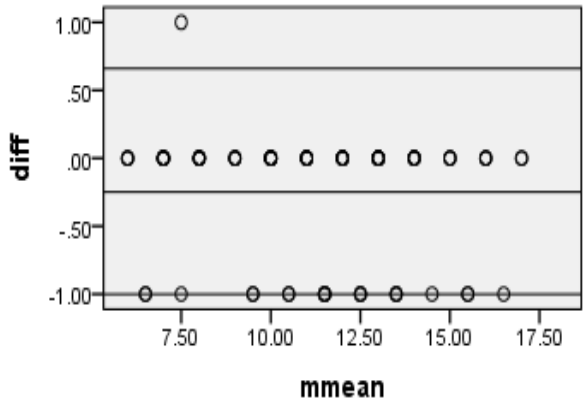
(A) Y=0.072+0.983x, r=0.990



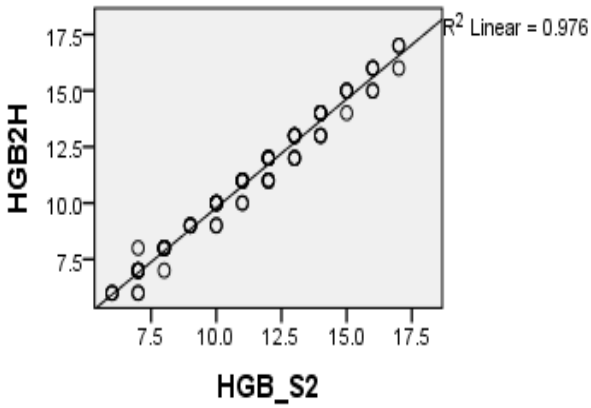
Mean=-0.14, SD=0.44, LoA (-1.0, 0.73)



(B) Y=0.086+1.005x, r=0.989



Mean=-0.25, SD=0.46, LoA (-1.16, 0.66)



(C) Y=0.138+1.01x, r=0.988

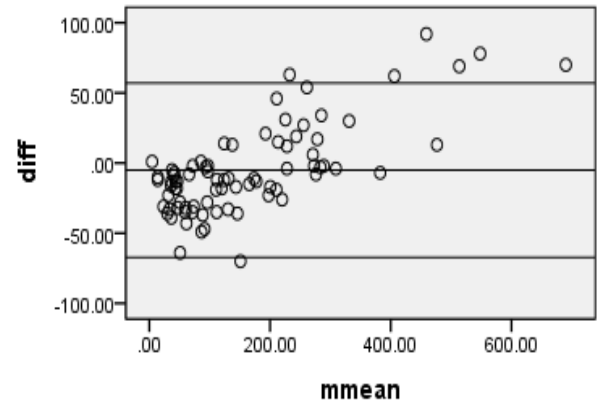
Figure 5: Bland-Altman and linear regression graph of HGB in low level on (A) Cell-Dyn and Horiba (B) Cell-Dyn and Sysmex (C) Horiba and Sysmex where diff and mmean, HGB2, HGB2H, HGB-S2 are difference and mean of the two measurement, HGB measured in Cell-Dyn, Horiba, and Sysmex respectively.

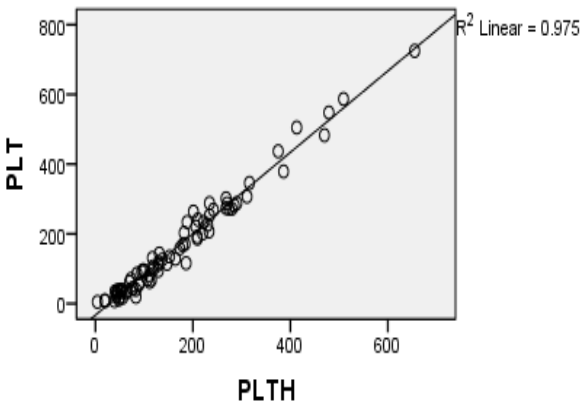
6.4.3 Agreement test of PLT in the low level

The agreement of Cell-Dyn and Horiba is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 95% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.987 between the two analyzers with the equation of y=1.17x-33.07, figure 6 (A).

The agreement of Cell-Dyn and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 96.25% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.992 between the two analyzers with the equation of y=3.059+0.98x, figure 6 (B).

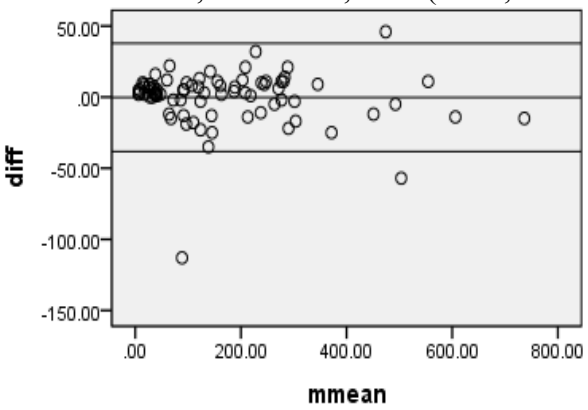
The agreement of Horiba and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 95% of cases and from the linear regression graph, we can see that there is an excellent correlation of r=0.985 with the equation of y=35.17+0.818x, figure 6 (C).

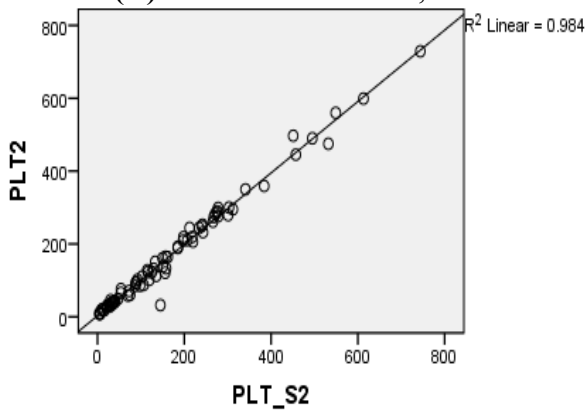




Mean=-5.09, SD=31.71, LoA (-67.2, 57.11)

(A) Y=1.17x-33.07, r=0.987

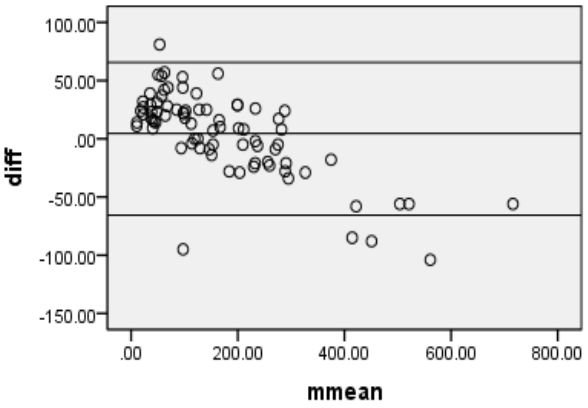


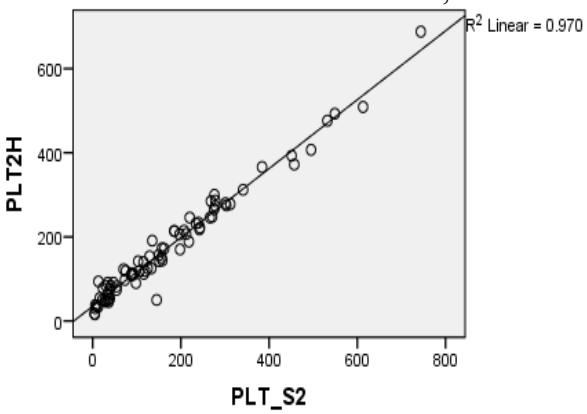


(B)

Mean=-0.27 SD=19.41, LoA (-38.3, 37.8)

Y=3.059+0.98x, r=0.992





Mean=4.56, SD=35.80, LoA (-65.6, 65.6)

(C) Y=35.17+0.818x, r=0.985

Figure 6: Bland-Altman and linear regression graph of PLT in low level on (A) Cell-Dyn and Horiba (B) Cell-Dyn and Sysmex (C) Horiba and Sysmex where diff and mmean, PLT(PLT2), PLTH(PLT2H), and PLTS2 are difference and mean of the two measurement, PLT measured in Cell-Dyn, Horiba and Sysmex respectively

6.4.4 Agreement test of WBC in the normal level

The agreement between Cell-Dyn and Horiba is acceptable because in the Bland-Altman plot, the difference is lying between LoA for at least 95% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.983 with the equation of y= 1.03x-1.27, figure 7 (A).

The agreement between Cell-Dyn and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 98.75% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.964 with the equation of y= 0.093+0.938x, figure 7 (B).

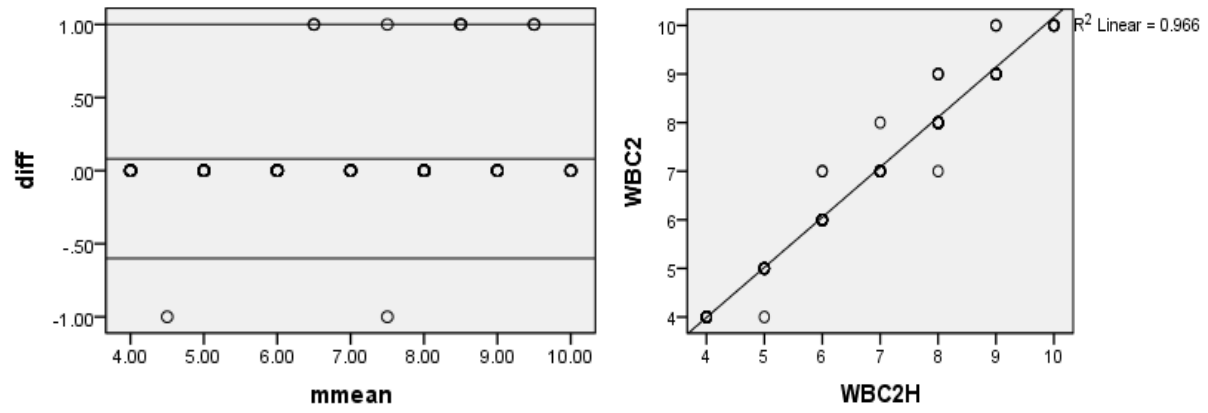
The agreement between Horiba and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 97.5% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.959 with the equation of y= 0.364+0.89x, figure 7 (C).

