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Comparison of Manual Platelet Estimates and Automated Platelet Count at Tikur Anbessa Specialized Hospital, Addis Ababa

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This is to certify that the thesis prepared by Elias Bisrat, entitled: ***Comparison of Manual Platelet Estimates and Automated Platelet Count at Tikur Anbessa Specialized Hospital, Addis Ababa*** 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
ALL	Acute Lymphocytic Leukemia
AML	Acute Myeloid Leukemia
CBC	Complete Blood Cell Count
CHD	Chronic Heart Disease
CLD	Chronic Liver Disease
CLL	Chronic Lymphocytic Leukemia
CML	Chronic Myeloid Leukemia
EDTA	Ethylene Diamine Tetra Acetic Acid
IDA	Iron Deficiency Anemia
MKs	Megakaryocytes
OIF	Oil Immersion Field
PBS	Peripheral Blood Smear
PI	Principal Investigator
PLT	Platelet
RBC	Red Blood Cell
RVI	Retroviral infection
SD	Standard Deviation
SOP	Standard Operating Procedure
TASH	Tikur Anbessa Specialized Hospital
UTI	Urinary tract infection
WBC	White Blood Cell

Operational definitions

Thrombocytosis - High Platelet count above the normal range (Platelet count >337 x 10^9/L).

Thrombocytopenia - Low Platelet count below the normal range (Platelet count <98 x 10^9/L).

Severe thrombocytopenia: Platelet count below 20×10^9 /L.

Platelet:RBC ratio: a ratio obtained from number of platelets/1000 RBC.

Platelet estimate: The number of platelets per 1000 RBC multiplied by automated RBC count in 10^6/uL to get an estimation of platelet count in 10^3/uL. This is done by counting the proportion of platelets relative to 1000 red cells in a thin part of a film made from an EDTA blood sample using the x100 oil immersion objective. Or the number of platelets in 10 OIF and multiplying the average number of platelets in an OIF by 20,000.

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 opposite. A 0.0 value indicates that the two measurements are unrelated.

Bland Altman plot: a difference plot which put the difference on the dependent axis scattered around the bias which is y- axis and the average of the two methods on the independent axis which is x- axis.

Limit of agreement: It represents the range of values in which agreement between methods will lie for approximately 95% of the sample.

Abstract

Background: The accuracy of automated platelet counts can be compromised when measuring severely thrombocytopenic samples. This is especially of concern because current clinical guidelines lowered the prophylactic platelet transfusion threshold to 10×10^9/L for patients without additional risk factors. This count is a threshold for background count of many hematological analyzers, thus making validation of the automated platelet count a crucial step.

Objective: To compare manual platelet estimates and automated platelet count by Sysmex KX 21N at Tikur Anbessa Hospital (TASH).

Methods: A hospital based cross-sectional comparative study was conducted in Tikur Anbessa Specialized Hospital from January to March 2017 on 320 blood samples. Platelet count was enumerated by sysmex KX 21 and by manual method. Number of platelets/1000 RBC in PBS was multiplied by automated RBC count in 10^6/uL to get an estimate of platelet count in 10^3/uL and the average number of platelet/10 oil emersion field (OIF) was multiplied by 20,000. The data were entered and analyzed using SPSS version 20 and interpreted accordingly. Statistical significance was determined at 95% confidence interval. Pearson correlation coefficient was used to determine correlation between the methods. Bland Altman plot was used to assess agreement between tests. Figures and tables were used for the description of the data.

Result: For normal platelet count the Paired t-test showed non-significant difference between automated and PLT estimated from average platelets/10 OIF ($p>0.05$), were positively correlated ($r=0.994$) and in the limit of agreement range (95%). For severe thrombocytopenic samples the two platelet estimation methods did not show significant difference when tested by paired t-test ($p>0.05$), were positively correlated ($r=0.869$) and in the limit of agreement range (97.5%).

Conclusions: The mean PLT count by automated and PLT estimated from PLT:RBC ratio for normal count did not show significant difference so the two methods could give the same PLT count result. The mean PLT count for severe thrombocytopenic patient samples did not show significant difference when analyzed by the two manual platelet estimation methods.

Key words: Peripheral blood smear, Platelet count, Platelet: red blood cell ratio, platelet estimate.

1. Introduction

1.1. Background

Platelets are small anucleated cell fragments that have a characteristic of discoid shape and a diameter ranging from 1 to 3 μm. They are formed from the cytoplasm of megakaryocytes (MKs), their precursor cells, which reside in the bone marrow [1]. Megakaryocytes are enormous cells that reside in the bone marrow and elsewhere during development and undergo highly complex processes to generate platelets. A single megakaryocyte can give rise to 1000- 3000 platelets [2].

The life span of a normal platelet is about 7-10 days, and they are destroyed by the macrophages in the spleen. The platelet in peripheral blood is heterogeneous with respect to size, density, and staining characteristics [3]. On films made from blood anticoagulated with the strong calcium chelating agent, EDTA and stained with Wright stain, platelets appear as small, bluish grey, oval to round bodies with several purple-red granules [4].

Platelets are extremely versatile effectors of hemostasis, inflammation, and immune activity with specialized roles in host defense, response to injury, and immune surveillance [5]. Normally the platelet count for Ethiopians ranges from 98 to 337×10^9 /L [6]. In a number of clinical conditions the platelet count may be increased (thrombocytosis) or decreased (thrombocytopenia). Both thrombocytosis and thrombocytopenia need to be evaluated and treated. Severe thrombocytopenia in which the platelet count falls below 20×10^9 /L present as internal and external bleeding and sometimes may be fatal. On the other hand, thrombocytosis may present as thrombotic events [7].

Platelets have proved to be more difficult to count than red or white cells. The methods commonly used for counting platelets are: Manual method using counting chamber; Examination of a peripheral blood smears (PBS); and Using automated hematology analyzers. An accurate and reproducible platelet count is essential for proper patient management. Manual method is time consuming, subjective and tedious with high levels of imprecision. The introduction of automated full blood counters using impedance technology resulted in a dramatic improvement in precision. However, impedance counts still have limitations as cell size analysis cannot discriminate platelets from other similar-sized particles. More recently, light scatter or

fluorescence methods have been introduced for automated platelet counting, but there are still challenges in obtaining accurate platelet count [8].

A peripheral blood smear (PBS) is usually performed to verify the automated platelet count, particularly if it is flagged by the analyzer for verification or if it is significantly lower than the lowest limit of the reference range. Many laboratories opt to verify the automated platelet count when it is below 100×10^9/L on a new patient or when a delta-check fails with a significant drop in the platelet count (≥ 50% drop) on follow-up blood counts. Verification of platelet count below 100×10^9/L is important because pseudo-thrombocytopenia of this magnitude may unnecessarily trigger a hematology consult, additional laboratory work-up, postponement of surgery/special procedure, and/or a platelet transfusion [9].

Estimation of platelet count from PBS is done by counting the number of platelets in 10 OIF and multiplying the average number of platelets in an OIF by 20000. This count is reasonably close to automated machine counts. If RBC by a semi-automated counter is available, it is possible to obtain an approximation of the platelet count. This is done by counting the proportion of platelets relative to 1000 red cells in a thin part of a film made from an EDTA blood sample using the x100 oil immersion objective. The number of platelets per 1000 RBC is multiplied by automated RBC count in 10^6/uL to get an estimation of platelet count in 10^3/uL. The estimation of platelet count from peripheral blood smears must be reviewed properly for erroneous count. It is well documented that even the most expensive and most effective machine is not able to replace human judgment of microscopic analysis [10].

1.2. Statement of the problem

Platelet count is an important element of the diagnostic and treatment process in many disorders. In patients with thrombocytopenia, especially in the case of platelet transfusion, the reliability of the platelet estimation is highly desired and necessary to provide appropriate treatment [11, 12].

Measurement of platelet counts using automated hematology analyzers is usually quite precise and accurate. However, the accuracy of automated platelet counts can be compromised when measuring very low platelet counts or in the presence of interference from non-platelet particles or platelet abnormalities [13]. Studies, mainly focusing on the counts of low levels of platelets, demonstrated that automated counts were not as accurate in severely thrombocytopenic samples. These findings are of concern because current clinical guidelines lowered the prophylactic platelet transfusion threshold to 10×10^9/L for patients without additional risk factors [14]. This count is a threshold for background count of many hematological analyzers, thus making validation of the automated platelet count a crucial step.

In patients with severe thrombocytopenia obtaining accurate and precise platelet counts by automated analyzers is still challenging especially when a clinical decision has to be made for platelet transfusion. A recent result of the international Biomedical Excellence for Safer Transfusion Collaborative study clearly indicated that significant inaccuracy exists when counting low levels of platelets using routine hematology analyzers. This inaccuracy might impact on over or under‐transfusion of platelet concentrates to patients at high‐risk of bleeding [15].

In addition to this limitation of the technology, automated platelet counts can be inaccurate even at normal or high platelet ranges owing to the characteristics of blood specimens. For example, inaccuracies are seen in specimens with a substantial amount of interfering particles, including WBC fragments, RBC fragments, immune complexes, bacteria, lipid droplets, or protein aggregates. WBC fragments can cause the spurious elevation of platelet counts in patients with acute leukemia at diagnosis and during chemotherapy [16, 17].

