Biomedical and Biopharmaceutical Research
Abbreviation: Biomed. Biopharm. Res.
Volume: 22: Issue: 02 |Year: 2025
Page Number: 438-451

CORRELATION OF PLATELET COUNT ESTIMATION: MANUAL VERSUS AUTOMATEDMETHOD - A CROSS-SECTIONAL STUDY

Correlation

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Article Received:27-06-2025

Article Accepted:20-07-2025

Abstract

BACK GROUND: Platelet count assessment is needed in clinical practice and in certain diseases. In hematology laboratories, various methods for platelet counting are used like automated hematology analyzer counting, platelet count estimation by peripheral blood smear(PBS) method and hemocytometer (neubauer chamber). The reliability of platelet count is required in cases where the platelet transfusion is necessary.

METHODS: This study involved collecting blood samples from 500 patients randomly selected, including both inpatients and outpatients, over the period of one and a half year. The samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes. Platelet counts were measured through automated platelet analysis and then compared with those obtained manually.

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RESULTS: The results revealed a subtle trend, showing that manual slide-derived platelet counts slightly exceeded those produced by automated analyzers.

CONCLUSION: The study found a clear correlation between automated and manual methods for assessing platelet counts. However, in cases of extremely high or low platelet counts, the manual method proved more reliable, as it avoids issues such as platelet clumping or uneven distribution, thereby ensuring greater accuracy.

Keywords: Peripheral blood smear, automated hematology analyzer and platelet count.

INTRODUCTION

Platelets are small (1- 4µm in diameter), anucleate and discoid circulating blood cells. They have a short-lifespan of about 7-12 days in circulation. They are formed in the bone marrow by a process of fragmentation of the cytoplasm of megakaryocytes. About 70% of the platelets are in circulation while 30% lie sequestered in the spleen. The platelets in peripheral blood are heterogenous with respect to size, density, and staining characteristics. In hemostasis, inflammation and immunological activity, platelets carry out both structural and molecular functions. They also have a specific role in host defense, injury response and immune surveillance.[1,2]

The evaluation of platelet count has a significant role in both research laboratories as well as in clinical practice. Common methods used in laboratories for estimating platelet counts include - manual evaluation on the peripheral blood smear (PBS), by hemocytometer and by utilization of automated hematology analyzer.

Platelet counting has proved to be more challenging than counting red cells or white cells. The PBS is a crucial and useful hematological technique for estimating platelet count in patient blood samples. Achieving accurate and consistent platelet count is essential for appropriate patient care. The manual counting method is labor-intensive, subjective and prone to high levels of variability. The advent of automated complete blood counters using impedance technology has significantly improved precision in platelet counting. However, impedance counts are still restricted since platelets cannot be distinguished from other particles of similar size using cell size analysis. Despite the recent introduction of fluorescence or light scatter techniques for automated platelet counting, challenges persist in achieving accurate platelet counts. With widespread use of automated analyzers nowadays, there has been enhanced accuracy in platelet counting. However, the results of automated counters cannot be totally relied in certain cases like in cases when there is delay in sample processing which can also cause pseudothrombocytopenia on automated analyzers.[3]

The normal range of platelet count in a healthy person is 1,50,000/mm^3 to 4,50,000/mm^3. According to reports, the average number of platelets observed per oil immersion field on a PBS slide are multiplied by 20,000 to estimate the platelet count per cubic millimeter.[4,5,6] Usually, a PBS is used to confirm the automated platelet count, particularly when the analyzer flags it for verification or when the count falls significantly below the lower limit of the reference range.