6.4.5 Agreement test of HGB in the normal level

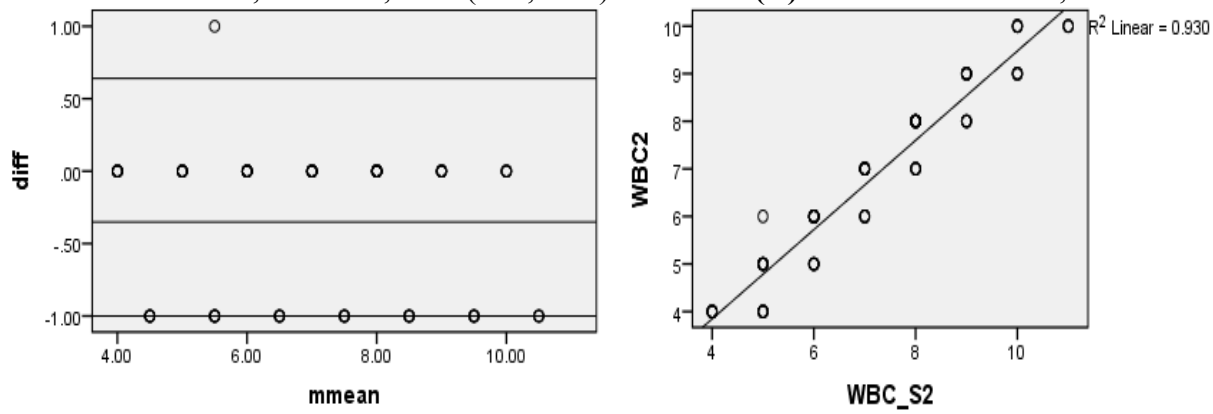
The agreement between Cell-Dyn and Horiba is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 95% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.958 with the equation of y= 0.633+0.96x, figure 8 (A).

The agreement between Cell-Dyn and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 95% of cases and from the linear regression graph we can see there is an excellent correlation of r=0.968 with the equation of y= 0.691+0.942x, figure 8 (B).

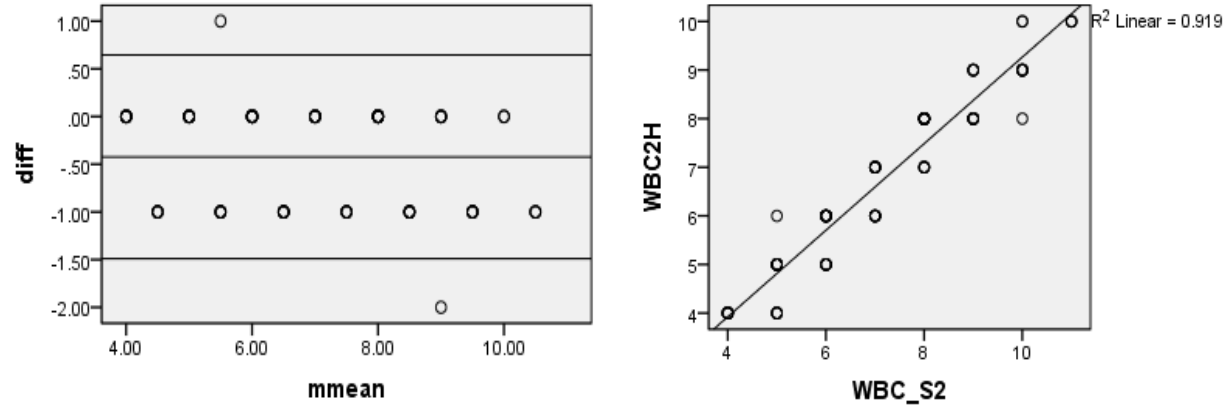
The agreement between Horiba and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between LoA for more than 98.75% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.951 with the equation of y= 0.901+0.923x, figure 8 (C).



Mean=0.08, SD=0.35, LoA (-0.6, 0.76) **(A)** Y=1.03x-1.27, r=0.983

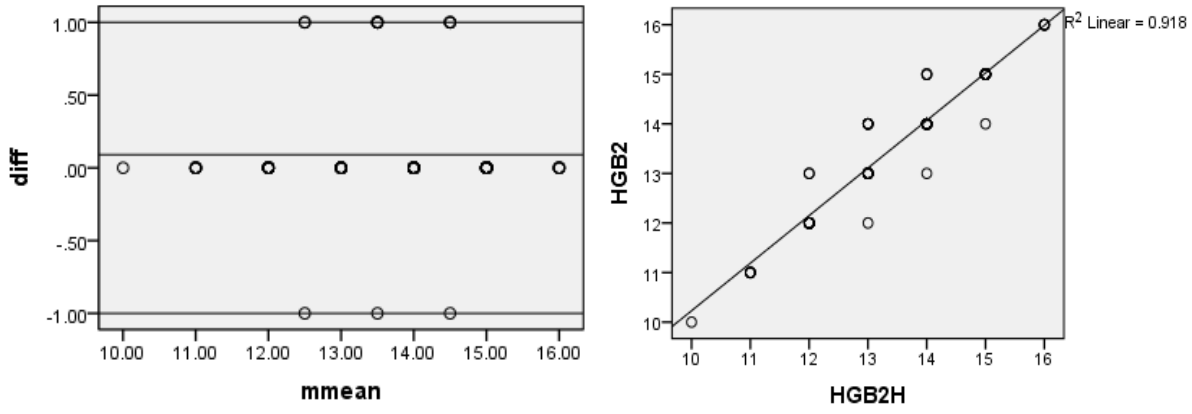


Mean=-0.35, SD=0.51, LoA (-1.34, 0.64) **(B)** Y=0.093+0.938x, r=0.964

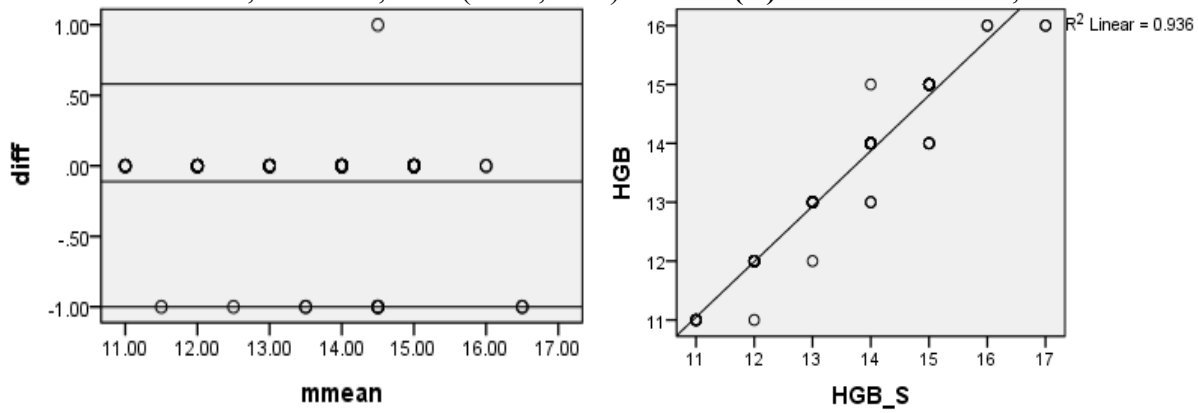


Mean=-0.43, SD= 0.55, LoA (-1.49, 0.645) **(C)** Y=0.364+0.89x, r=0.959

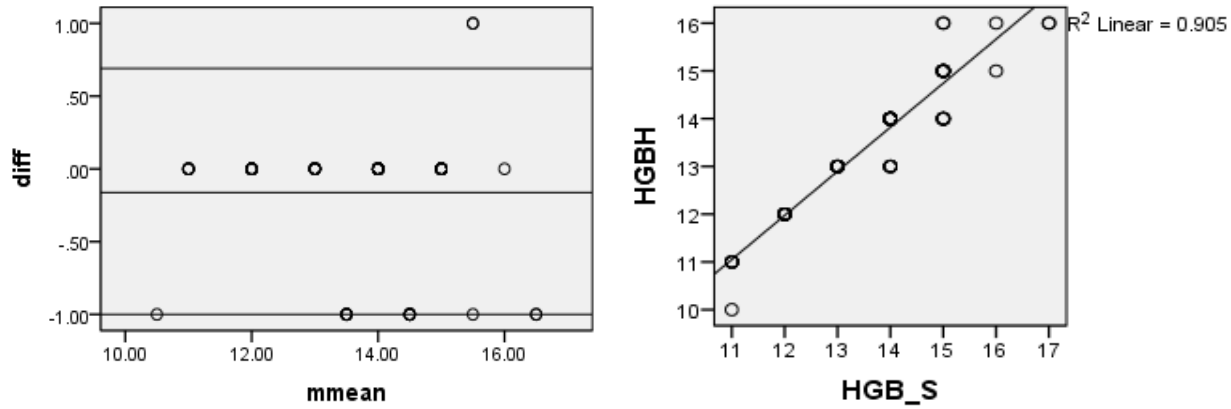
Figure 7: Bland-Altman and linear regression graph of WBC in normal level on (A) Cell-Dyn and Horiba (B) Cell-Dyn and Sysmex (C) Horiba and Sysmex where diff and mmean, WBC2, WBC2H, and WBC-S2 respectively are difference and mean of the two measurement, WBC measured in Cell-Dyn, Horiba, and Sysmex.



Mean=0.09, SD=0.39, LoA (-0.69, 0.87) **(A)** Y=0.633+0.96X, r=0.958



Mean=-0.11, SD=0.36, LoA (-0.81, 0.58) **(B)** Y=0.691+0.942x, r=0.968



Mean=-0.16, SD=0.43, LoA (-1.01, 0.69) **(C)** Y=0.901+0.923x, r=0.951

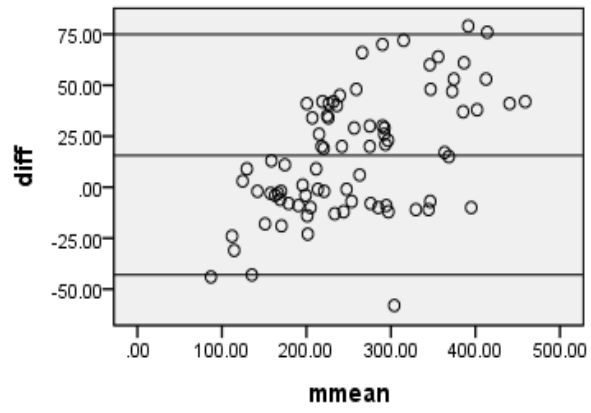
Figure 8: Bland-Altman and linear regression graph of HGB in normal level on (A) Cell-Dyn and Horiba (B) Cell-Dyn and Sysmex (C) Horiba and Sysmex where diff and mmean, HGB(HGB2), HGBH(HGB2H), and HGBS respectively are difference and mean of the two measurement, HGB measured on Cell-Dyn, Horiba and Sysmex.