False decreases in the impedance platelet count will occur in the presence of large platelets and if there is platelet clumping as seen with pseudo-thrombocytopenia by EDTA dependent agglutinins [13].

The International Council for Standardization in Hematology and the International Society for Laboratory Hematology have recommended the use of labeled platelets in a fluorescence flow cytometer, together with a semi-automated, single channel aperture impedance counter as the reference method for platelet counting, but few centers are able to afford this [18].

Some authors recommend calculating the average number of platelets counted in 10 oil immersion fields; the adequate values are included between 8 to 20 platelets per field. The average number of platelets is then multiplied by a factor of 20,000 for wedge preparations or 15,000 for monolayer in order to obtain the platelet count per micro litter [19-21]. As to my knowledge, no study evaluated the use of platelet estimates, thus, this study is planned to compare manual platelet estimates and automated platelet count.

1.3. Significance of the study

This study was carried out to provide information about manual platelet estimation and to verify the reliability of these methods by comparing with the automated platelet count. TASH is the highest level referral center for patients coming from all over Ethiopia including severely thrombocytopenic patients requiring platelet transfusion. The finding of this study will help improve the clinical management of such patients. The TASH laboratory managers will be benefited as the finding helps to develop appropriate guideline for platelet estimation. In addition, as far as my literature review goes, there is no published research from Ethiopia; thus, the study findings can be used as base line data for further studies.

2. Literature review

A study was conducted in India by Bajpai, *et al* in 2015 to compare the estimation of platelet count done by peripheral smear method and by automated cell counter in 92 EDTA samples of patients. The result showed no significant difference of values between peripheral blood smear (PBS) method of platelet estimation when compared with that of automated cell counter platelet value [22].

Another cross-sectional study by Asim Momani *et al* from India conducted in 2015 evaluated the accuracy of manual platelet count estimates by comparing to automated counter results. They randomly selected blood specimens of patients attending the Clinical Laboratory during August through October 2013 and did their evaluation using the stained thin blood film and automated analyzer for the same sample at the same time in the Hematology department. The manual method of platelet count estimates on blood smears were not significantly different from the counts by Sysmex KX 21 automated counter. Despite that platelet count estimates with the manual method in general are slightly higher than the automated method, it is a reliable technique and appears to provide platelet count estimates to be used in quality assurance [23].

A prospective study was conducted in a tertiary care hospital on 200 EDTA anticoagulated blood samples in India during October-November 2015 by Umarani MK *et al*. They estimated and verified the reliability of platelet count indirectly from peripheral blood smear (PBS) on the basis of platelet: red blood cell (RBC) ratio using automated RBC count in comparison to automated platelet counts. The result showed automated platelet count ranged from 20 – 688 x 10^3/uL and had a mean value of 308 x 10^3 / uL. Platelet count estimated by the method used in this study had a range of 15 – 695 x 10^3 / uL and the mean were 309 x 10^3 /uL. Two sample t-tests showed no significant difference between the two methods. Pearson correlation of the two methods gave a value of 0.978, showing that the two methods are highly positively correlated [24].

Abid BF *et al* from Iraq conducted a study in 2009 to verify the reliability of the estimation technique of platelet count on the basis of red cell: platelet ratio. Platelet count range using the manual method was 100- 499x10^3/µl, the mean count was 263.11±104.07 x10^3/µl, and the median was 247.5 x10^3/µl. Using the automated method, platelet count ranged between 95-484 x10^3 /µl, the mean was 258.43 x10^3 /µl, and the median was 242.5 x10^3 /µl. There was no

significant difference in results of platelet count using both methods. Regression analyses gave the following equation by comparing the automated (y) to the manual method (x): y=0.9893x - 1.8621 (r= 0.966). The paired t test showed no significant difference between the two methods. The Intra-class Correlation Coefficient (ICC) was equal to 0.988. The plot of the differences between the automated and manual values against their means showed that the difference mean was 2.116 with a standard deviation SD= 40.215. It was noticed that 93% of the differences were within the agreement limits (mean±2SD) [25].

In Aden – Yemen, a cross sectional study was conducted at the National Center of Public Health Laboratories (NCPHL), during October-December 2011 by Bakhubaira S *et al* with an overall aim to compare the results of platelet count by the automated versus the manual methods. The mean platelets count estimated by the manual method was 225.2 ± 95.4 × 10^9 /L, while that estimated by the automated method was 245.7 ± 109.8 × 10^9 /L, with no significant statistical difference between both means. The Pearson correlation test showed significant positive correlation between both methods (r: 0.563). This correlation remained significant for samples of normal count by the two methods (r: 0.359). But there was insignificant negative correlation for samples of low or high counts determined by the two methods (r: -0.151 and -0.559), respectively. The study concluded that platelet count is not varied when done by manual or automated methods, but in every method, it should be accompanied by platelet estimate, especially with abnormal counts [26].

In 2015, in Oman Mohamed-Rachid B *et al* conducted a cross sectional study in a university hospital with the objective to evaluate the performance of three methods (impedance, optical light, immunological) along with the manual technique in thrombocytopenic patients. Compared to other techniques, impedance method provided an overestimation of platelet count and failed to show a result in 15% of cases with low platelet counts (<15 × 10^9/l). Good to excellent correlations and reliability values were evidenced among study methods, but a poor reliability was noticed between the impedance and immunological methods with an intraclass correlation coefficient of 0.49 (confidence interval: 0.15–0.73; P = 0.003). In the bias analysis, the impedance method showed the highest levels of disagreement with other techniques.

This study concluded that the optical or immunological technique appear to be superior to the impedance method in estimating low platelet counts. As such, the health care staff and the

physicians must be aware of this limitation, especially in the presence of severe thrombocytopenia, when a decision of platelet transfusion has to be made [27].

In Algeria, Brahimi *et al* conducted a study in 2008 to estimate the platelet count indirectly by using the automated red blood cell (RBC) and calculating the platelet count on the basis of the red cell: platelet ratio in a stained blood film and to verify the reliability of this technique. The regression analyses for the entire data set collected in this study with the two laboratory methods gave the following least squares equation by comparing the automated (y) to the manual method (x): $y=0.8548x + 12.013$ ($r=0.908$). The paired t-test showed no significant difference between the two methods and the Intra-class Correlation Coefficient (ICC) was equal to 0.905. The plot of the differences between the automated and manual values against their means according to Band and Altman design showed that the difference mean was 3.209 with a standard deviation SD=46.331. It was noticed that 93% of the differences were within the agreement limits (mean±2SD), and that 77% of the differences were less than 20,000 platelets/µl [28].

All in all, most of the published data indicated the usefulness of platelet estimates while no published study exist in Ethiopia as far as my literature search goes. Hence, necessitating the current study in a tertiary care teaching and referral hospital of the country, where most of platelet transfusions are taking place.

3. Objectives

3.1.General objective

- To compare manual platelet estimates against the automated platelet count by Sysmex KX 21N at Tikur Anbessa Hospital.

3.2. Specific objective

- To compare automated platelet count and platelet estimate from PBS based on PLT:RBC ratio.
- To compare automated platelet count and platelet estimate based on average platelets/10 oil immersion field (OIF).
- To compare platelet estimate from PBS based on PLT:RBC ratio and platelet estimate based on average platelets/10 OIF.

4. Hypothesis

There will be strong agreement between results from manual platelet estimates and automated platelet count using Sysmex KX 21N.

5. Materials and methods

5.1. Study area

This study was conducted at Tikur Anbessa Specialized Hospital (TASH) which is located in Lideta sub city in Addis Ababa. TASH was established in 1973 G.C to serve the needs of the whole country as specialized referral hospital and also as the main teaching hospital of the School of Medicine under Addis Ababa University with bed capacity of more than 700. The faculty is the oldest and the largest among the health training institutions in the country, staffed with the most senior specialists. The Hospital has 201 doctors, 627 nurses, 55 lab technologists and >115 other health professionals dedicated to providing the health care services. The hospital also has >1300 permanent and contract administrative staffs to support the hospital activities. TASH provides diagnosis and treatment for approximately 370,000-400,000 patients per year [29].

TASH laboratory has different departments and the hematology department is one of them. This department is equipped with different hematology analyzers and equipment used to perform different hematological tests. The hematology laboratory receives in the day time in average 350-380 samples with different tests requested.

5.2. Study design and period

Cross-sectional method comparison study was conducted from January through March 2017.

5.3. sample

5.3.1. Source sample

The source samples were all blood samples of patients that were collected for CBC analysis in 5ml EDTA tube.

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

Mislabeled, overfilled, inadequate, clotted and hemolyzed blood samples.

5.4. Study variables

5.4.1. Dependent variable

Platelet count.

5.4.2. Independent variables

Age, sex, platelet count category (Severe Thrombocytopenia, Thrombocytopenia, Normal, Thrombocytosis), clinical diagnosis.

5.5. Measurement and data collection

5.5.1. Sample size determination

For the method comparison studies, as stated in Clinical and Laboratory Standard Institute (CLSI) 2013 guideline a minimum of 40 specimens are recommended. Thus, a minimum of 40 samples for each of the platelet categories (normal, thrombocytopenia, severe thrombocytopenia, and thrombocytosis) was included in this study. Using convenient sampling techniques, a total of 320 specimens were analyzed in duplicate for each method [30].

5.5.2. Sampling method

Convenient sampling method was used.