Platelet count less than 1,50,000/mm^3 is known as thrombocytopenia.[7] It is associated with abnormal bleeding that includes spontaneous skin purpura, mucosal hemorrhages and prolonged bleeding following trauma. The underlying causes of thrombocytopenia can be either due to hyperdestructive thrombocytopenia (dengue, immune thrombocytopenic purpuraetc) or due to hypoproliferative thrombocytopenia (anemia, pancytopenia, acute leukemia etc). The other causes are due to abnormal splenic sequestration of the platelets causing splenomegaly and due to EDTA induced thrombocytopenias.[8]

Platelet count more than 4,50,000/mm^3 is known as thrombocytosis.[9] Thrombocytosis can arise from - primary/clonal and secondary/reactive causes. Primary causes include essential thrombocythemia, polycythemia vera and chronic myeloid leukemia, among others. Secondary causes are - post hemorrhage, post splenectomy and chronic myeloid leukemia etc.

With automated hematology analyzers platelet counts are often measured with a high degree of precision and accuracy. Yet, when assessing extremely low platelet counts or when non platelet particles or aberrant platelets are present, the precision of automated platelet counts may be jeopardized.[13]

Platelet count estimated by automated analyzer should be verified against the platelet count on PBS in cases where the cell size analysis cannot distinguish platelets from other similarly sized particles[10] (like fragmented RBC's) and in some severe thrombocytopenia cases.[11] Moreover, the presence of large platelets exceeding the upper limit may lead to underestimated platelet counts. There has been improvement in the ability to discriminate platelets with the use of multiple light scatter parameters , electrical resistance other than impedance alone in automated analyzers.[12] Automated analyzers also provide estimation of platelet indices like Plateletcrit, MPV, Platelet Distribution Width, which together helps in evaluation of functional integrity of platelets.

MATERIAL AND METHODS

Our study was conducted in the patients attending Hematology wing of the Department of Pathology, Government Medical College, Patiala, for routine CBC counts including platelet count. Blood samples from 500 randomly selected patients over a span of one and a half year, were collected in the EDTA tubes and were evaluated by automated analyzer (Medsource Alpha Count 60) and manual methods for platelet count. Clearance was obtained from Institutional Ethics Committee for our present study.

STUDY DESIGN : Cross- sectional study.

SELECTION OF PATIENTS:

a) **INCLUSION CRITERIA** : Patients referred to the Pathology Department, from various clinical departments of the hospital for routine CBC (including platelet count).

b) **EXCLUSION CRITERIA :**Thehemolyzed, clotted blood samples and inadequate blood samples where the patients could not be accessed for repeatsampling were excluded.

COLLECTION :

2 ml venous blood samples were drawn and collected in EDTA vials. Each blood sample vial was properly labeled and mixed on a blood shaker. The samples were analyzed within four hours of collection

PROCESSING :

Hematology Automated Analyzer:

Blood samples were processed in a fully automated hematology analyzer (Medsource Alpha Count 60) to determine the platelet counts. It's a quantitative, automated hematology analyzer and leukocytes differential counter used in our laboratory.

FIGURE 1: HEMATOLOGY AUTOMATED ANALYZER (MEDSOURCE ALPHACONT 60) USED IN OUR LABORATORY

Typical parameters reported by analyzer include RBC count, WBC count (total and differential), Hb concentration, platelet count, HCT, MCV, MCH, and MCHC alongwith platelet indices like MPV, PDW, PCT and P-LCR. The analyzer was maintained and calibrated as per guidelines by the manufacturer

Various principles on which Automated hematology analyzers work are :

i. Electrical impedance
ii. Light scatter
iii. Fluorescence
iv. Light absorption
v. Electrical conductivity.

Majority of the analyzers are based on a combination of various principles. Medsource Alpha Count 60 automated cell analyzer is based on the principle of electrical impedance for platelet counting.

Peripheral Blood Smear

The peripheral blood smears were made simultaneously (after processing the samples in hematology analyzer) and stained with Leishman's stain. The PBS was examined under microscope under oil immersion lens (100 X) for platelet count.

Principle

The PBS is a laboratory technique used to examine blood cells under a microscope. The polychromic staining solution (Wright, Leishman) contain methylene blue and eosin. These basic and acidic dyes generate multiple colors when applied to cells. Methanol serves as a solvent as well as a fixative. The fixative causes the cells to adhere to the glass slide and prevents them from any further changes.