6.4.6 Agreement test of PLT in the normal level

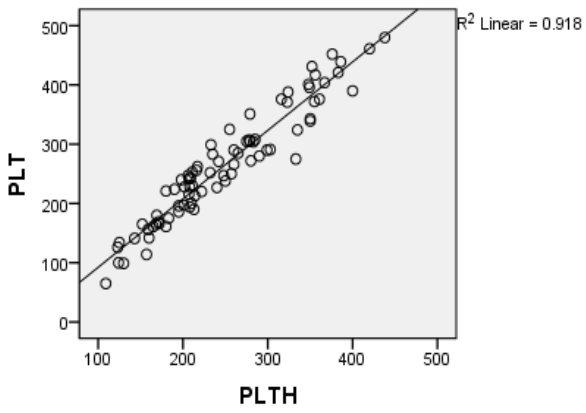
The agreement between Cell-Dyn and Horiba is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 96.25% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.958 with the equation of y= 1.16x-23.29, figure 9 (A).

The agreement between Cell-Dyn and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 95% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.983 with the equation of y= 12.57+0.937x, figure 9 (B).

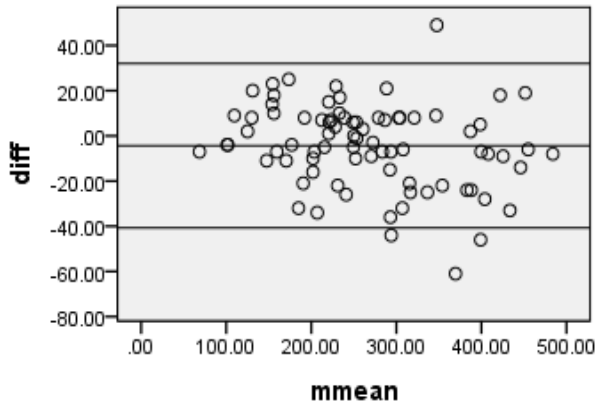
The agreement between Horiba and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 96.25% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.964 with the equation of y= 0.44.24+0.762x, figure 9 (C).



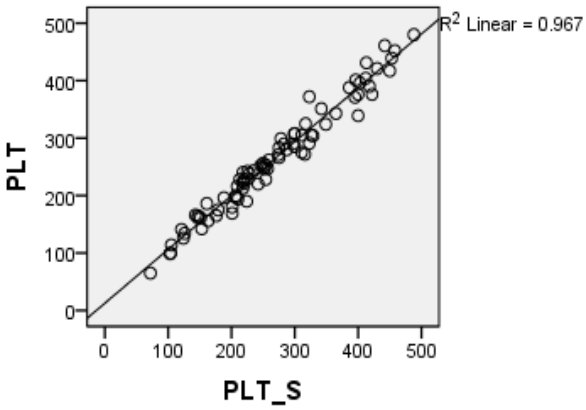
Mean=15.6, SD=30.01, LoA (-43, 74.9)



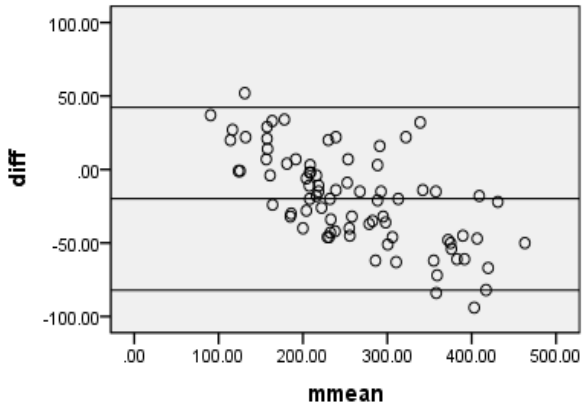
(A) Y=1.16x-23.29, r=0.958



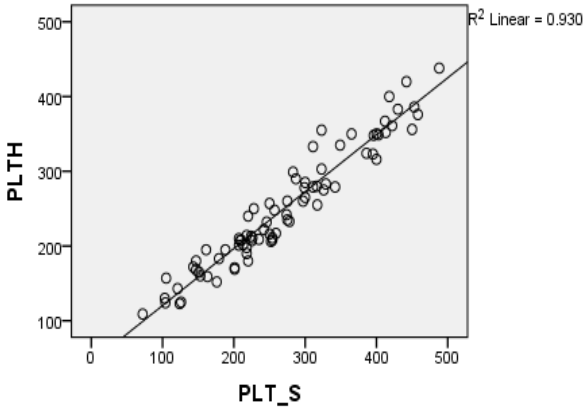
Mean=-4.33, SD=18.55, LoA (-40.7, 32.04)



(B) Y=12.57+0.937x, r=0.983



Mean=-19.91, SD=31.76, LoA (-82.15, 42.33)



(C) Y=44.24+0.762x, r=0.964

Figure 9: Bland-Altman and linear regression graph of PLT in normal level on (A) Cell-Dyn and Horiba (B) Cell-Dyn and Sysmex (C) Horiba and Sysmex, where diff, mmean, PLT, PLTH, and PLTS respectively are difference of the two measurement, mean of the two measurement, PLT measured in Cell-Dyn, Horiba, and Sysmex.

6.4.7 Agreement test of WBC in the high level

The agreement between Cell-Dyn and Horiba is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 97.5% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.985 with the equation of y= 0.084+1.016x, figure 10 (A).

The agreement between Cell-Dyn and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 95% of cases and from the linear regression graph we can see there is an excellent correlation of r=0.986 with the equation of y= 2.218+0.883x, figure 10 (B).

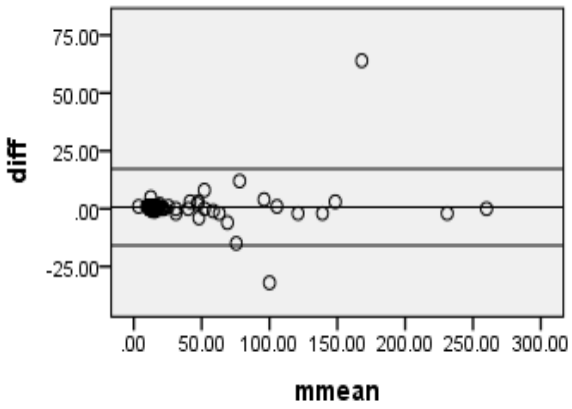
The agreement between Horiba and Sysmex is acceptable because, in the Bland-Altman plot the difference is lying between LoA for 97.5% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.966 with the equation of y= 3.32+0.839x, figure 10 (C).

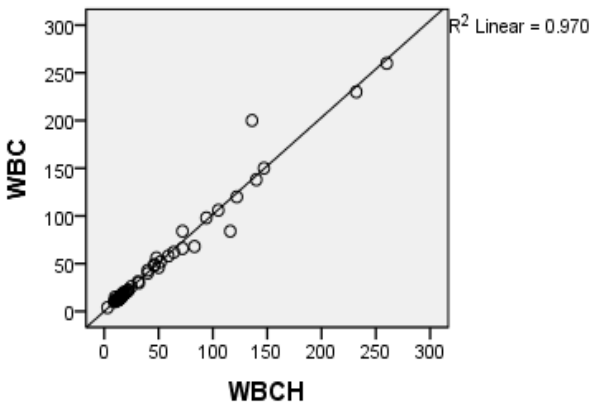
6.4.8 Agreement test of HGB in the high level

The agreement between Cell-Dyn and Horiba is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 98.75% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.917 with the equation of y= 2.242+0.845x, figure 11 (A).

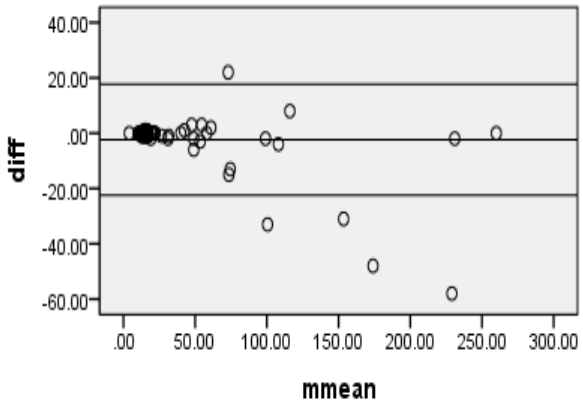
The agreement between Cell-Dyn and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 98.75% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.983 with the equation of y= 0.635+0.948x, figure 11 (B).

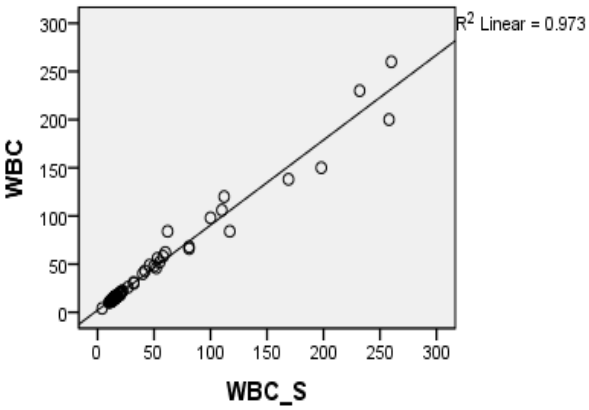
The agreement between Horiba and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 98.75% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.920 with the equation of y= 0.088+0.963x, figure 11 (C).



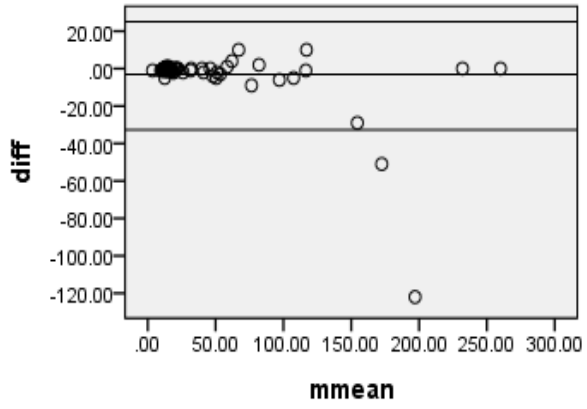


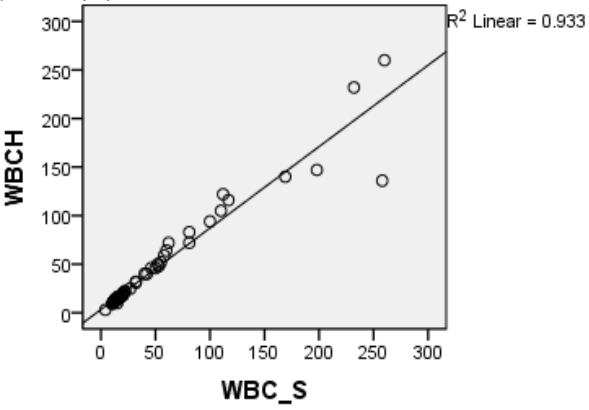
Mean=0.65, SD=8.48, LoA (-15.9, 17.25) **(A)** Y=0.084+1.016x, r=0.985





Mean=-2.39, SD=10.27, LoA (-22.5, 17.7) **(B)** Y=2.218+0.883x, r=0.986





Mean=-3.04, SD=15.13, LoA (-32.68, 26.6) **(C)** Y=3.32+0.839x, r=0.966

Figure 10: Bland-Altman and linear regression graph of WBC in high level on (A) Cell-Dyn and Horiba (B) Cell-Dyn and Sysmex (C) Horiba and Sysmex where diff and mmean, WBC, WBCH, and WBCS respectively are difference and mean of the two measurement, WBC measured on Cell-Dyn, Horiba, and Sysmex.