5.5.3. Data collection procedure

Fresh whole Blood Samples were collected from patients of any age and gender with any diagnosis at TASH laboratory reception for CBC evaluation by phlebotomists following SOP's and transported to the hematology section. No additional samples were collected for this study as left over sample was used. Data of age, sex and clinical diagnosis of patients were taken from the request forms.

5.5.4. Laboratory analysis

CBC was performed using Sysmex KX 21N Hematology analyzer. Whereas manual platelet estimation was done on PBS stained with Wright stain. Number of platelets/1000 RBC in PBS was multiplied by automated RBC count in 10^6/uL to get an estimate of platelet count in 10^3/uL. In addition the average number of platelet/10 oil emersion field (OIF) was multiplied by 20,000. The standard operating procedures are found in Annex I and Annex II.

Principle of SYSMEX KX 2N

The KX-21N performs speedy and accurate analysis of 18 parameters including a 3-part WBC differential, plus histograms for RBC, PLT and WBC in blood. 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 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 noncyanide hemoglobin method.

The principle is that the blood sample is aspirated and measured to predetermined volume, diluted at a specific ratio, and fed into each transducer. The transducer chamber has 2 mini holes called aperture. Blood cells suspended in the diluted sample pass through an aperture causing a change in the direct current resistance between electrodes. The size of the blood cell is detected as electric pulses. The number of blood cells is calculated by counting the pulses (31).

Principle of wright stain technique

Preparation of blood film involves placing a drop of well-mixed, EDTA anticoagulated blood at one end of the slide. A second slide is used as a spreader to spread the blood across the base slide width, and to push the blood along the base slide's length. After the blood smear is dried, it was stained by wright stain. Wright's stain is a polychromatic stain consisting of a mixture of eosin and methylene blue. When applied to blood cells, the dyes produce multiple colors based on the ionic charge of the stain and the various components of the cell. The eosin ions are negatively charged and stain basic cell components giving them an orange to pink color. The methylene blue ions are positively charged and stain the acid cell components in varying shades of blue.

The neutral components of the cell are stained by both components of the dye producing variable colors.

5.6. Data quality assurance

Blood samples quality were ensured by collecting and processing according to the standard operating procedures. High, normal and low quality control materials were run every morning for SYSMEX analyzer before patient samples are run and the results were kept confidentially. Quality of the Wright's stain was checked by preparing one differential slide daily using a patient sample with a normal MCV, MCH, MCHC and total white count. The stained slides were reviewed for meeting color specifications of the different white cells, red cells and platelets under normal conditions. It was also checked for the presence of any precipitation and/or contamination. All analyses were done in duplicates. Data was recorded and documented properly by using instrument print out for the automation and careful transcription for the manual methods.

5.7. Data analysis and interpretation

The data obtained from the tests were entered and analyzed by using statistical package for social science (SPSS version 20) computer software and interpreted accordingly. Statistical significance was determined at 95% confidence interval. Pearson correlation coefficient was used to determine correlation between platelet estimates on PBS and automated platelet count. Bland Altman plot was used to assess agreement between the tests. In the Bland Altman method the differences in platelet estimates versus the automated count was plotted against mean of the two methods. Agreement considered acceptable when the difference is lying between mean $\pm$ two standard deviation (Mean$\pm$1.96SD) for 95% and above of cases. P-Values < 0.05 considered statistically significant. Figures and tables were used for the description of the data.

5.8. Ethical considerations

The study obtained ethical clearance from the Department Research and Ethics Review Committee (DRERC) of Addis Ababa University, College of Health Sciences, School of Allied

Sciences, Department of Laboratory Sciences. All information collected in this study was given code numbers and no name was recorded. The key to this code numbers kept in a locked file and was accessible to the authorized staff only.

5.9. Dissemination of the result

The finalized paper of this study will be presented and submitted to Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Laboratory science. A copy of this material will be given to TASH laboratory and will be used to improve the laboratory's practice by developing guideline. The finding will also be communicated to Ministry of Health, Addis Ababa Health Bureau, and respective hospitals. The result will also be disseminated through publication in peer reviewed local and international journals and through presenting it in relevant workshop, seminars and scientific conferences.

6. Result

6.1. Socio-demographic characteristics and clinical diagnosis of the study population related to platelet estimates and automated platelet count

A total of 320 patients' blood samples were analyzed during the study. Of them, 156 (48.8%) were from females and 164 (51.2%) were from males. The mean age ± SD of the studied patients was 38.78±17.6 and the majority was between 31-45 years (33.8%) (Table1). When relating the mean platelets count by the three methods to the sex of the studied patients, it was found that the estimated mean platelets count did not show significant difference ($P>0.05$). The paired t test for 320 samples by the three methods showed statistically significance difference ($p<0.05$) and were positively correlated when tested by a correlation test.

Table1: socio-demographic characteristics of the study population

Variables	Frequency	Percent (%)
Sex		
Male	164	51.2%
Female	156	48.8%
Age (years)		
Under 15	37	11.6%
16-30	67	20.9%
31-45	108	33.8%
40-60	71	22.1%
61 and older	37	11.6%

About 15 clinical conditions were identified in the patients from whom blood samples were obtained for this study. The majorities were having CML (15.6%) followed by AML (11.3%) and ALL (10.6%). Very low platelet count values were observed in patients with CLD and Aplastic Anemia. ALL, AML and CLL patients have low platelet counts. Normal Platelet counts were observed in CML, CHD, UTI and RVI. Bladder cancer, Breast Cancer, Cervical Cancer, IDA, Polycythemia Vera and Prostate Cancer showed high mean platelet count. The mean platelet count by the three methods did not show significant difference by clinical conditions of patients (P>0.05) (Table 2).

Table2: The mean platelet count by automated, PLT estimation based on PLT:RBC ratio and PLT estimation based on AVR PLTS/10 OIF methods according to clinical diagnosis in TASH ,January – March, 2017 (n=320).

Variable	No	Automated PLT count ($x10^9$/L)		PLT estimation based on PLT:RBC ratio		PLT estimation based on AVR PLTS/10 OIF	
Clinical diagnosis*		Mean	SD	Mean	SD	Mean	SD
ALL	34	49	92	44	91	43	90
AML	36	33	23	28	22	26	22
Aplastic Anemia	17	18	22	13	18	13	18
Bladder Cancer	11	488	153	479	149	481	156
Breast Cancer	28	379	139	375	138	371	139
Cervical Cancer	21	356	166	352	166	351	165
CHD	10	290	164	289	151	282	158
CLD	7	13	4	7	3	7	2
CLL	36	91	127	87	129	84	128
CML	50	131	157	124	156	122	153
IDA	9	379	560	364	521	361	516
Polycythemia Vera	22	654	318	633	295	628	292
Prostate Cancer	5	437	200	429	199	421	195
RVI	17	257	122	254	121	253	121
UTI	17	285	129	282	127	282	129

*ALL=Acute lymphocytic leukemia; AML= Acute myelocytic leukemia; CHD=chronic heart disease; CLD=chronic liver disease; CLL=chronic lymphocytic leukemia; CML=chronic myelocytic leukemia; IDA=Iron deficiency anemia; RVI-Retroviral infection; UTI=Urinary tract infection.

6.2. Comparison of platelet count according to platelet categories

6.2.1. Comparison of Automated PLT count and PLT estimated from PBS based on PLT:RBC ratio

When relating automated and PLT estimation based on PLT:RBC ratio according to PLT categories, the following results were obtained. For severe thrombocytopenic patients, the paired t-test showed significant difference between the methods ($P<0.05$). The correlation test was applied and showed positive correlation between the results of both methods ($r=0.825$, $p<0.001$) (figure1A). The plot of difference between the two methods against their means according to Bland and Altman design showed that the mean difference was 5.43 with a standard deviation SD=3.12 (figure2A). It was noticed that 95% of the difference was with in the agreement limit (mean±2SD).

For thrombocytopenic patient samples, as in the severe cases, the paired t-test showed significant difference ($P<0.05$). The correlation test was applied and showed positive correlation between the results of both methods ($r=0.969$, $p<0.001$) (figure1B). The plot of difference between the two methods against their means according to Bland and Altman design showed that the mean difference was 6.6 with a standard deviation SD=5.8 (figure2B). It was noticed that 97.5% of the difference was in the agreement limit (mean±2SD).

In relation to normal PLT count, the paired t-test showed significant difference ($P<0.05$). The correlation test was applied and showed positive correlation between the results of both methods ($r=0.99$, $p<0.001$) (figure1C). The plot of difference between the two methods against their means according to Bland and Altman design showed that the difference mean was 1.35 With a standard deviation SD=9.176 (figure2C). It was noticed that 97.5% of the difference was in the agreement limit (mean±2SD).

For high PLT count patient samples, the paired t-test showed significant difference ($p<0.05$). The correlation test was applied and showed positive correlation between the results of both methods ($r=0.997$, $p<0.001$) (figure1D). The plot of difference between the two methods against their means according to Bland and Altman design showed that the difference mean was 13.14 With a standard deviation SD=23.8 (figure2D). It was noticed that 96.25% of the difference was in the

agreement limit (mean±2SD). Table 3 showed the mean and the SD of platelet count by the three methods according to platelet category.