NEUBAUER CHAMBER

The Neubauer chamber, also known as a hemocytometer, is a device used for counting cells, particularly in blood or other bodily fluids. In our study Neubauer chamber was used for platelet count in a few randomly selected patients especially with low platelet count.

PRINCIPLE:

The principle of the Neubauerchamber is based on the:**Grid and Depth Calibration**: The Neubauer chamber has a specific grid incised on its surface, that is used to count cells under a microscope. The grid is precisely calibrated in terms of both area and depth. The depth of the chamber allows a predetermined volume of the fluid to be placed between the chamber and the cover slip, ensuring a standardized area for counting.

OBSERVATIONS

The present study was a prospective cross sectional study conducted in the blood samples collected from 500 random patients, both outdoor and indoor patients, who were sent to Hematology wing of Pathology department for estimation of their CBC count including platelet count. The platelet count estimation was done by hematology analyzer (Medsource Alpha Count 60), from PBS examination under oil immersion objective lens and by using Neubauer chamber in few thrombocytopenic patients (< 1,50,000/mm^3 platelet count), randomly selected, amongst study patients. The estimated platelet counts from these methods were later correlated with each other and the following observations were noted from our cross sectional study.

TABLE 1: AGE DISTRIBUTION OF STUDY POPULATION

Age Groups (years)	Number	Percentage
< = 20	83	16.6%
21 – 40	212	42.4%
41 – 60	142	28.4%
>60	63	12.6%
Total	500	100%
Mean ± S.D	38.24 ± 18.46	

In this cross sectional study, patients were divided into different age groups. The maximum number of patients were seen in 21-40 years age group: 212 (42.4%) and lowest were in >60 years age group: 63 (12.6 %). The mean age recorded was 38.24 +/- 18.46.

TABLE 2: COMPARISON OF PLATELET COUNT BY AUTOMATED AND MANUAL (PBS) METHOD

Automated (/mm^3)	Manual (PBS) /mm^3			Total
	< 150000	150000 - 450000	> 450000	
< 150000	54	16	0	70
150000 – 450000	3	366	3	372
> 450000	0	16	42	58
Total	57	398	45	500
Kappa	0.801			
P value	<0.001			
Significance	HS			

In this cross sectional study comparison was done in platelet estimation by automated and manual (PBS) methods. Platelet count calculated was categorized in 3 groups – low count (<150000 /mm^3), normal range (150000- 450000)/mm^3 and high count (>450000 /mm^3).

Platelet count calculated in 70 patients by automated method came out in low count group, when compared with manual method - 54 patients also had low platelet count while 16 patients had normal range platelet count.

Similarly 372 patients had normal range of platelet count by automated method but when compared with manual method – 366 patients had within normal range values while 3 patients had low count and high count values respectively.

High platelet count value was estimated in 58 patients by automated method which when done by manual method came out to be high platelet count in 42 patients while 16 patients had normal range platelet value.

Overall Kappa correlation coefficient value and P value came out to be 0.801 and <0.001 respectively and were highly significant statistically.

TABLE 3: COMPARISON OF PLATELET COUNT BY: NEUBAUER WITH AUTOMATED METHOD

Automated (/mm^3)	**Manual (NEUBAUER) /mm^3**			**Total**
	< 150000	150000 - 450000	> 450000	
< 150000	46	1	0	47
150000 – 450000	3	0	0	3
> 450000	0	0	0	0
Total	49	1	0	50
Kappa	-0.031			
P value	0.799			
Significance	NS			

Table 3 shows comparison done in platelet estimation by automated and manual (NEUBAUER) methods in 50 random patients amongst the 500 study population patients. Platelet count calculated was categorized in 3 groups – low count (<150000 /mm^3), normal range (150000- 450000)/mm^3 and high count (>450000 /mm^3).