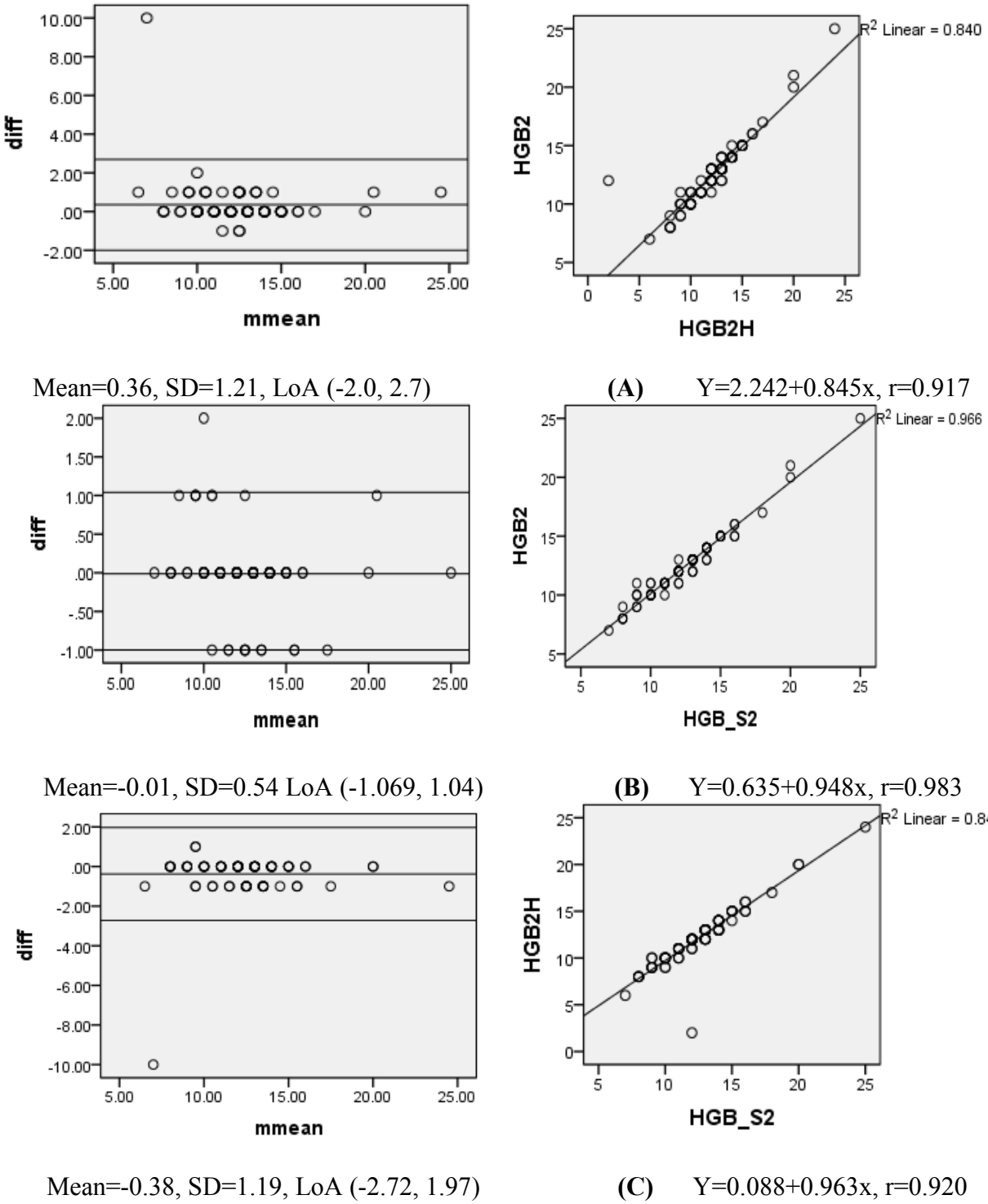


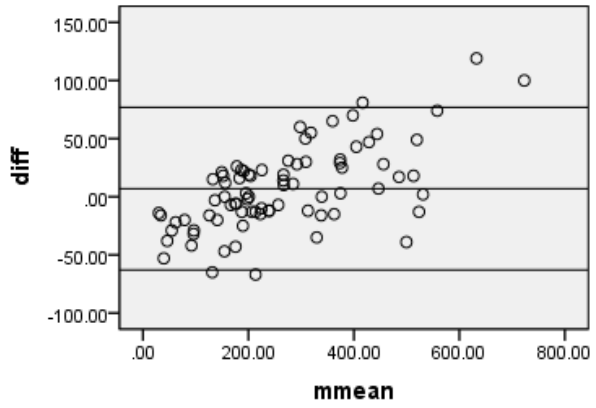
Figure 11: Bland-Altman and linear regression graph of HGB in high level on (A) Cell-Dyn and Horiba (B) Cell-Dyn and Sysmex (C) Horiba and Sysmex where diff and mmean, HGB2, HGB2H, HGBS2 respectively are difference and mean of the two measurement, HGB measured on Cell-Dyn, Horiba, and Sysmex.

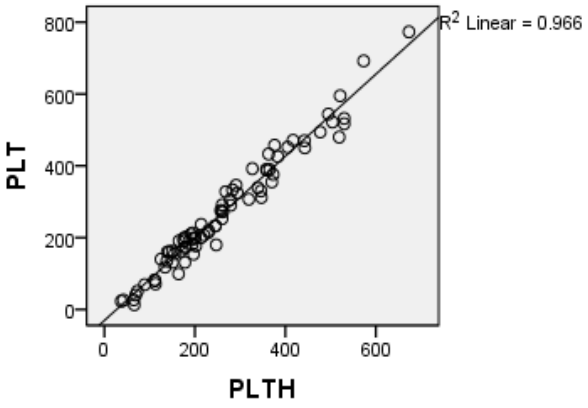
6.4.9 Agreement test of PLT in the high level

The agreement between Cell-Dyn and Horiba is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 95% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.983 with the equation of y= 1.143x-30.582, figure 12 (A).

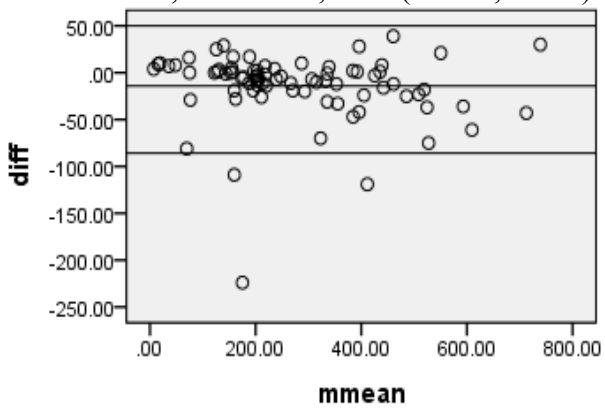
The agreement between Cell-Dyn and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 96.25% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.975 with the equation of y= 0.2+0.95x, figure 12 (B).

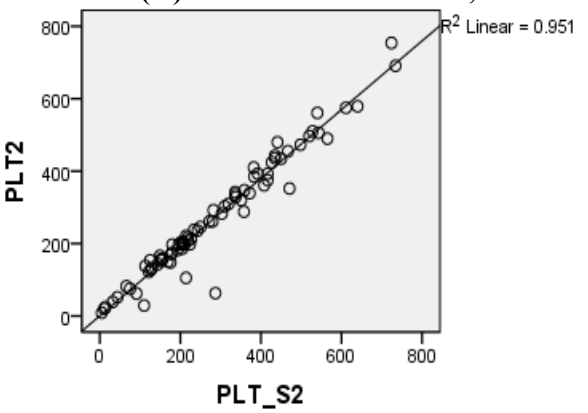
The agreement between Sysmex and Horiba is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 95% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.934 with the equation of y= 28.43+0.815x, figure 12 (C).



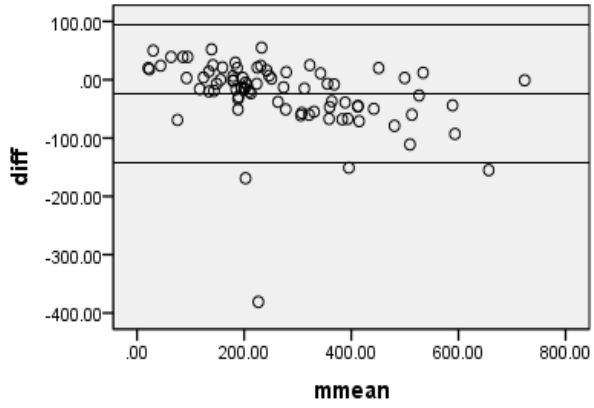


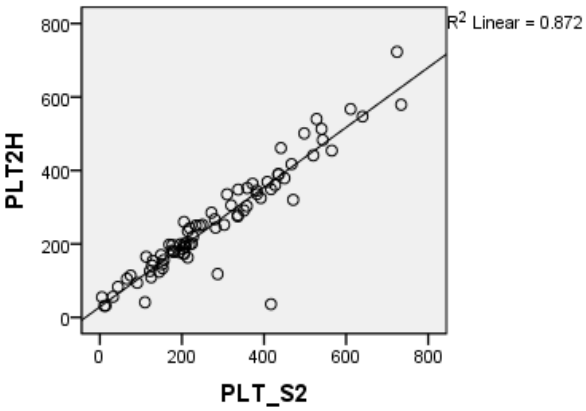
Mean=7.00, SD=35.67, LoA (-62.92, 76.92) **(A)** Y=1.143x-30.582, r=0.983





Mean=-14.13, SD=36.60, LoA (-85.87, 57.625) **(B)** Y=0.2+0.95x, r=0.975





(C)

Mean=-24.13, SD=60.36, LoA (-142.4, 94.185) Y=28.43+0.815x, r=0.934

Figure 12: Bland-Altman and linear regression graph of PLT in high level on (A) Cell-Dyn and Horiba (B) Cell-Dyn and Sysmex (C) Horiba and Sysmex where diff and mmean, PLT(PLT2), PLTH(PLT2H), and PLTS2 respectively are difference of the two measurement, PLT measured in Cell-Dyn, Horiba, and Sysmex.