Table3: The mean platelet count by automated, PLT estimation based on PLT:RBC ratio and PLT estimation based on AVR PLTS/10 OIF methods according to platelet category in TASH ,January – March, 2017

	automated		PLT count based on PLT:RBC ratio		PLT count based on AVR PLT/10OIF	
	Mean	SD	Mean	SD	Mean	SD
Severe thrombocytopenia	12.3	5.4	6.9	3.9	6.5	3.3
Thrombocytopenia	53.2	22.9	46.6	21.1	44.4	21
Normal	231	65	230	65.1	226.5	65
Thrombocytosis	562.6	235	549.4	217	546.8	214.5

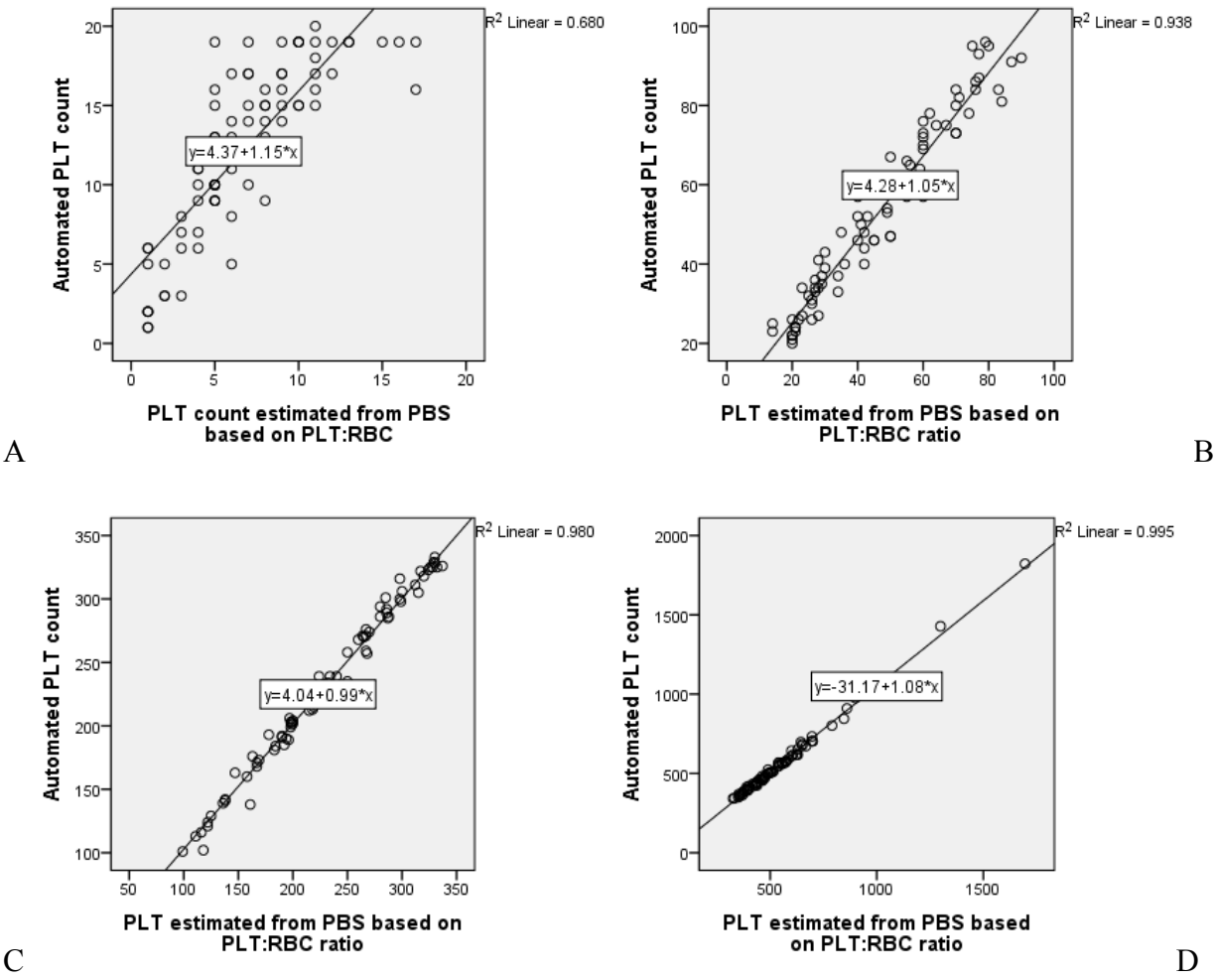

Figure 1. Correlation of automated versus PLT estimated from PLT:RBC ratio (A. Sever thrombocytopenia, B. Thrombocytopenia, C. Normal, D. Thrombocytosis) in TASH, January – March, 2017 (n=80).

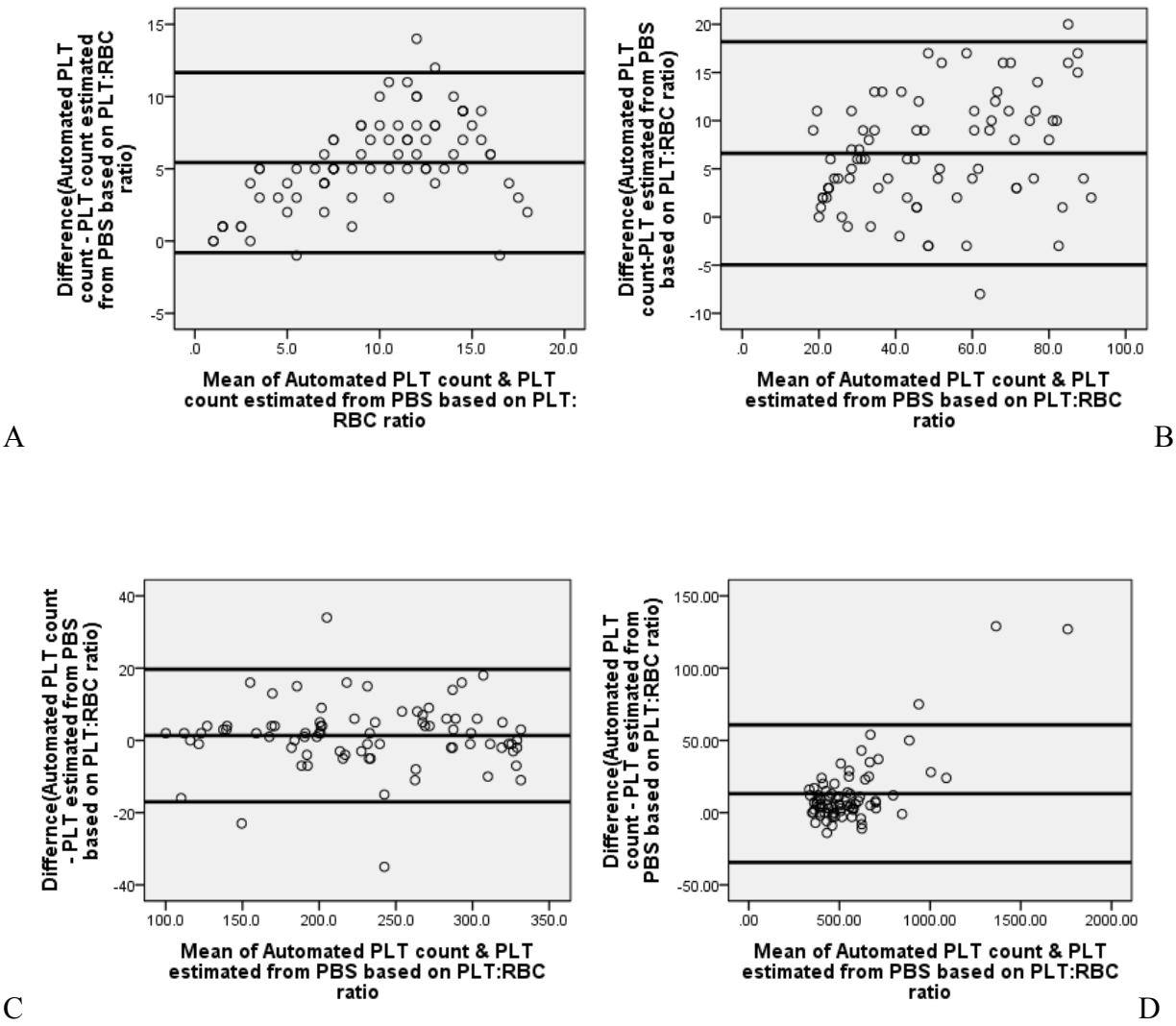

Figure 2. Differences versus mean plots for automated and PLT estimated from PLT:RBC ratio according to Bland and Altman design (A. Sever thrombocytopenia, B. Thrombocytopenia, C. Normal, D. Thrombocytosis) in TASH, January – March, 2017 (n=80).

6.2.2. Comparison of Automated PLT count and PLT estimated from PBS based on AVR PLTS/10 OIF according to PLT categories

For severe thrombocytopenic patients, the paired t-test showed significant difference ($P<0.05$). The correlation test was applied and showed positive correlation between the results of both methods ($r=0.911$, $p<0.001$) (figure3A). The plot of difference between the two methods against their means according to Bland and Altman design showed that the difference mean was 5.78

With a standard deviation SD=2.747 (figure4A). It was noticed that 97.5% of the difference was in the agreement limit (mean±2SD).