Platelet count calculated in 47 patients by automated method came out in low count group, when compared with neubauer method - 46 patients also had low platelet count while 1 patient had platelet count within normal range.

Similarly 3 patients had normal range of platelet count calculated by both automated and neubauer method.

Their Kappa value and P value came out to be – 0.031 and 0.799 respectively and were not significant statistically.

TABLE 4: COMPARISON OF PLATELET COUNT BY: NEUBAUER WITH PBS METHOD IN STUDY POPULATION

Manual (PBS) /mm^3	**Manual (NEUBAUER)/ mm^3**			**Total**
	< 150000	150000 - 450000	> 450000	
< 150000	49	0	0	49

150000 – 450000	0	1	0	1
> 450000	0	0	0	0
Total	49	1	0	50
Chi Square	1.00			
P value	<0.001			
Significance	HS			

In this table 4 - comparison was done in platelet estimation in 50 random patients from the study population by manual – PBS and neubauer methods . Platelet count calculated was categorized in 3 groups – low count (<150000 /mm^3), normal range (150000- 450000)/mm^3 and high count (>450000 /mm^3).

Platelet count calculated in 49 patients by both methods came out in low count group .

Similarly in 1 patient normal range of platelet count was calculated by both methods.

Their Chi square value and P value came out to be 1.00 and <0.001 respectively and were highly significant statistically.

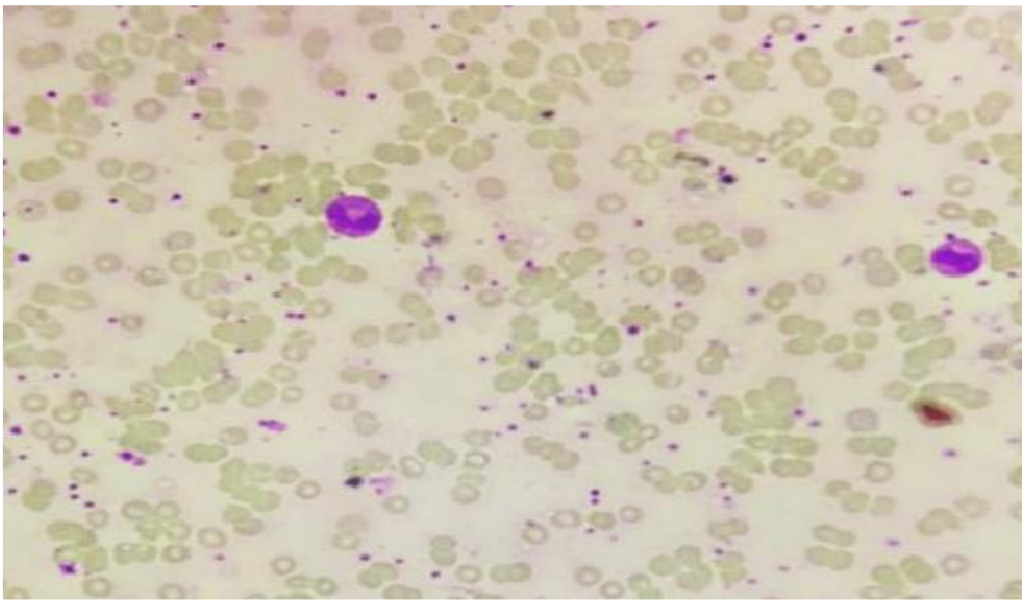

FIGURE 1: PBS SLIDE SHOWING RBC'S, WBC'S, PLATELETS

(400X, LEISHMAN STAIN)