6.5 Agreement study of WBC differentials

6.5.1 Agreement test of GRAN %

The agreement between Cell-Dyn and Horiba is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 95% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.988 with the equation of y= 1.559+0.948x, figure 13 (A).

The agreement between Cell-Dyn and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 95% of cases and from the linear regression graph we can see there is a good correlation of r=0.924 with the equation of y= 8.699+0.861x, figure 13 (B).

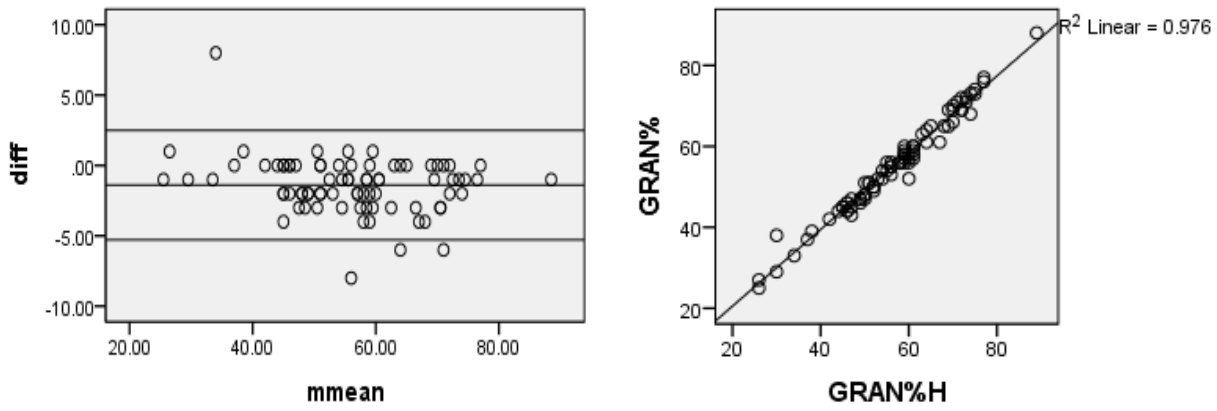
The agreement between Horiba and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 95% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.933 with the equation of y= 7.698+0.905x, figure 13 (C).

6.5.2 Agreement test of LYM %

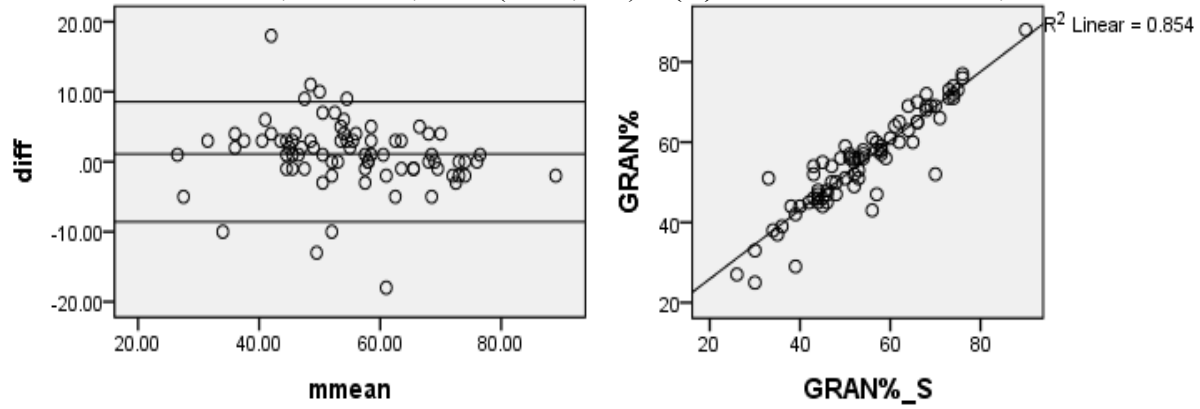
The agreement between Cell-Dyn and Horiba is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 95% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.990 with the equation of y= 1.785+0.954x, figure 14 (A).

The agreement between Cell-Dyn and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 96.25% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.974 with the equation of y= 2.95+0.93x, figure 14 (B).

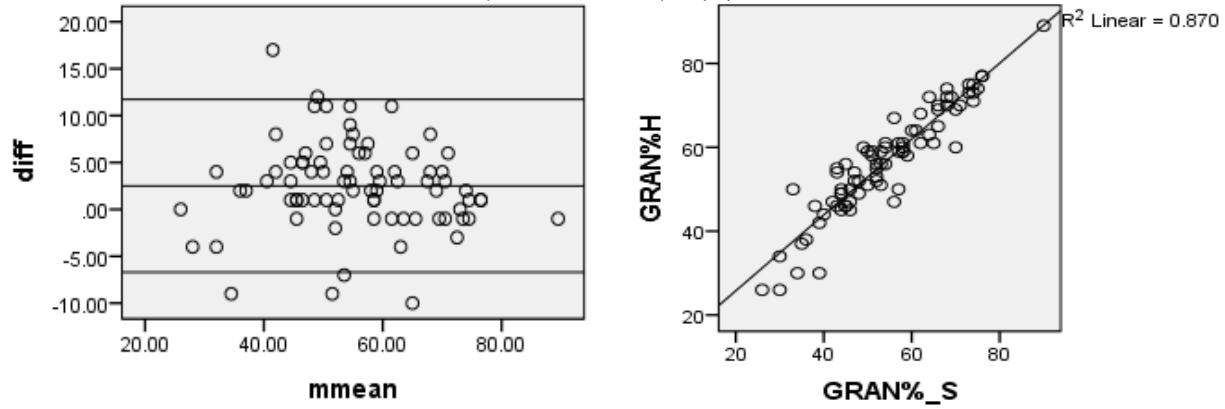
The agreement between Horiba and Sysmex is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 96.25% of cases and from the linear regression graph, we can see there is an excellent correlation of r=0.972 with the equation of y= 1.62+0.964x, figure 14 (C).



Mean=-1.39, SD=1.99, LoA (-5.28, 2.5) **(A)** Y=1.559+0.948x, r=0.988



Mean=1.13, SD=4.95, LoA (-8.579, 8.575) **(B)** Y=8.699+0.861x, r=0.924



Mean=2.51, SD=4.71, LoA (-6.71, 11.73) **(C)** Y=7.698+0.905x, r=0.933

Figure 13: Bland-Altman and linear regression graph of GRAN % on (A) Cell-Dyn and Horiba (B) Cell-Dyn and Sysmex (C) Horiba and Sysmex, where diff and mmean, GRAN%, GRAN%H, GRAN%S respectively are difference and mean of the two measurement, granulocyte measured in Cell-Dyn, Horiba, and Sysmex.

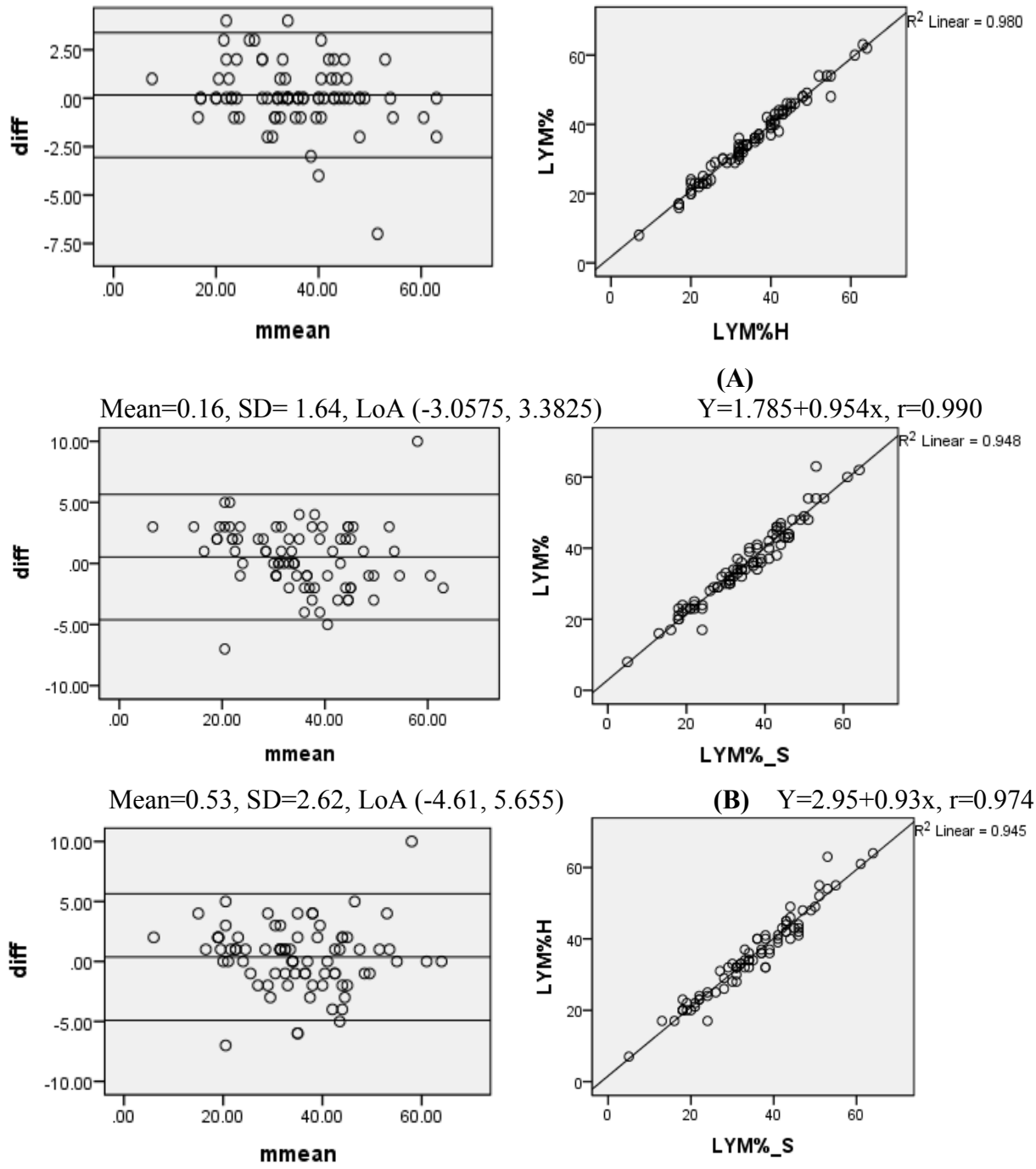


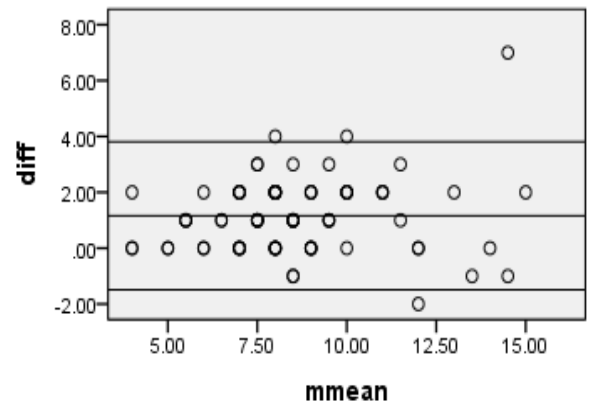
Figure 14: Bland-Altman and linear regression graph of LYM % on (A) Cell-Dyn and Horiba (B) Cell-Dyn and Sysmex (C) Horiba and Sysmex where diff and mmean, LYM%, LYM%H, and LYM%-S respectively are difference and mean of the two measurement, lymphocytes as measured in Cell-Dyn, Horiba, and Sysmex