For thrombocytopenic patient samples, the paired t- test showed significant difference ($p<0.05$). When the two methods tested by correlation test, they were positively correlated ($r=0.978$), $p<0.05$) (figure3B). The plot of difference between the two methods against their means according to Bland and Altman design showed that the difference mean was 8.79 With a standard deviation SD=4.934 (figure4B). It was noticed that 92.5% of the difference was in the agreement limit (mean±2SD).

The paired t-test showed non significant difference between the two methods in patients with normal PLT count ($p>0.05$). Positive correlation was observed by the results of the two methods when tested by correlation test ($r=0.994$, $p<0.01$) (figure3C). The plot of difference between the two methods against their means according to Bland and Altman design showed that the difference mean was 4.35 with a standard deviation SD=7.123 (figure4C). It was noticed that 95% of the difference was in the agreement limit (mean±2SD).

Statistically a significant difference was observed by a paired t-test when we related the two methods in patients with high PLT count ($p<0.05$). Positive correlation was observed when the methods were tested by correlation test ($r=0.997$, $p<0.001$) (figure3D). The plot of difference between the two methods against their means according to Bland and Altman design showed that the difference mean was 15.8 with a standard deviation SD=25.88 (figure4D). It was noticed that 96.25% of the difference was in the agreement limit (mean±2SD).

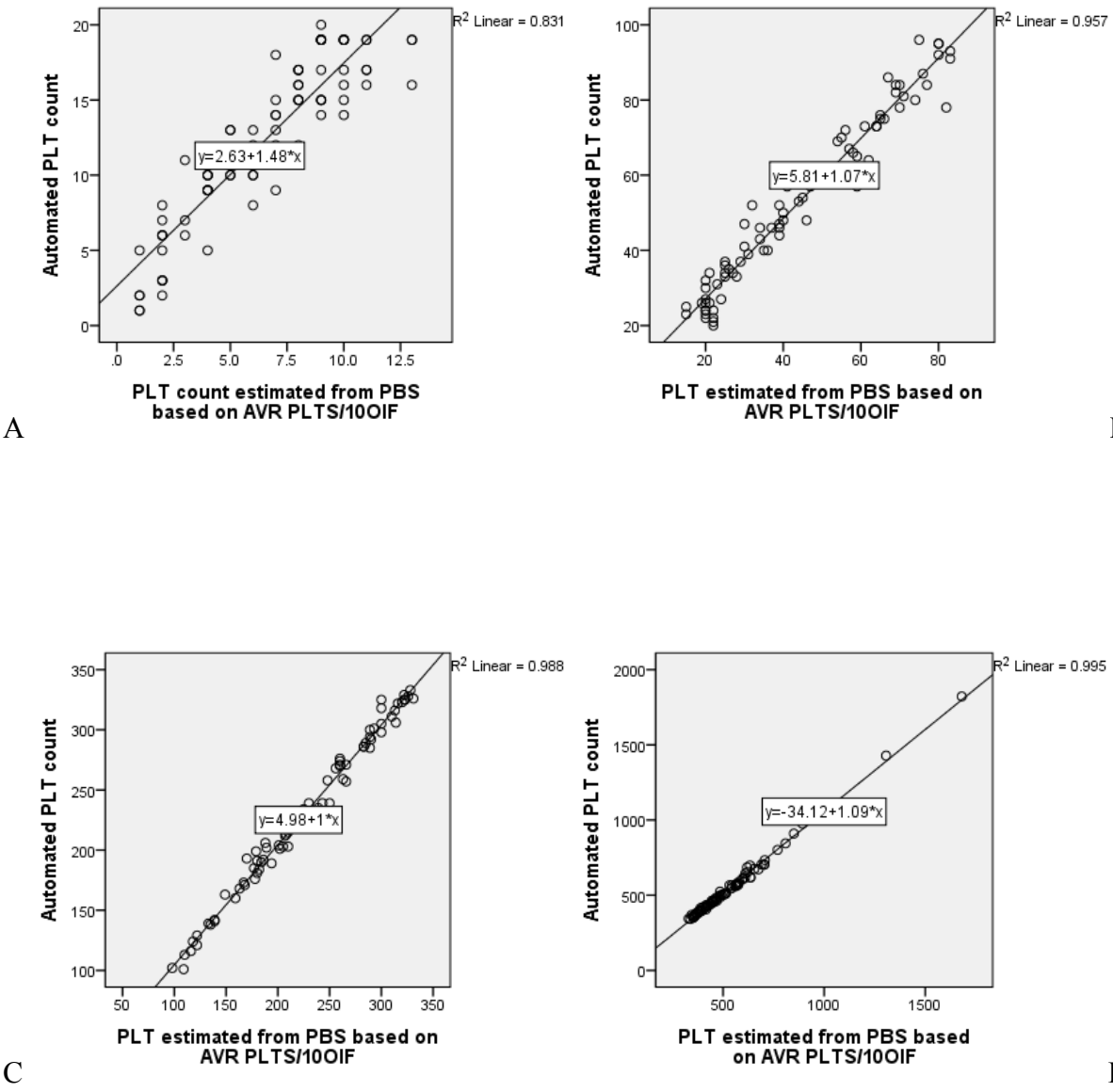

Figure 3. Correlation of automated versus PLT estimated from PBS based on AVR PLTS/10OIF (A. Sever thrombocytopenia, B. Thrombocytopenia, C. Normal, D. Thrombocytosis) in TASH, January – March, 2017 (n=80).

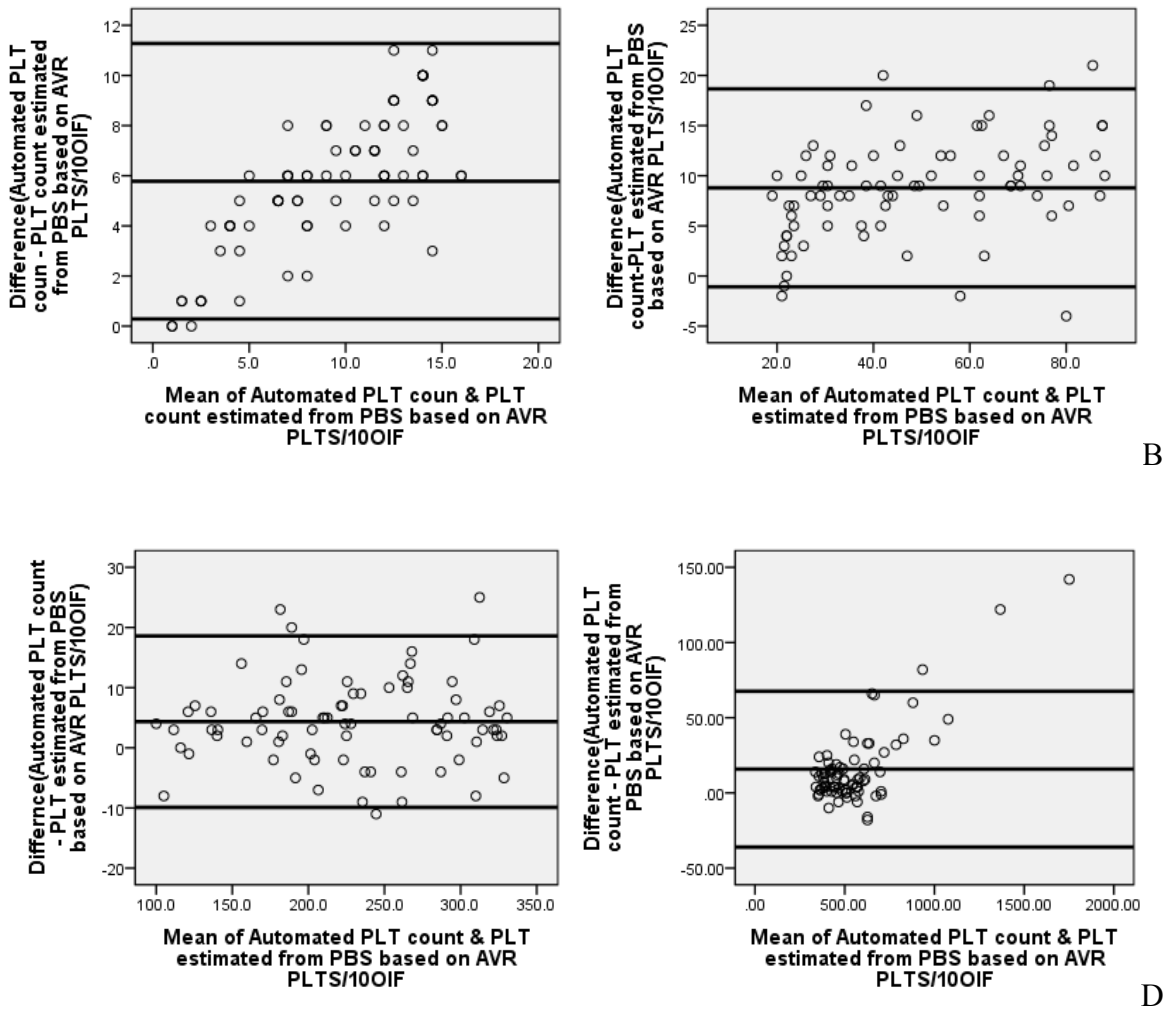

Figure 4. Differences versus mean plots for automated & PLT estimated from PBS based on AVR PLTS/10OIF according to Bland & Altman design (A. Sever thrombocytopenia, B. Thrombocytopenia, C. Normal, D. Thrombocytosis) in TASH, January – March, 2017 (n=80).