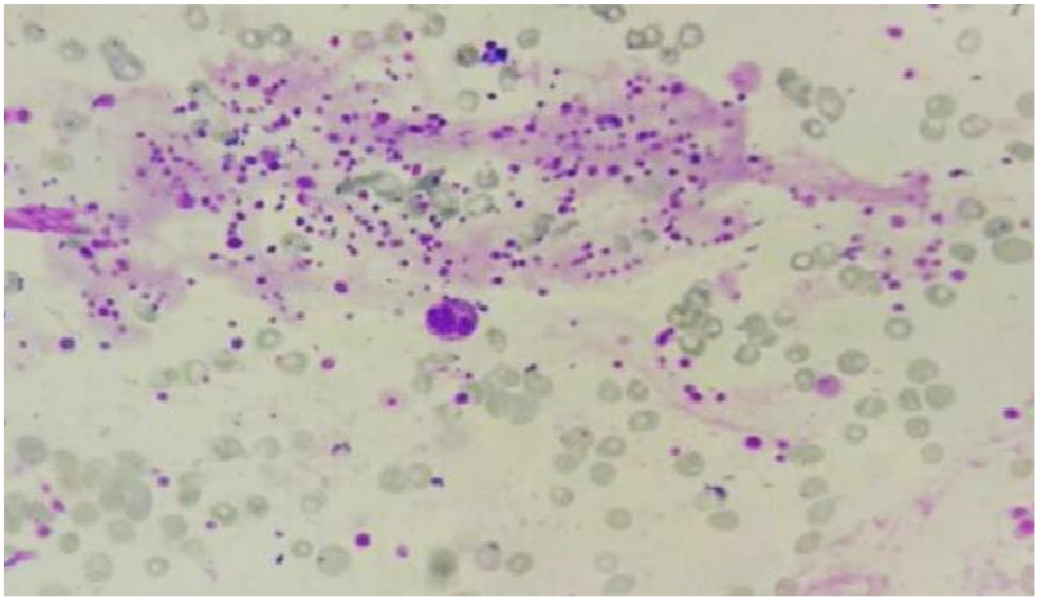

FIGURE 2 : CLOTTED BLOOD SAMPLE ON PERIPHERAL SMEAR FIBRIN STRANDS FORMATION-LOW PLATELET COUNT ON ANALYZER (400X, LEISHMAN STAIN)

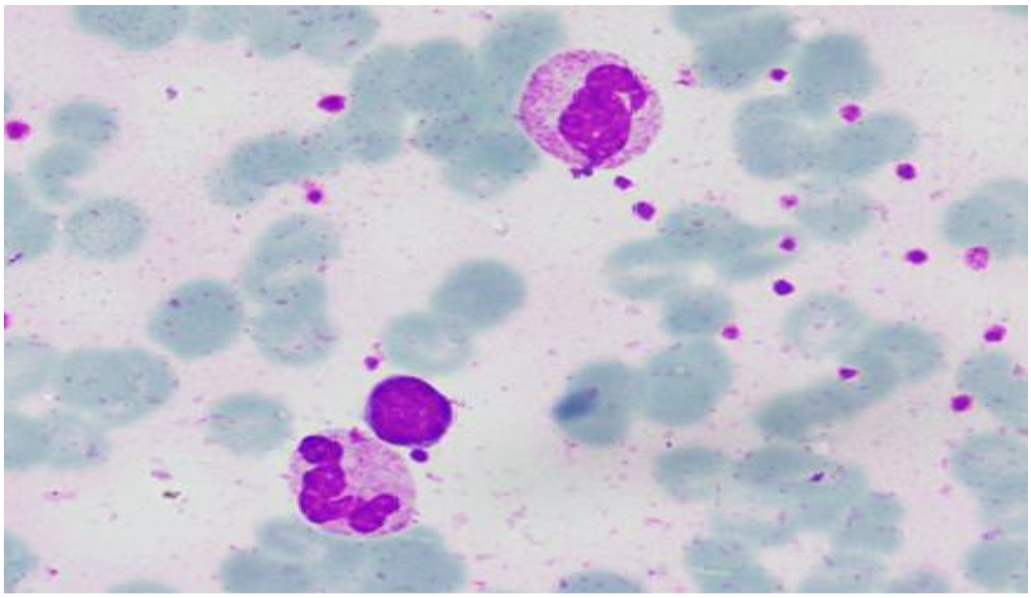

FIGURE 3: LEISHMAN STAINED PERIPHERAL SMEAR SHOWING RBC'S, WBC'S AND PLATELETS (OIL IMMERSION VIEW)