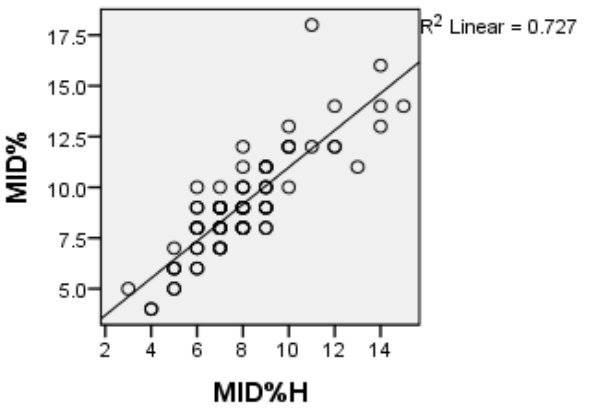
6.5.3 Agreement test of MXD cells %

The agreement between Cell-Dyn and Horiba is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 95% of cases and from the linear regression graph, we can see there is good correlation of r=0.853 with the equation of y= 1.873+0.911x, figure 15 (A).

The agreement between Cell-Dyn and Sysmex is not acceptable because, in the Bland-Altman plot, the difference is lying between LoA for 93.75% of cases which is less than 95% (less than the Bland-Altman rule) and from the linear regression graph we can see there is poor correlation of r=0.051 with the equation of y= 6.66+0.233x, figure 15 (B).

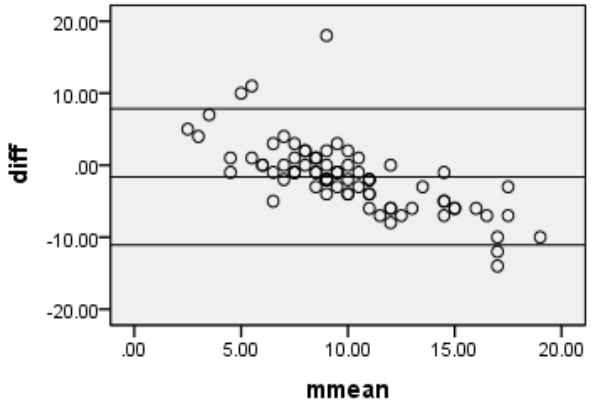
The agreement between Horiba and Sysmex is not acceptable because, in the Bland-Altman plot the LoA is wider and covers only 92.5% of cases which is < 95% (less than the Bland-Altman rule). From the linear regression graph we can see there is also poor correlation of r value=0.0563 with the equation of y= 5.413+0.241x, figure 15 (C).

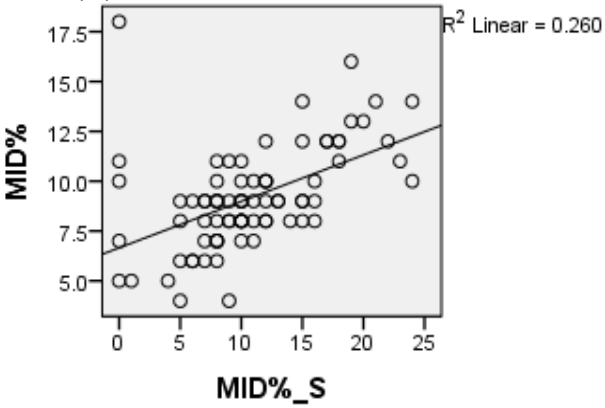




Mean=1.16, SD=1.35, LoA (-1.49, 3.81)

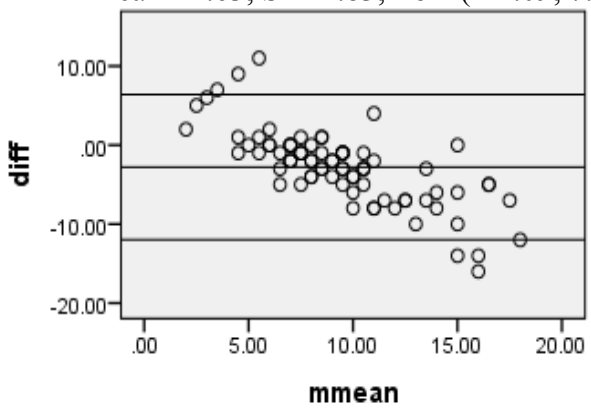
(A) Y=1.873+0.911x, r=0.853

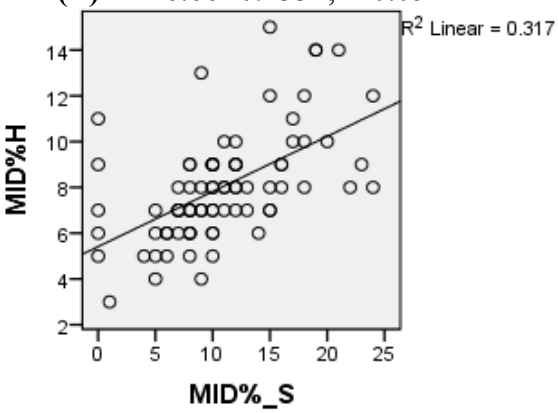




Mean=-1.63, SD=4.83, LoA (-11.09, 7.85)

(B) Y=6.66+0.233x, r=0.051





Mean=-2.79, SD=4.69, LoA (-11.98, 6.41)

(C) Y=5.413+0.241x, r=0.056

Figure 15: Bland-Altman and linear regression graph of MXD % on (A) Cell-Dyn and Horiba (B) Cell-Dyn and Sysmex (C) Horiba and Sysmex where diff and mmean, MID%, MID%H, and MID%-S are difference and mean of the two measurement, mixed cells measured by Cell-Dyn, Horiba, and Sysmex.

7. Discussion

In this study, three modern hematology analyzers- Cell-Dyn 1800, Horiba ES60, and Sysmex KX-21 were evaluated and compared, regarding CBC and DLC on different levels of reference ranges (low, normal, high). It was demonstrated that the three analyzers comply with the companies' recommendation in terms of precision, linearity and carryover. Fernandez *et al* in 2001 in Florida, USA, evaluated Coulter LH-750 against the Gen S system analyzer by regression analysis and the mean differences between methods. WBC, RBC, HGB, MCV, PLT, and RDW r value of > 0.980 and MPV showed r value of > 0.960. In our study even if we compared different analyzers from the above authors, we found the same result except r value of MPV > 0.600. This difference in correlation of MPV could be due to the technology difference applied on the analyzers. Sample size difference might have little role for this differences, they used a total of 383 samples [20] slightly greater than ours which was 267. An excellent performance of automated differential count was showed by Fernandez *et al* in 2001 at Florida [19]. Their r value for WBC was > 0.920, while for neutrophils, lymphocytes, and eosinophil, the r values were > 0.990; for monocytes r > 0.970 and for basophils r > 0.800. In spite of analyzer difference between us, this was also the same with our finding except for monocyte and basophil because we could not specifically measure these parameters on our 3 part differential analyzers.

A study by Rui Q *et al* in 2013 verified the performance of Beckman Coulter LH-750, Mindray BC-5800 and Sysmex XE-2100 in clinical laboratories. They used a total of 15 fresh whole blood specimens. Except platelet precision of BC-5800 analyzers, the rest parameters complied with specification of each analyzer. Even though we studied different analyzers from the above authors, all our precision of the directly measured parameters (WBC, RBC, HGB, MCV, PLT, and MPV) comply with the manufacturer's specification on all the three analyzers. This variation on platelet precision on one of their analyzers may be due to the reason that they may use flagged samples but in our case we used normal and non-flagged samples only for precision study [22].

In a comparative study, the three analyzers showed a good correlation (r > 0.900) with each other for CBC parameters. The Precision study of DLC was good in our study with all the analyzers fulfilling the manufacturers claim. Precision was also good for CBC parameters. We did the precision study only for normal level samples as per the manufacturer's user manual. But the study by Letho T and Hedberg P on the performance evaluation of Abbott Cell-Dyn Ruby

though different analyzer from us, in Finland [24], the precision was done for all levels. The comparison study of neutrophils and lymphocytes of the analyzers was also excellent with r > 0.920. This is the same finding by the above authors in different analyzers [25].

An inter-instrument agreement for most parameters was good, although small numbers of discrepancies observed by Leers MPG, a study conducted in Germany between Abbott Cell-Dyn and Sysmex XT-2000i [26]. They found systematic biases for MCV, PLT and MPV and also found that WBC subpopulation counts were in good agreement with no major outliers [26]. We found comparable results nonetheless in different analyzers from the above authors in that there was a good agreement on most of the analyzers for most parameters and small discrepancies observed on some parameters between Cell-Dyn with Horiba and Sysmex at the normal level but in the low level and high level all the parameters had good agreements with limits of agreements lie mean$\pm$1.96SD for > 95% of cases. There was also moderate correlation of MPV(r > 0.600) for the analyzers except between Horiba and Sysmex (r=0.572) at high levels only. These systematic bias on this specific parameter is also the same with the above study who got systematic bias on MPV and a study conducted by Guerti K and his colleagues in 2009 [28] but with different analyzers from the current study.