6.2.3. Comparison of PLT estimated from PBS based on PLT:RBC ratio and PLT estimated from PBS based on AVR PLTS/10 OIF according to PLT categories

For severe thrombocytopenic patient samples, there was no significant difference (p=0.105). Positive correlation was observed by the results of the two methods when tested by correlation test (r=0.869, p<0.001) (figure5A). The plot of difference between the two methods against their means according to Bland and Altman design showed that the difference mean was 0.35 with a standard deviation SD=1.91 (figure6A). It was noticed that 97.5% of the difference was in the agreement limit (mean±2SD).

For thrombocytopenic patient samples, the paired t- test showed significant difference ($p<0.05$). When the two methods tested by correlation test, they were positively correlated ($r=0.978$), $p<0.05$) (figure5B). The plot of difference between the two methods against their means according to Bland and Altman design showed that the difference mean was 2.18 With a standard deviation SD=5.725 (figure6B). It was noticed that 96.25% of the difference was in the agreement limit (mean±2SD).

In relation to normal PLT count, the paired t-test showed significant difference ($P<0.05$). The correlation test was applied and showed positive correlation between the results of both methods ($r=0.986$, $p<0.001$) (figure5C). The plot of difference between the two methods against their means according to Bland and Altman design showed that the difference mean was 3 With a standard deviation SD=10.9 (figure6C). It was noticed that 93.75% of the difference was in the agreement limit (mean±2SD).

For high PLT count patient samples, the paired t-test showed significant difference ($p<0.05$). The correlation test was applied and showed positive correlation between the results of both methods ($r=0.999$, $p<0.001$) (figure5D). The plot of difference between the two methods against their means according to Bland and Altman design showed that the difference mean was 2.7 With a standard deviation SD=10.3 (figure6D). It was noticed that 93.75% of the difference was in the agreement limit (mean±2SD).

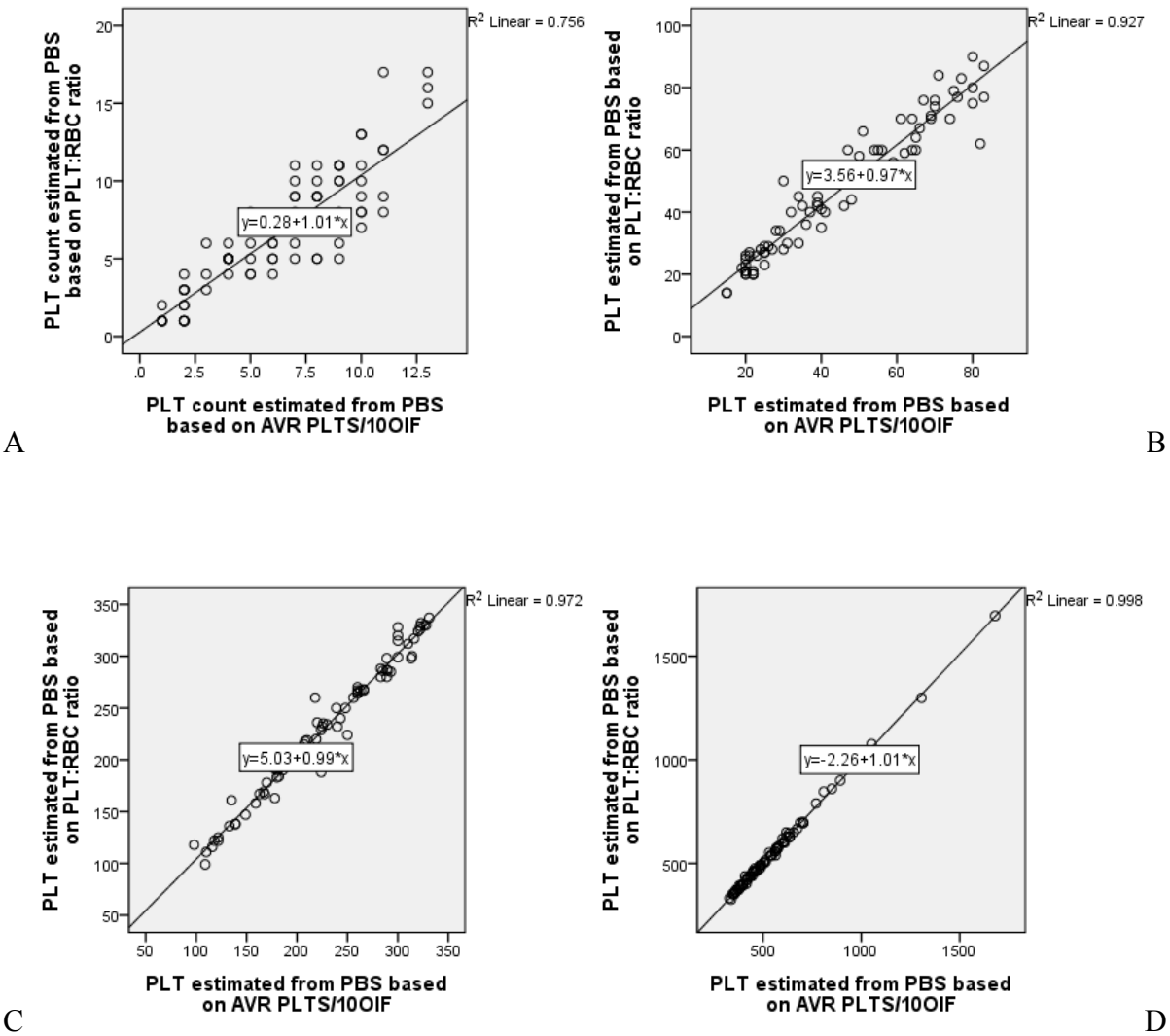

Figure 5. Correlation of PLT estimated from PBS based on PLT:RBC ratio versus PLT estimated from PBS based on AVR PLTS/10OIF (A. Sever thrombocytopenia, B. Thrombocytopenia, C. Normal, D. Thrombocytosis) in TASH, January – March, 2017 (n=80).

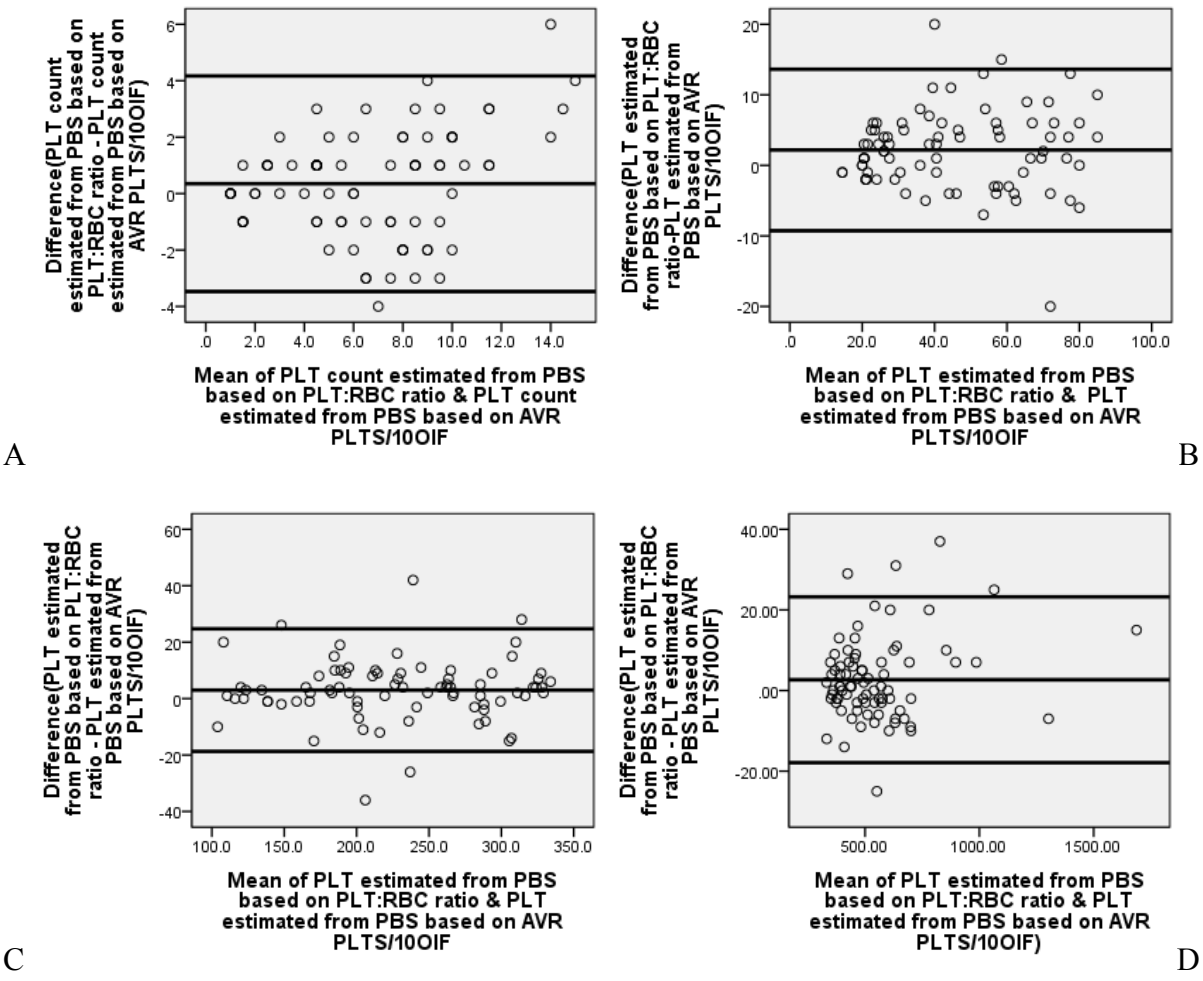

Figure 6. Differences versus mean plots for PLT estimated from PLT:RBC ratio & PLT estimated from PBS based on AVR PLTS/10OIF (A. Sever thrombocytopenia, B. Thrombocytopenia, C. Normal, D. Thrombocytosis) in TASH, January – March, 2017 (n=80).

