

# Comparison of Platelet Counts Estimated by Peripheral Blood Smear Examination and Automated Haematology Analyzer

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## Abstract:

**Introduction:** Platelet count estimation is one of the common as well as important investigations in clinical practice to diagnose many diseases like dengue, malaria, etc. Different methods for platelet estimations are available. These methods are manual counting semi-automated and automated hematology analyzer counting, platelet count estimation by peripheral blood smear (PBS) method, etc. Semi-automated or automated analyzers may produce erroneous results in the presence of particles or light scatter like giant platelets, fragmented red blood cells, and platelet clumps, so alternative methods like PBS examination can be used for validation.

**Aim:** To compare platelet count estimation performed by the automated cell counter method and the PBS examination method.

**Objective:** Peripheral Blood smear examination acts as a good quality control tool to validate the results produced by the automated cell counter.

**Materials and Methods:** The present study was carried out in the Department of Pathology, Akshaya Health Centre, Urban Bangalore. The study included 100 random blood samples collected into ethylenediaminetetraacetic acid (EDTA) vacutainers over 3 months. These were analyzed by both peripheral blood smear and automated cell counter for platelet estimation. The statistical analysis was done for test performances and their comparisons by using the coefficient of variation (CV), linear regression, and mean differences with SPSS software.

**Results:** No significant difference ( $p = 0.06635$ ) was observed between the manual peripheral blood smear (PBS) method (platelets average per 100x, multiplied by 15000/ $\mu$ l) of platelet estimation ( $207.13 \pm 15.898 \times 1000/\mu$ l) and that of automated cell counter platelet value ( $206.53 \pm 16.278 \times 1000/\mu$ l). A significant positive correlation was observed between the results of both methods ( $r=0.9995$ ,  $p < 0.001$ ) when analyzed by the Pearson correlation test.

**Conclusion:** The peripheral blood smear platelet estimation results are comparable with automated analyzer results. Hence, the PBS examination serves as a quality control tool in assessing the results of the automated cell counters.

**Keywords:** Platelet Count; Automated Method; Manual Method; Peripheral Blood Smear.

## INTRODUCTION

Platelets (thrombocytes) play a key role in homeostasis and thrombosis in the body. They are one of the formed elements of blood measuring 2-3 $\mu$ m. They are anucleated cells with their cytoplasm filled

with granules. Normally the platelet count ranges from  $150$  to  $450 \times 10^3$  /ul. Platelet estimation is one of the critical parameters in diagnosis, treatment and the patient care. Platelet counts can be estimated by manual methods using counting chamber and a peripheral blood smear (PBS) examination. However, automation, haematology analyzers are being used widely in laboratory practices from semi - automated to completely automated analyzers, based on the principles of impedance, flow cytometry and optical fluorescence. Automated method is reliable, simple, fast, and most widely used [1].

However, the accuracy of platelet count by automated cell counter is compromised while processing blood samples with giant platelet, platelet clumps or presence of RBC or WBC fragments etc.. Also, inadequate quality control and calibration affects the auto-analyzer readings. PBS examination evaluates the results of automated cell counters in such condition and to confirm the auto analyzer readings [2]. In a Leishman's stained peripheral blood preparation, they can be identified as small purple coloured bodies having irregular borders (Image 1). Our aim is to study and compare the platelet count by automated analyzer and Leishman stained peripheral smear examination method.