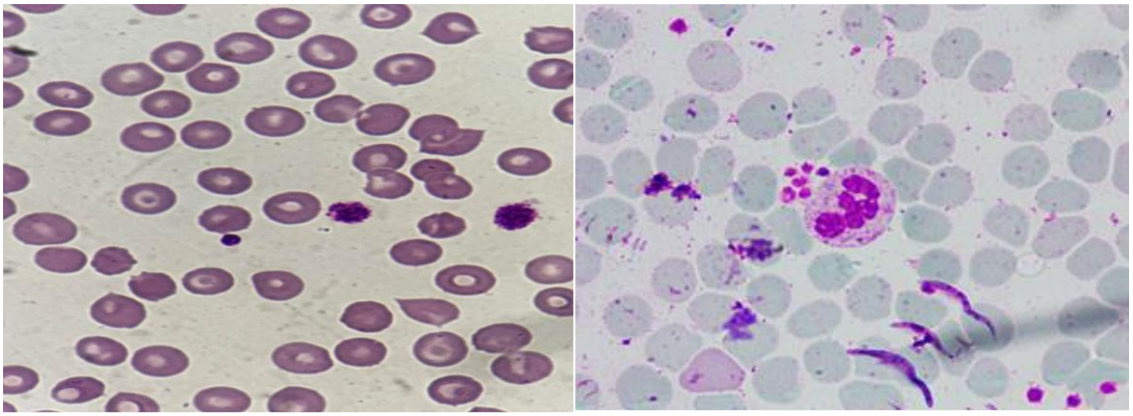

FIGURE 4 & 5: OIL IMMERSION VIEW OF LEISHMAN STAINED PERIPHERAL SMEAR SHOWING GIANT PLATELETS AND PLATELET SATELLITISM RESPECTIVELY

DISCUSSION

Platelet counts are typically conducted in the laboratories using methods such as examination of PBS, counting with Neubauer chamber or utilizing automated hematology analyser. In a wet preparation, platelets appear as colorless, disc shaped or elliptical refractile bodies. Platelets appear as circular, oval or rod shaped structures and have a light blue color when stained with Leishman's stain on a PBS. In addition to their many uses platelets are essential for wound healing, thrombosis and hemostasis.

Age-wise distribution

Aashna et al(2019)[19]	Lavanya et al(2019)[18]	Present study
Age distribution was between 1 and 84 years	Age distribution was between 1 and 85 years	In our study, the age distribution was between 1 and 82 years
Mean age observed was 26.76 years (± 21.37 years)	Median age was 45 years in this study	In our present study mean age was observed to be 38.24 ± 18.46 years

Automated analyzers indeed offer numerous advantages in clinical settings including speed, efficiency in handling large sample volumes, accuracy and precision in quantitative tests etc. However there are few significant disadvantages associated with automated analyzers-

1. Flagging issues- Like platelet histogram in a CBC report may show flags such as- PL flag (Abnormal height at lower discriminator), PU flag (Abnormal height at upper discriminator) and MP (Multi Peak) flag for platelet anisocytosis. Therefore it often requires manual examination of a blood smear to further confirm or investigate the result.
2. High Cost- Automated analyzers are typically expensive to purchase, maintain and operate.
3. Erroneous results due to various interfering factors as described below:

Platelet aggregates in EDTA anticoagulated blood can result in falsely low platelet counts. Similarly platelet satellitism (platelets adhering to and encircling neutrophils) can also cause inaccurate counts.

Some of the pre analytical errors can occur while using automated hematologyanalyzers. One of the example is spurious thrombocytopenia which may erroneously be reported because of inadequate filling of the test tubes and/or insufficient inversion of the tube during sampling.