7. Discussion

The whole 320 samples showed significant different results ($p<0.05$) between the methods but they were positively correlated. This is in line with an earlier report by Ike SO *et al* [32] who reported that PLT count by automated and manual estimation methods have statistically significant difference ($p<0.0001$) and are positively correlated ($r=0.7795$). The mean difference between automated and PLT estimated from PLT:RBC ratio, automated and PLT estimated from AVR PLT/10 OIF, and PLT estimated from PLT:RBC ratio and PLT estimated from AVR PLT/10OIF is 6.3, 8.4 and $2x10^9$/L respectively. This indicates that the automated hematology analyzer (Sysmex KX-21N) readings correlated well with the manual estimation methods. The result of the present study is in contrast with an earlier report by Bajpai R *et. al* [22] who reported that automated and manual PLT estimated from AVR PLT/10 OIF have no significant difference ($p=0.69$). However, in this present study some statistical variations observed between the automated and manual methods.

when the samples are separated according to sex and age of patients, we did not obtain any significant difference in counting by automated or manual platelet estimate in males or in females, in younger or in older patients. This is in line with a previous study conducted by Bakhubaira S [26].

For sever thrombocytopenic patient samples, automated and PLT estimated from PLT:RBC ratio, and automated and PLT estimated from AVR PLTS/10 OIF produced a significant different results with a mean difference of 6.6 and $8.7x10^9$/L respectively. Automated method and the two estimation methods were positively correlated ($r=0.825$, $r=0.911$ respectively) and were in a good agreement. The two PLT estimate techniques did not show significant difference with a mean difference of $<1x10^9$/L, positively correlated (0.869) and were in a good agreement (96.25%). Similarly, Mohammed-Rachids *et al* showed that the impedance method provided an over estimation of platelet counts compared to other methods and showed the highest level of disagreement in severe thrombocytopenic samples [27].

A recent result of the international Biomedical Excellence for Safer Transfusion Collaborative study clearly indicated that significant inaccuracy exists when counting low levels of platelets using routine hematology analyzers. This inaccuracy might impact on over or under‐transfusion

of platelet concentrates to patients at high‑risk of bleeding [15]. The present study showed that the automated PLT count give a mean PLT count of $12.3x10^9$/L which is higher than the mean PLT count by PLT estimated from PLT:RBC ratio and PLT estimated from AVR PLTS/10 OIF (6.9 and $6.5x10^9$/L respectively) for sever thrombocytopenic samples. Collectively, these findings clearly suggest that impedance method will not give the best platelet count at low levels and in a number of cases the counts would be falsely high, which may possibly affect the clinical decisions of platelet transfusions.

For thrombocytopenic patient samples, the three methods showed significantly different results but positively correlated. Automated and PLT estimated from PLT:RBC ratio were in a good agreement (97.5%). Similarly Asim M *et al* showed in their earlier report that the manual method for performing platelet count estimates on blood smears gives estimates that are significantly different from the counts by the automated method on the Sysmex KX21 automated counter at $p < 0.05$ [23].

In normal PLT count patient samples, automated and PLT estimated from AVR PLT/10 OIF showed non significant result ($p>0.05$), positively correlated (0.994) and were in the agreement limit (95%). This result is similar with a study conducted by Bakhubaira S who reported that statistically, there is no signifcant difference between the number of normal PLT count by the two methods ($p>0.05$) [26].

In high PLT count patient samples, the three methods were statistically significant ($p<0.05$). When tested by a correlation test all the three methods were positively correlated. Agreement was noticed between automated and PLT estimated from PLT:RBC ratio (96.25%) and also in automated & PLT estimated from AVR PLTS/10OIF (96.25%). No agreement was indicated between PLT estimated from PLT:RBC ratio and PLT estimated from AVR PLTS/10 OIF (93.75%). Similarly Bakhubaira S in his study by samples with high count, he observed signifcant statistical difference between automated and PLT estimated from AVR PLT/10 OIF ($p<0.05$) [26].

8. Strength and Limitation

8.1 Strength

- For each platelet category the recommended sample size was 40 but we doubled the sample size.
- Samples were run in duplicate.

8.2 Limitation

- Unable to use reference method (immunoplatelet counting/ flowcytometrey) for platelet counting.

9. Conclusion and recommendation

9.1. Conclusion

The mean PLT count by automated and PLT estimated from PLT:RBC ratio for normal count did not show statistically significant difference so the two methods could give the same PLT count result. The mean PLT count for severe thrombocytopenic patient samples did not show significant difference when analyzed by PLT estimates based on PLT:RBC ratio and PLT estimates based on AVR PLTS/10 OIF. So the two manual PLT estimation methods give could give the same result.

9.2. Recommendation

Possible to use manual PLT estimate based on PLT:RBC ratio and manual PLT count based on AVR PLT/10 OIF to verify automated platelet count for severely thrombocytopenic patients. TASH being a highest level referral center for patients with hematologic abnormalities and most of platelet transfusion is taking place could use these methods.

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Annex I : English Versions of Participant Information sheet and consent form

Participant Information sheet

TikurAnbesa Specialized Hospital

Laboratory Department

You are invited to participate in a study to be conducted in TASH by Elias Bisrat a Master's student of Addis Ababa University at the Department of Medical Laboratory Sciences. Please read the following statements and ask any unclear points before you agree to participate.

Introduction

The topic of the study is "Comparison of manual platelet estimates and automated platelet count. The study will be carried out on leftover blood sample that you or your child provides for routine examination. Participation in this study is exclusively voluntarily and you can withdraw anytime from the study.

If you are not interested to participate, there will be no consequences. If you decide to participate, we will use the leftover sample.

What is expected from me as participant of the study?

As a participant of this study, there is no additional blood sample collected from you/your child. The leftover sample will be used for this study.

Potential benefits to participant and/or to the society

Based on the results obtained, corrections will be taken in reporting of automated plateletcount whenever there is abnormal result by estimating platelets from peripheral blood smear. Hence, you are indirectly benefiting other patients and the society.

Compensation for participation

You will not receive any payment for your or your child's participation in this research study.

Confidentiality

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.

Person to contact

Please direct any questions you may encounter during this study to the principal investigator.

Elias Bisrat

Department of Medical Laboratory Sciences, College of Health Sciences Addis Ababa University

Cell phone - +251913356208

Email – eliasbisratt@gmail.com

Consent form

This page contains an agreement signature to participate in the study entitled "Comparison of manual platelet estimates and automated platelet count". So, please read the following points and sign your signature at the end in the space provided.

1. I understand the objective of the study is to compare manual platelet estimates and automated platelet
2. I know that the left over sample (blood) that I/my child gave is going to be used for this study only.
3. I understand that, all the information and the results are confidential.
4. I understand that I will not get any money for my/my child's participation.
5. All the information is explained by the phlebotomist and Principal investigator
6. I understand that my/my child's participation is voluntary and can withdraw anytime from the study and this will not affect the service I am/my child getting from the hospital.

Therefore, with full understanding of the situations I agree the leftover sample can be used for this study.

Signature of the participant: _____

Address of the participant: _____

Date: _____

Parent consent for children aged 12-17 years: I agree the leftover sample from my child can be used for this study provided my child gives assent. Parent signature:_____

Assent from children aged 12-17 years: With full understanding of the situations I agree the leftover sample can be used for this study provided my parents give their consent.

Signature of the participant: _____

Address of the participant: _____

Date: _____

Annex II: Amharic version of Participant Information sheet and consent form
በአዲስ አበባ ዩኒቨርሲቲ ፤የጤና ሳይንስ ኮሌጅ

የህክምና ላቦራቶሪ ት/ክፍል

በአዲስ አበባዩኒቨርሲቲ፤የጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ትምህርት ክፍል የሁለተኛ ዲግሪ ተማሪ በሆኑት አልያስ ብስራት የመመረቂያ ጥናት ላይ እንዲሳተፉ ተጋብዘዋል፡፡እባክዎ በዚህ ጥናት ላይ ከመሳተፍዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡ / ይመልሱ፤ግልፅ ያልሆነ ነገር ካጋጠመዎት ይጠይቁ፡፡

መግቢያ

የጥናቱ ርዕስ "የፕላትሌት ቆጠራን በማይክሮስኮፕ ተቆጥሮ የሚገኘውን የፕላትሌት ቁጥር ከደም መቁጠሪያ ማሽን ጋር ማወዳደር" እርስዎ በዚህ ጥናት ላይ የሚኖሮት ተሳትፎ ሙሉ ለሙሉ በበጎ ፈቃደኝነት ላይ የተመሠረተ ነዉ፡፡በዚህ ጥናት ዉስጥ ላለመሳተፍ ከወሰኑ በዚህ የህክምና ቦታ ዉስጥ የሚሰጥዎት አገልግሎት አይቅዋረጥም፡፡በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሑፍ ወይም በጣት ፊርማዎትን ማስቀመጥ ይጠበቅቦታል፡፡

የጥናቱ ተሳታፊ በመሆኔ የሚጠበቅብኝ ምንድንነዉ?