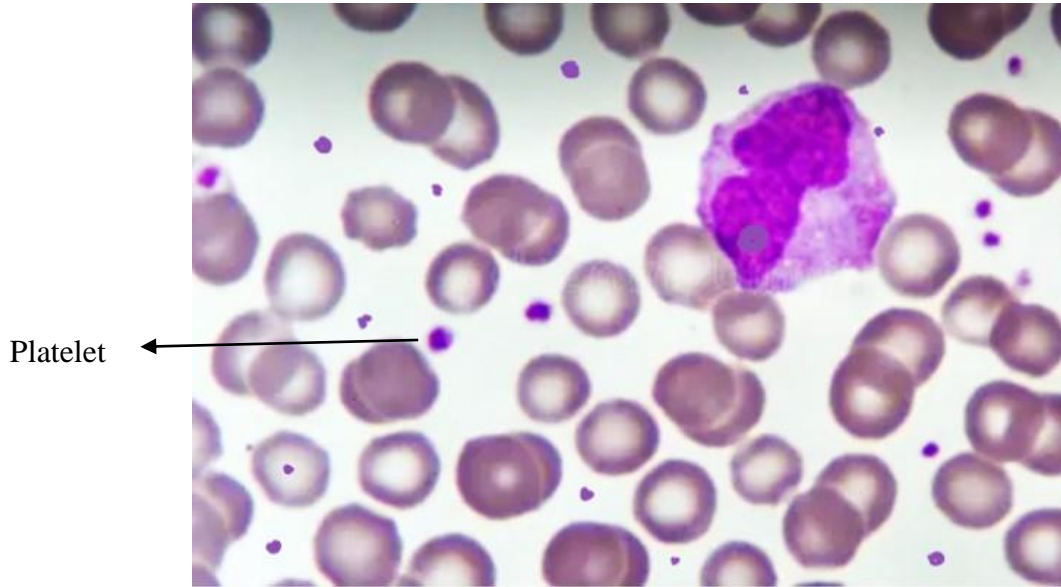
## MATERIALS AND METHODS

The present study was carried out in the Department of Pathology at a diagnostic centre, Akshaya Health Centre in urban Bangalore, over the period of 3 months (July – September,2023). The blood samples were collected from 100 patients referred from different hospitals and clinics for blood investigations for diagnosis or follow up. The patient's samples were randomly selected with any medical diagnosis. Venous blood samples were collected for all the patients in EDTA vacutainers tube and were stored at room temperature until they were analyzed within two hours. The inadequate samples, haemolysed samples and clotted samples blood samples were excluded from the study.

Each blood sample was mixed properly with automated mixer for ten minutes. The platelet count was estimated by processing blood samples in an automated hematology analyzer **UNITRON BIO-MEDICALS (UBM) Fx-19T automated cell counter** (Image 2). The quality control, calibration and maintenance of the analyzer were done as recommended by the manufacturer. The blood samples were processed with hematology analyzer and the same blood samples were used to prepare air dried PBS and was stained manually with Leishman's stain. The PBS was examined under light microscopy with x100 oil immersion lens. If PBS of blood sample showed platelet aggregates, the respective blood sample was excluded. The platelets were counted in ideal zone of PBS where RBC's just touching each other with fairly even distribution of platelets and WBC's. Platelets were counted in 10 ideal zone. The average number of platelets was calculated and was multiplied by fifteen thousand. In an ideal zone of blood film,each platelet on an average 100x oil immersion field represents 15,000 platelets /  $\mu$ l. Thus, the final platelet count estimation was done from PBS [3].

The statistical analysis was done for test performances and their comparisons by using coefficient of variation (CV), linear regression and mean differences with SPSS software.

**Image 1. Peripheral blood smear (PBS) under 100x oil immersion showing platelet.**



**Image 2. UNITRON BIO-MEDICALS (UBM) Fx-19T automated cell counter.**



**RESULT**

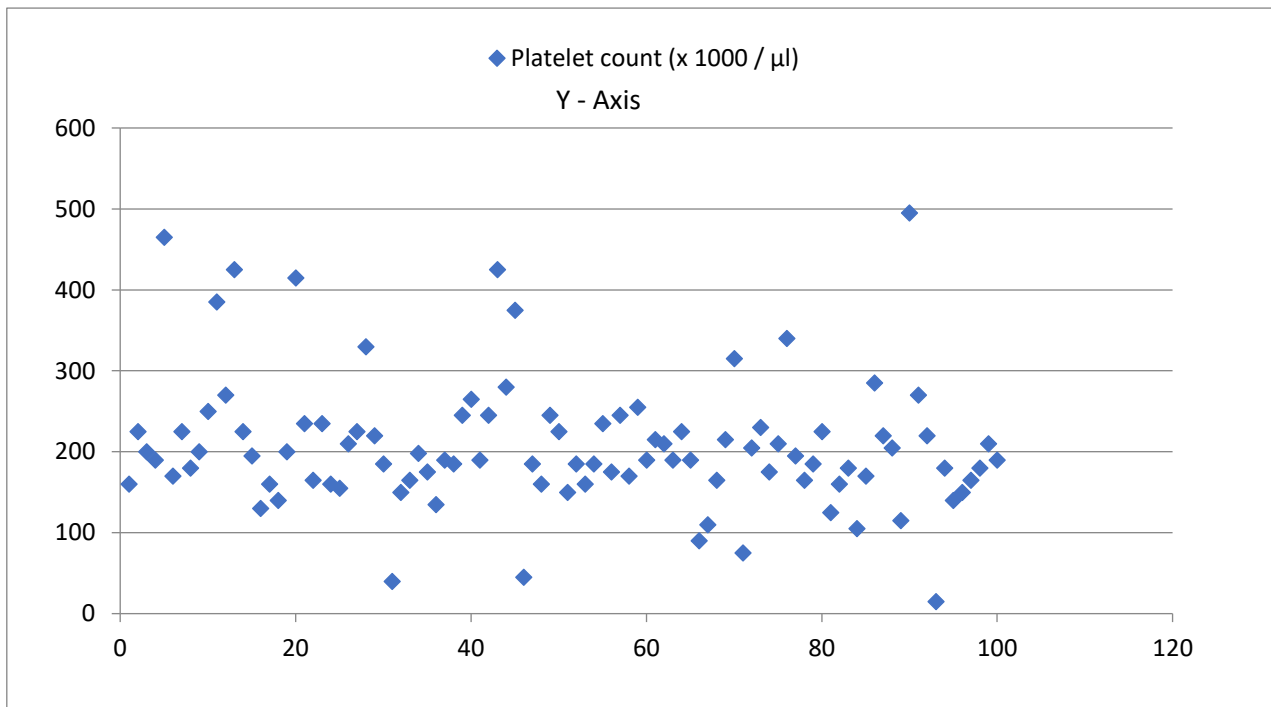
We analyzed the data by paired t-test obtained by two different methodologies studied on 100 samples and observed no significant difference ( $p = 0.06635$ ) between manual peripheral blood smear (PBS) method (platelets average per 100x, multiplied by 15000/  $\mu$ l) of platelet estimation ( $207.13 \pm 81.11 \times 1000/ \mu$ l) and that of automated cell counter platelet value ( $206.53 \pm 83.05 \times 1000/ \mu$ l). (Fig. 1, 2 and 3) (Table 1)