For the comparison of CBC parameters, each machine was compared with the mean difference measurements by correlation and Bland-Altman analysis. No large differences were detected for all major CBC parameters. We found that good carryover < 0.5% and excellent linearity for WBC, HGB, and platelet counts (r > 0.900) and RBC in the normal range (r > 0.800) for all the analyzers tested. Precision was good for the major CBC parameters: CV% being ≤ 2.0 for most of the analyzers, except for platelet count with CV % of 2.9 %, 2.9 % and 4.9 % for Cell-Dyn, Sysmex and Horiba respectively and WBC with CV % of 2.2 % and 2.9 % for Cell-Dyn and Horiba respectively. Correlation between Cell-Dyn 1800, Horiba ES60, and Sysmex KX-21 was excellent (r > 0.900) for all major CBC parameters at all levels. These findings are alike with those of other authors Shu G *et al* in 2013 [29] even though the compared analyzers were different from the current study.

The three analyzers showed good correlation (r > 0.900) for WBC, HGB, and PLT. For RBC, the r values were > 0.800 at the normal level and > 0.900 at the low and high levels. In the DLC, the three analyzers showed good correlations for neutrophil (r > 0.920) and lymphocyte (r > 0.972).

Correlation coefficients were very low for mixed cells (r =0.040) between Cell-Dyn and Sysmex and (r=0.005) between Horiba and Sysmex. These results are comparable with findings reported by Devreese K *et al* in 1991 [30] still with different analyzers from the current study.

Carryover, precision, linearity and agreement were performed on the three analyzers. Carryover and precision were within the manufacturer specifications and linearity met the specified range on all parameters studied on all the three analyzers. The three analyzers showed excellent correlation and agreement with each other for WBC, Neutrophil %, Lymphocyte %, RBC, HGB, and PLT for all levels, correlation being > 0.900 and LoA lies between mean$\pm$1.96SD for > 95% of cases for most Bland-Altman plots. This was akin with a study by Gould N *et al* in1999 [31] however the current study was done in different analyzers from the above authors.

In our study, we compared CBC and DLC in a total of 240 samples from different levels of reference ranges. All the analyzers exhibited very good correlation and agreement among each other for most CBC parameters. Neutrophils and lymphocytes also showed very good correlations (r > 0.900). But lymphocytes were correlated fairly in a study conducted by Meintker L *et al* in 2013 [32]. In our study, we got excellent correlation of lymphocyte between the analyzers still and all with different analyzer from the above authors. This variation between lymphocyte correlation could be due to a difference in sample size or due to the differences in the technology of the automated analyzers used, they used 5 part differential analyzers while all the analyzers used in the current study were three part differential.

The performance of the three analyzers was evaluated and compared with each other. Bland-Altman plot and linear regression were analyzed for the major CBC parameters and DLC. The Bland-Altman plots showed that the three analyzers were in good agreement with each other because the limit of agreement lies within mean$\pm$1.96SD for > 95% cases. Before the agreement and correlation study, we did linearity, precision and carry-over to check the analyzers performance. The % CVs of precision were < 3% for most CBC parameters and < 5% for the DLC except mixed cells (% CV>5%), though it fulfills the manufacturers specification. The results obtained using these analyzers were well correlated and agreed with each other with r value of > 0.900 for most CBC parameters for all levels studied and r > 0.924 for DLC except mixed cells (r > 0.005) especially between Cell-Dyn and Sysmex and between Horiba and Sysmex. The correlation of mixed cells between Cell-Dyn and Horiba was moderate for all

levels (r > 0.600). This finding was the same with the study conducted by Jo Y *et al* in 2013 [33] though the comparison was done between different analyzers in the current study.

The agreement study was done on different levels of reference ranges. One of this was the evaluation and comparison of low levels of different parameters on the three analyzers. In the low level study, for example, WBC was one of our major targets of study. All results obtained with the three analyzers showed good agreement and correlation with each other with most of the parameters. The r values were > 0.900 for most CBC parameters except MPV (r > 0.600) and r > 0.924 for DLC except mixed cells (r=0.400). The low WBC level gave a result of neutrophil with r > 0.920 and lymphocyte with r > 0.970. So, the low level samples were in good agreement on the different analyzers on low level WBC and differential except mixed cells with r=0.040. This result is in accordance with a study conducted by Young L *et al* in 2015 [34] that said between different analyzers with the authors.

In Ethiopia, a study conducted by Lulit Hailu, used the Bland-Altman method to compare Cell-Dyn 1800 (3 part differential) and Sysmex XT-2000i (5 part differential) for the analysis of different hematological parameters at TASH. The study documented that all parameters agreed and hence concluded that the two analyzers can be used interchangeably. Although the study by Lulit did not check if the two instruments comply with the manufacturer's specification, the finding of agreement between the two analyzers was similar with our study [36]. Similarly, even if, the study by Tibebe Adinew at St Paul's hospital did not check agreement between the two analyzers (Sysmex KX-21 and Cell-Dyn 1800) [37], the findings of verification study between the two analyzers agree with our study.

8. Strength and limitation of the study

8.1 Strength

- ✓ Each sample was run in duplicates for the agreement studies.
- ✓ Total number of samples for the agreement study was made doubled from that of the minimum CLSI guideline requirement.
- ✓ The agreement study was made on each level (Low, Normal, High) of reference ranges.

8.2 Limitation

- ✓ To categorize low, normal, and high levels of CBC parameters, we used the already input reference range on each analyzer, but we considered the Ethiopian reference ranges as well [39].
- ✓ Limited studies were available on similar analyzer models, making comparison difficult.
- ✓ Accuracy was not made due to lack of standard materials.

9. Conclusion and Recommendation

9.1 Conclusion

Cell-Dyn 1800, Sysmex KX-21, and Horiba ES60 comply with the manufacturer's specification and the CBC values performed by these analyzers are in good agreement as well as the analyzers can also be used interchangeably with care for MPV and MXD cells. TASH being the tertiary level referral center for hematological disorders and having several patients on follow up, the current finding gives confidence on the reliability of the results, since patients' blood may not always be tested by the same machine.

9.2 Recommendations

- ✓ The differential count on mixed cells must be confirmed by using manual count.
- ✓ Regular verification against the manufacturer's specifications should be made for all analyzers.
- ✓ Evaluation and agreement should also be done on other analyzers in the hospitals and when new analyzers are introduced.
- ✓ MPV and mixed cells showed relative variation among the different instruments, therefore the value of these parameters should be interpreted carefully since majority of the patients are on regular follow up at the hospital.

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11. Annexes

Annex I. English Version of Participant Information Sheet

Addis Ababa University College of Health Sciences School of Allied Health Science

Department of Medical Laboratory Sciences

Title: Verification and comparison of Sysmex KX-21N, Cell-Dyn 1800 And Horiba ABX Micros ES 60 automated hematology analyzers at Tikur Anbessa Specialized Hospital Addis Ababa, Ethiopia.

Introduction

This information sheet is prepared by the principal investigator to clarify the study that you are asked to take part in. If there is any unclarity before you decide to participate or not, you can ask freely.

Purpose

We have planned to conduct a study with the objective of verifying performance and comparing agreement of Cell-Dyn 1800, Sysmex KX-21N, and Horiba ABX ES 60 hematology analyzers at Tikur Anbessa specialized hospital Addis Ababa, Ethiopia.

Confidentiality

Any information that we are going to collect about you during this research will be kept confidential. Your name and identity on the request paper will be changed to confidentiality code for the purpose of this study. Samples and information given by the participants will serve only for this research not for any other purpose.

Benefit

Findings from this study will be utilized to improve patient care by managers, administrators, and policy makers. So, you are indirectly benefiting yourself, other patients and the society at large by involving into this quality related laboratory research.

Person to contact

Please direct any questions or problems you may encounter during this study to the principal investigator:

Henok Kebede,

Department of Medical Laboratory Tikur Anbessa Specialized Hospital laboratory technologist.

Tel: +251-913035170 **email:** henikeb@ yahoo.com

Annex II Amharic Version of Participant Information Sheet

አዲስ አበባ ዩኒቨርስቲ ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ትምህርት ክፍል

ርዕስ፡ በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል ህክምና ላቦራቶሪ ክፍል ውስጥ የሚገኙትን የሲስሜክስ KX-21፣ የሴል ዳይን 1800 እና የሆሪባ ABX micros ES 60 ሄማቶሎጂ መመርምሪያዎችን በአምራቾቹ መመሪያ መሰረት መገምገም እና እርስ በእርስ ያላቸውን ስምምነት ማወዳደር፡፡

መግቢያ

ይህ የመረጃ ወረቀት እንዲሳተፉበት የተጠየቁትን ጥናት ግልጽ ለማድረግ ሲባል በተመራማሪው በኩል ተዘጋጅቶ የቀረበ ጽሁፍ ነው፡፡ በጥናቱ ለመሳተፍ ወይም ላለመሳተፍ ከመወሰንዎ በፊት ማንኛውም ያልገባዎት ነገር ካለ በግልጽ መጠየቅ ይችላሉ፡፡

ዓላማ

በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል ህክምና ላቦራቶሪ ክፍል ውስጥ የሚገኙትን የሲስሜክስ KX-21፣ የሴል ዳይን 1800 እና የሆሪባ ABX micros ES 60 ሄማቶሎጂ መመርምሪያዎች ለማወዳደር የሚያስችል ጥናት ለማካሄድ አቅደናል፡፡

የተሳታፊዎች ምስጢር ስለመጠበቅ

በዚህ ጥናት ስለእርስዎ የምንሰበስበው ማንኛውም መረጃ ሚስጥራዊነቱ ይጠበቃል፡፡ለጥናቱ ሲባል በመጠየቂያው ወረቀት ላይ ያለውን የእርስዎ ስምም ሆነ ማንነት ወደ ሚስጥራዊ ቁጥር ይቀየራል፡፡ እንዲሁም የሰጡት ናሙናም ሆነ መረጃ ከዚህ ጥናት ውጪ ለሌላ አላማ ጥቅም አይውልም፡፡

ጥቅም

ከዚህ ጥናት የሚገኘው ውጤት የህሙማንን ጤና ለማሻሻል፣በአስተዳዳሪዎች እና በመመሪያ አውጪዎች ጥቅም ላይ ይውላል፡፡ ስለዚህ እርስዎም ከላቦራቶሪ ውጤት ጥራት ጋር በተገናኘ የላቦራቶሪ ጥናታዊ ምርምር ላይ በመሳተፍዎ በቀጥታ እራስዎትን፣በተዘዋዋሪ ደግሞ ሌሎች ህሙማንን፣ እና በአጠቃላይ ህብረተሰቡን ይጠቅማሉ ማለት ነው፡፡

ጥያቄ ካለዎት

ይህን ጥናት በተመለከተ ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ ችግሮች ወይም ጥያቄ ካለዎት በሚከተለዉ አድራሻ ይጠቀሙ፡፡