TABLE 5: PLATELET COUNT ESTIMATION AND ITS MEAN VALUES BY ANALYZER IN OUR STUDY POPULATION

Platelet count category	No. of Patients	Percentage (%)	Mean Platelet count by analyser (+/- SD) (x 10^9/L)
Thrombocytopenia (<150 x 10^9/L)	70	14	87.46 (± 37.55)
Normal Platelet count (150-450 x 10^9 /L)	372	74.4	281.85 (± 77.60)
Thrombocytosis (>450 x 10^9/L)	58	11.6	560.46 (± 98.22)
Total	500	100	

This table depicts categorization of the patients in our cross sectional study on the basis of platelet count estimation by hematology analyser and thereby categorised as :

14% (70) patients had thrombocytopenia.

74.4% (372) patients had normal platelet count.

11.6% (58) patients had thrombocytosis.

TABLE 6 : PLATELET COUNT ESTIMATION AND ITS MEAN VALUES BY MANUAL METHOD (PBS) IN 500 STUDY PATIENTS

Platelet count category	No. of Patients	Percentage (%)	Mean Platelet Count by manual method (+/- SD)
Thrombocytopenia (<150 x 10^9/L)	57	11.4	85.74 (± 34.28)
Normal Platelet count (150-450 x 10^9 /L)	398	79.6	283.17 (± 79.82)
Thrombocytosis (>450 x 10^9/L)	45	9.0	528.40 (± 73.90)
Total	500	100	

Table 6 depicts how individuals in our cross sectional study were categorized, on the basis of platelet estimation by manual method (PBS) :

In our study 11.4% (57) patients had thrombocytopenia.

79.6% (398) patients with normal platelet count.

9.0% (45) patients had thrombocytosis.

TABLE7 : VARIOUS STUDIES COMPARING THE AUTOMATED WITH MANUAL PLATELET COUNT METHOD OF STUDY POPULATION

Study	Study population	N	Name of Analyzer	Mean PLT count by analyzer (x 10^9/L)	Mean PLT count by manual method	P value	Correlation coefficient value

					(x 10^9/L)		
Malok M et al 2007.[5]	Randomly selected hospitalisedpatients	184	Coulter LH750	268	269	0.87	0.9
Ike SO et al 2010.[13]	Healthy adults as well as in patients	60	Sysmex KX-21N	265.5 +/- 18.94	251.7 +/- 18.58	<0.001	0.779
Babadoko AA et al 2016.[16]	Randomly selected hospitalised patients	100	Swelab Alpha, Sweden	278.10 +/- 162	244.80 +/- 171.80	0.043	0.531
Aashna et al 2019.[19]	Randomly selected hospitalised patients	200	Mindray BC-5800	210.59 +/- 161.65	272.60 +/- 163.81	<0.001	0.857
Present Study	**Randomly selected opd patients and indoor patients**	**500**	**Medsource Alpha Count 60**	**286.95 +/- 141.67**	**282.33 +/- 124.35**	**<0.001**	**0.801**

N: number of patients

Some other studies also found significant correlation between automated and manual method of platelet estimation like Castromayor et al found statiscally significant difference between manual and automated platelet count results with p value <0.05.[31]

Balakrishnan et al also found significant correlation between manual and automated platelet count (p=0.50).[28]

TABLE 8 : MEAN PLATELET VOLUME (MPV) AND PLATELET DISTRIBUTION WIDTH (PDW) IN THROMBOCYTOPENIC PATIENTS (BY ANALYZER)

Category	No. of patients	Percentage (%)	Mean PLT count (+/- SD) (x 10^9/L)	Mean of MPV (+/- SD) (fL)	Mean PDW (+/- SD) (%)
Thrombocytopenic patients (<150 x 10^9)	70	14	87.46 (± 37.55)	14.35 (± 3.06)	20.92 (± 3.31)

In this table - mean platelet count, mean MPV and mean PDW in the thrombocytopenic patients (by analyzer platelet count estimation) of the study population is depicted.