የጥናቱ ተሳታፊ በመሆንዎ ምንም ዓይነት ተጨማሪ የደም ናሙና እንዲሰጡ አይጠየቁም።እርስዎ ለምርመራ በሚሰጡት ደም ጥናቱ የሚካሂድ ይሆናል እንጂ አዲስ ናሙና እንዲሰጡ አይጠየቁም፡፡

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች

በጥናቱ ውጤት መሰረት የላብራቶሪ ውጤቶቹን በመረዳት ማስተካከያ ይደረግበታል፡፡ስለዚህም በማሽኖች ምክንያት የሚመጣውን ውጤት ትክክለኝነቱን ለማረጋገጥ ያስችላል፡፡በጥናቱ በመሳተፍዎ በተዘዋዋሪ መንገድ ለሌሎች ህሙማን ብሎም ለህብረተሰቡ ይጠቅማሉ ማለት ነው።

በዚህ ጥናት በመሳተፍ የሚከፈል ክፈያ

በዚህ ጥናት ስለተሳተፉ ምንም ዓይነት ክፍያ አይከፈልዎትም፡፡

የተሳታፊዎች ሚስጢር ስለመጠበቅ

ለጥናቱ ሲባል በመጠየቂያው ወረቀት ላይ ያለውን የርስዎን ስምም ሆነ ማንነት ወደ ሚስጥራዊ ቁጥር ይቀየራል. እንዲሁም የሰጡት ናሙናም ሆነ መረጃ ከዚህ ጥናት ውጪ ለሌላ አላማ/ጥቅም አይውልም፡፡

ጥያቄ ካሎዎት

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

የሕክምና ላብራቶሪ ሳይንስ ት/ክፍል፤ የጤና ሳይንስ ኮሌጅ፤አዲስ አበባ ዩኒቨርሲቲ

አልያስ ብስራት

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የስምምነት መጠየቅ ያቅጽ

የጥናቱ ተሳታፊ መለያቁጥር፡_____

የዚህ ጥናት ርእስ ““የፐላትሌት ቆጠራን በማይክሮስኮ ፕተቆጥሮ የሚገኘውን የፐላትሌት ቁጥር ከደም መቁጠሪያ ማሽን ጋር ማወዳደር“ ጥናቱ የሚካሄደው በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል ነው።እባክዎትን ከዚህ በታች የተዘረዘሩ ነጥቦች በጥሞና ያንቡቡ እና በመጨረሻ በተሰጠው ክፍት ቦታ ይፊርሙ::

1. “የፐላትሌት ቆጠራን በማይክሮስኮፕ ተቆጥሮ የሚገኘውን የፐላትሌት ቁጥር ከደም መቁጠሪያ ማሽን ጋር ለማወዳደር የሚካሄደውን የጥናት ዓላማ ተረድቻለሁ።
2. እኔ እና ልጄ የምንሰጠው ናሙና አስፈላጊው ምርመራ ከተካሄደ በኋላ ለዚህ ጥናት እንደሚዉል አውቂያለሁ።
3. ለጥናቱ የምሰጠው ናሙና እንዲሁም ውጤቱ በሚስጥር እንደሚያዝ ተረድቻሆሁ።
4. እኔ እና ልጄ በጥናቱ በመሳተፋችን የሚከፈለን ክፍያ እንደሌለ ኣውቂያለሁ።
5. ሁለም የሚያስፈልገው ነገር በተመራማሪው በኩል ተብራርቶልኛል።
6. በዚህ ጥናት ላይ እኔ እና ልጄ የሚኖረን ተሳትፎ ሙለ በሙሉ በበነ ፈቃደኝነት ላይ የተመሠረተ መሆኑን፣ በማንኛውም ጊዜ ማቋረጥ እንደምንችልና በዚህ ጥናት ዉስጥ በመሳተፋችን በዚህ የህክምና ቦታ ዉስጥ የሚሰጠን አገልግሎት እንደማይቋረጥ ተረድቻሆሁ::

ስለዚህ ከላይ የተጠቀሱትን ነጥቦች በመረዳት ናሙና (ደም) ለመስጠት ተስማምቻለሁ።

የተሳታፊ ፌርማ፡_____

ቀን፡_____

እድሜአቸው ከ 12-17 ለሆኑ ልጆች ቤተሰብ ፍቃድ የሚሰጡበት

ልጄ በዚህ ጥናት ላይ ለመሳተፍ ፉቃዱን የሚሰጥ ከሆነ ከልጄ የሚወሰደው የደም ናሙና ለዚህ ጥናት እንዲውል ተስማምቼአለው::

የቤተሰብ ፊርማ፡_____

እድሜአቸው ከ 12-17 ለሆኑ ልጆች ፍቃድ የሚሰጡበት

ቤተሰቦቼ በዚህ ጥናት ላይ እኔ እንድሳተፍ ከተስማሙ አኔ ስለ ጥናቱ ሁኔታ በመረዳት ለመሳተፍ ፍቃደኛ ነኝ::

የተሳታፊው ፊርማ፡ _____

የተሳታፊው አድራሻ፡ _____ ቀን፡_____

Annex I: Standard operating procedure and reagents for SYSMEX KX 21N

Specimen requirements

About 3-4 ml of venous blood collected into EDTA tubes.

Procedure

1. Turn ON the power switch on the right side of the unit. Self-check, auto rinse, and background check will be automatically performed, and the "Ready" (ready for analysis) will appear.
2. When auto rinse and background check are normally completed, "Ready" is displayed.
3. Perform quality control analysis on 3 levels of control blood material (low, normal and high) to verify that the instrument is performing within the specified ranges of the quality control material.
4. If the result of quality control in acceptable range run the blood samples.
5. Press [SAMPLE No.] key in the Ready status.
6. Entering patient ID, sample ID, Patient name.
7. Press [ENTER] key,This will fix the sample No. and the status becomes ready for analysis.
8. Mix the sample sufficiently before analysis.
9. Set the tube to the sample probe, and in that condition, press the start switch.
10. When the LCD screen displays "Analyzing," remove the tube.
11. After that, the unit executes automatic analysis and displays the result on the LCD screen.
12. Analysis result can be printed out on the built-in printer.

Reagents of SYSMEX KX-21

CELLPACK: is ready to use for impedance and photoelectrical analysis of whole blood, its ingredients are: sodium chloride, boric acid, sodium tetra borate, EDTA-2K.

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: non ionic surfactant, organic quaternary ammonium salt.

CELLCLEAN: is a strong alkaline detergent to remove lysing reagents, cellular residuals and blood proteins remaining in the hydraulics of sysmex analyzer. Ingredients: sodium hypochlorite.

Annex II: Standard operating procedure for blood smear wright staining

Specimen requirements

About 3-4 ml of venous blood collected into EDTA tubes

Procedure

1. Place a drop of blood, about 2 mm in diameter approximately inch from the frosted area of the slide.
2. Place the slide on a flat surface, and hold the narrow side of the non frosted edge between your left thumb and forefinger.
3. With your right hand, place the smooth clean edge of a second (spreader) slide on the specimen slide, just in front of the blood drop.
4. Hold the spreader slide at a 30 angle, and draw it back against the drop of blood.
5. Allow the blood to spread almost to the edges of the slide.
6. Push the spread forward with one light, smooth, and fluid motion. A thin film of blood in the shape of a bullet with a feathered edge will remain on the slide.
7. Label the frosted edge with patient name, ID# and date.
8. Allow the blood film to air-dry completely before staining.
9. Place the air dried smear film side up on the staining rack
10. Cover the smear with Wright stain and leave for 2 minutes
11. Dilute with buffer for three minutes.
12. Wash the smear with tap water
13. Air dry the smear
14. Count the platelets under 100x objective
    - The platelets are counted in the ideal zone of a smear stained with wright stain where blood cells did not overlap, and there is fairly even distribution of white blood cells and platelets.

Quality control

1. Prepare one differential slide daily using a patient sample with a normal MCV, MCH, MCHC and total white count. Stain the slide as indicated in procedure section. Review the slide for Color, Precipitation and Contamination.

2. If the color does not meet the specifications identified in the SOP or precipitation and/or contamination are present, the quality is determined to be unsatisfactory. Indicate unsatisfactory on the Quality Control (QC) form and replace the stain as indicated in the following section. Document the problem and corrective action in the appropriate section of the QC form. If the stain is determined to be satisfactory, indicate satisfactory on the QC sheet

Declaration

Assurance of Principal Investigator

The undersigned agrees to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and conditions of the research publications office in effect at the time of grant is forwarded as the result of this application.

Name of the student: Elias Bisrat (Bsc, Msc candidate)

Date _____ Signature _____

Approval of Advisors:

Aster Tsegaye, MSc, PhD

Date_____ Signature _____

Mintewab Hussein, MSc

Date_____ Signature _____

Melatwork Tibebu, MSc

Date _____ Signature _____