ሄኖክ ከበደ

በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል ላቦራቶሪ ክፍል ላቦራቶሪ ቴክኖሎጂስት፡፡

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Annex III. English Version of Informed Consent Form

Consent form

I, the undersigned, confirm that, as I give consent to participate in the study, it is with a clear understanding of the objectives and conditions of the study and with recognition of my right to withdraw from the study if I change my mind. I give consent to include me in the proposed research. I have been given the necessary information about the research. I have also been assured that I can withdraw my consent at any time without penalty or loss of benefits. The proposal has been explained to me in the language I understand**.**

Name ----------

Signature: ----------

Annex IV Amharic Version of Informed Consent Form

ስለ ጥናቱ ማረጋገጫ

እኔ ስሜ ከታች የተገለጸው የጥናቱ ተሳታፊ ለመሆን ስወስን የጥናቱ አላማ፣ አሰራሮች እና ቅድመ ሁኔታዎች በግልጽ በመረዳት እና ለጥናቱ ተሳታፊነት ፍቃደኝነቴን በማንኛውም ደረጃ የማንሳት መብቴን በማረጋገጥ ነው፡፡ በመሆኑም በጥናቱ ተሳታፊ ለመሆን ስወስን በጥናቱ ሳቢያ ሊከሰቱ የሚችሉ አደጋዎች በሚገባ የተረዳሁና ከጥናቱ በማንኛውም ደረጃ እራሴን ለመሰረዝ ብወስን ተገቢ የሆኑ እገዛዎች ሁሉ እንደማይነፈጉኝ በማመን ነው፡፡ እነዚህን መረጃዎች ሁሉ በሚገባ በምረዳው ቋንቋ የተገለጸልኝ መሆኑን በፊርማዬ አረጋግጣለሁ፡፡

ሙሉ ስም ---------- ፊርማ----------

Annex V Standard Operating Procedure for Cell-Dyn 1800

Reagents

- Cell-Dyn 1800 Diluent of 20 liter
- Cell-Dyn 1800 Detergent of 20 liter
- Cell-Dyn 1800 Lyse of 3.8 or 5 liter
- Cell-Dyn 1800 Enzymatic cleaner
- Cell-Dyn 1800 Calibrator
- Cell-Dyn 1800 tri- level control reagents

Reagents preparation:

- Reagents are commercially prepared

Reagents stability and storage:

- All the above three reagents are stored at room temperature and the last three reagents are stable at 2-8°C up to their expiry date.

Supplies

- ✓ Disposable glove
- ✓ BD Vacutainer tube (EDTA anticoagulant tube)
- ✓ Dry gauze
- ✓ Cotton Swab
- ✓ Vacutainer
- ✓ Needle with holder
- ✓ 70 % Ethanol alcohol
- ✓ Tourniquet

Equipments

- ✓ Cell-Dyn 1800 hematology analyzer
- ✓ Key Board
- ✓ Electrical blood mixer
- ✓ Electrical power stabilizer (500 or 1000 w)
- ✓ Printer

Sample

Whole blood specimen, collected in K_2EDTA anticoagulant

Amount required

¾ of the lavender collection tube (3 ml)

Transport and Storage: 2-8 ^{0}C

Procedure for sample run

1. Prior to running patient specimens, perform DAILY Start-up procedure
2. When the READY message is displayed on the RUN Screen, the instrument is ready to run specimens. To run patient specimens, proceed as follows
3. With the cap tightly secured on the specimen tube, slowly invert the tube 10 to 15 times
4. Remove the cap from the pre-mixed specimen tube
5. Place the tube under the aspiration probe and raise the tube so that the end of probe is deeply immersed in the specimen
6. Press the Touch Plate to activate the run.
7. When the sample has been aspirated from the tube, the probe will move up through the Wash Block. Remove the specimen tube and Recap the tube
8. After the cycle is completed, run results are displayed on screen and the aspiration probe moves into position to accept a new specimen. The current run data is saved to the data log
9. If Automatic Graphics Printout has been specified in the SETUP menu, a report is Printed according to the parameters selected during the setup procedure
10. If Automatic Graphics printout has not been specified in the SETUP menu, press [PRINT REPORT] to obtain a copy of the results. The print report format is the only method to be used for reporting patient results

Annex VI Standard Operating Procedure for Sysmex KX-21N

Reagents

Cell Pack: - is ready to use for impedance and photoelectrical analysis of whole blood and its ingredients are: sodium chloride, boric acid, sodium tetra borate, and K_2EDTA.

Stromatolyzer WH: - is ready to use lysing reagent to analyze the leucocytes by lysing the RBC and left the WBC free and easy to count; whole blood sample by resistance measurement and photometric measurement and its ingredients are: nonionic surfactant, organic quaternary ammonium salt.

Cell Clean: - is a strong alkaline detergent to remove lysing reagents, cellular residuals and blood proteins remaining in the hydraulics of Sysmex analyzer.

Reagents preparation: Reagents are commercially prepared.

Reagents stability and storage: All reagents are stable at room temperature up to their expiry date.

Supplies

- ✓ Disposable glove
- ✓ BD Vacutainer tube (EDTA anticoagulant tube)
- ✓ Dry gauze
- ✓ Cotton Swab
- ✓ Vacutainer
- ✓ Needle with holder
- ✓ 70% Ethanol alcohol
- ✓ Tourniquet

Equipments:

- ✓ Sysmex KX-21N
- ✓ Electrical blood mixer
- ✓ Electrical power stabilizer (500 or 1000 w)

Sample

Whole blood specimen collected in K_2EDTA anticoagulant tube.

Amount required

¾ of the lavender collection tube (3 ml)

Transport and Storage: 2-8 ^{0}C

Aspiration

- Whole blood mode- Approximately 50µl
- Pre-diluted mode-Approximately 20µl

Dilution

In pre-dilution mode a sample is diluted in to 1:26 before analysis.

E.g. 20µl of blood in 500µl of cell-pack.

The pre-dilution mode is used in analyzing a small amount of child's blood which is collected from the earlobe or finger.

Instrument dilution

In whole blood mode

- The dilution for HGB and WBC 1:500
- The dilution for RBC 1:25000

In pre-dilution mode

- The dilution for HGB and WBC is 1:1000
- The dilution for RBC is 1:25000

Analytical procedure

1. Mix the sample sufficiently
2. Remove the plug while taking care not to allow blood scatter
3. Set the tube to the sample probe and in that condition, press the start switch
4. The buzzer sounds two times - "beep, beep" - and when the LCD screen displays "Analyzing," remove the tube. After that, the unit executes automatic analysis and displays the result on the LCD screen. Then the unit turns to the Ready status, becoming ready for analysis of the next samples.
5. When the LCD screen displays "Ready," prepare the next samples and repeat the above procedures.

- ✓ To analyze the sample in Pre diluted Mode, first switch the analyzer to Pre diluted mode and follow the procedure as Whole Blood analysis.

Annex VII Standard Operating Procedure for Horiba ABX micros ES $_{60}$

Reagents

ABX Minidil & Minidil LMG

This reagent is necessary for the process involving Stabilizing, Counting, and Differentiating the blood cells.

ABX Minilyse and/or Alphalyse

This reagent is necessary for the lysing of the red blood cells, stabilizing, counting, and differentiating the white blood cells along with determining the hemoglobin concentration.

ABX Miniclean

This reagent is necessary for the washing of protein buildup from the counting apertures and chambers.

ABX Minoclair

This reagent is necessary for the concentrated cleaning procedure. A diluted bleach solution may also be used for this procedure as well.

Reagents preparation: Reagents are commercially prepared.

Reagents stability and storage: All reagents are stable at room temperature up to their expiry date.

Supplies

- ✓ Disposable glove
- ✓ BD Vacutainer tube (EDTA anticoagulant tube)
- ✓ Dry gauze
- ✓ Cotton Swab
- ✓ Vacutainer
- ✓ Needle with holder
- ✓ 70% Ethanol alcohol
- ✓ Tourniquet

Equipments:

- ✓ Horiba ABX micros ES $_{60}$
- ✓ Electrical blood mixer
- ✓ Electrical power stabilizer (500 or 1000 w)

Sample

- ✓ Whole blood specimen collected in K_2EDTA anticoagulant tube

Amount required

- ✓ $^3\!/_4$ of the lavender collection tube (3 ml)

Transport and Storage: 2-8 ^{0}C

Analytical Procedure

Power “ON” the instrument by pressing the ON/ OFF switch, located on the back, lower center of the rear panel. The LCD display will show the following: PLEASE WAIT FOR 3 MIN ESCAPE: ESC this indicates an instrument warm-up period for the internal electronics. Once the instrument warm-up phase is complete, the front panel LED will turn from “Red” to “Green” indicating that the initialization phase is complete. The ABX MICROS 60 will now automatically run a STARTUP CYCLE, if and only if the instrument has been programmed for an AUTO-Startup cycle. Check and verify the background counts. Blood samples must be gently and thoroughly mixed with a rocking or tilting motion just before placing the sample into the tube holder for the analysis cycle. Before analyzing any patient blood samples, it is recommended that the operator performs quality control analysis on 3 levels of control blood material, (Low, Normal, and High) to verify that the ABX MICROS 60 is performing within the specified ranges of the quality control material.

Sample analysis

Select the position of the tube holder.

• Mix the sample gently and thoroughly.

• Place the sample in that position of the tube holder.

• Close the tube holder door.

• The analysis cycle will begin if and only if “Auto-Start” was selected in the set-up menu.

• Press the “START” key if and only if “Manual-Start” was select in the set-up menu. The analysis cycle will take approximately 60 seconds. At the end of the cycle, the result prints out, the LED on the front panel turns from “Red” to “Green” and the ABX MICROS 60 is ready for the next analysis.

Annex VIII Instrument summary

Analyzer	Throughput per hour	Operating temperature	Reagent consumption per cycle
Cell-Dyn 1800	60 samples	18^0C-30^0C	Diluent= 27 ml, lyse=5 ml, detergent=32 ml
Horiba ES60	55 samples	18^0C-32^0C	Diluent=27 ml, lyse=11.6 ml, auto clean=32.2 ml, concentrated clean=18.5 ml
Sysmex KX-21	60 samples	15^0C -30^0C	Diluent=30 ml,*WH lyse=1ml

***WH=WBC and HGB lyse**

Annex IX: Declaration

I the undersigned, declare that this is my original work and has not been presented for a degree in this or any other university and all sources of materials used for this thesis have been acknowledged.

Name: **Henok Kebede**

Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia

Date of submission _____

This thesis has been submitted with our approval as University Advisors

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Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia

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Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia

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