In present study an inverse relationship was seen between estimated platelet count (analyzer) and the MPV and PDW.

In 1982, David Bessman demonstrated a correlation between MPV and megakaryocyte ploidy. Patients with immune thrombocytopenia exhibited low platelet count, MPV above the normal limit and high megakaryocyte ploidy. In contrast, patients with reactive thrombocytosis showed high platelet count, low MPV and low

megakaryocyte ploidy. Similar relation was seen in our study between MPV and platelet count (low platelet count with high MPV and vice-versa). This result was further supported by Till Ittermann et al in 2019 using impedance-based count on Sysmex XE 5000, affirming that MPV demonstrates an inverse correlation with the platelet count in study participants.

TABLE9 : COMPARISON OF VARIOUS STUDIES IN CALCULATION OF PLT COUNT ON A PERIPHERAL BLOOD SMEAR

Malok et al (2007)[5]	Study by Momodu I (2016)[17]	Anchinmane et al (2017)[24]	Present Study
This study Found strong correlation (r=0.90) of PLT count estimation on a PBS with by using 20000 as multiplication factor.	This study showed better results of PLT count estimation on a PBS using 20000 as Multiplication Factor.	Strong Correlation (r=0.9789) was found in their study by multiplying PLT count with 20000 on a PBS with analyser report.	In our present study 20000 was used as multiplication factor for PLT count on a PBS and results were correlated with analyzer , showing positive correlation coefficient value of 0.801

Despite being considered a best manual counting technique, the Neubauerchamber can still be prone to errors due to the random distribution of the cells across its grid. This variability in cell distribution adheres to the Poisson's law.

In our present study, few of the randomly selected blood samples of the patients (especially of thrombocytopenic patients) were used for PLT count estimation by Neubauer chamber and the results were correlated with those of analyzer method and PBS method. A highly significant result was found (p value<0.001, chi square value=1) when the neubauer PLT count compared with the PLT count on a PBS.

However, a not significant result (p=0.799) was observed in the current study while comparing analyzer and manual (neubauer) method of PLT count estimation. This is consistent with an earlier report by Bajpai R et al who reported no significant difference (p=0.69) between automated and manual PLT estimated from AVR PLT/10 OIF.[15]Anitha et al in their study also revealed no significant (p=0.4) difference in values between manual slide method of platelet estimation (2.76±0.71 lakhs/mm^3) when compared with that of automated cell counter platelet value (2.64±0.73 lakhs/mm^3).[30]

Despite the advances in hematology automation and application of molecular techniques, the PBF remains a very important diagnostic test to the hematologist.[17]

CONCLUSION

While automated hematology analyzers are essential for rapidly generating results from a large number of blood samples, the accuracy of peripheral blood smear platelet estimation is similarly reliable. This study demonstrated a significant positive correlation between the manual method (PBS) and automated analyzer. However in cases showing notable discrepancies in platelet counts like extremely high or low counts, the manual method remains reliable, ensuring accuracy by avoiding issues such as platelet clumping or uneven distribution. A key finding from our cross sectional study highlights the critical role of advanced hematology analyzers in swiftly and precisely evaluating complete blood counts. Additionally before finalizing the report, any samples showing abnormal platelet count on the analyzer should undergo reassessment using by manual method (PBS). This additional verification step enhances the accuracy of platelet count as the diagnostic conclusions are based on the reliable data. Manual platelet counting using Neubauer chamber is labor intensive and time consuming. It becomes impractical for laboratories (like our laboratory at GMC Patiala)

handling large sample sizes, to use this method. Therefore in cases where platelet count from automated analyzers requires verification, mostly platelet count estimation from manual method of leishman-stained peripheral smears is opted.

In essence the combination of manual and automated methods for platelet count estimation enhances our understanding and mastery of this crucial diagnostic parameter. This synergy contributes to improved clinical decision making and the delivery of optimal patient care.

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