We observed significant positive correlation between the two methods ( $r=0.9995$ ,  $p < 0.001$ ), when analysed by Pearson correlation test.

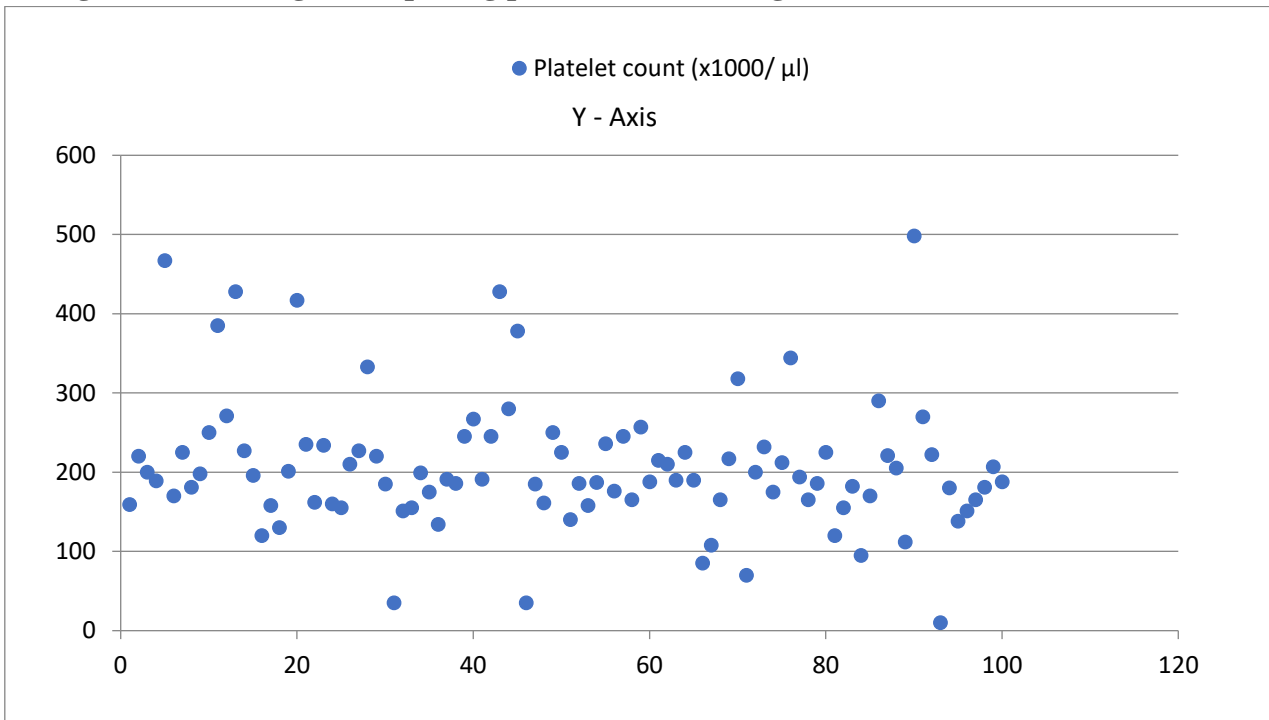
**Table 1: Mean and standard deviation values of platelet estimation by manual peripheral blood smear examination and automated cell counter.**

Variables	Number Of Samples (n)	Mean (x1000/ $\mu$ l)	Standard Deviation	t value (Paired t-test)	p value
Manual Method	100	207.13	81.11	-1.8565	<0.001
Automated Method	100	206.53	83.05		

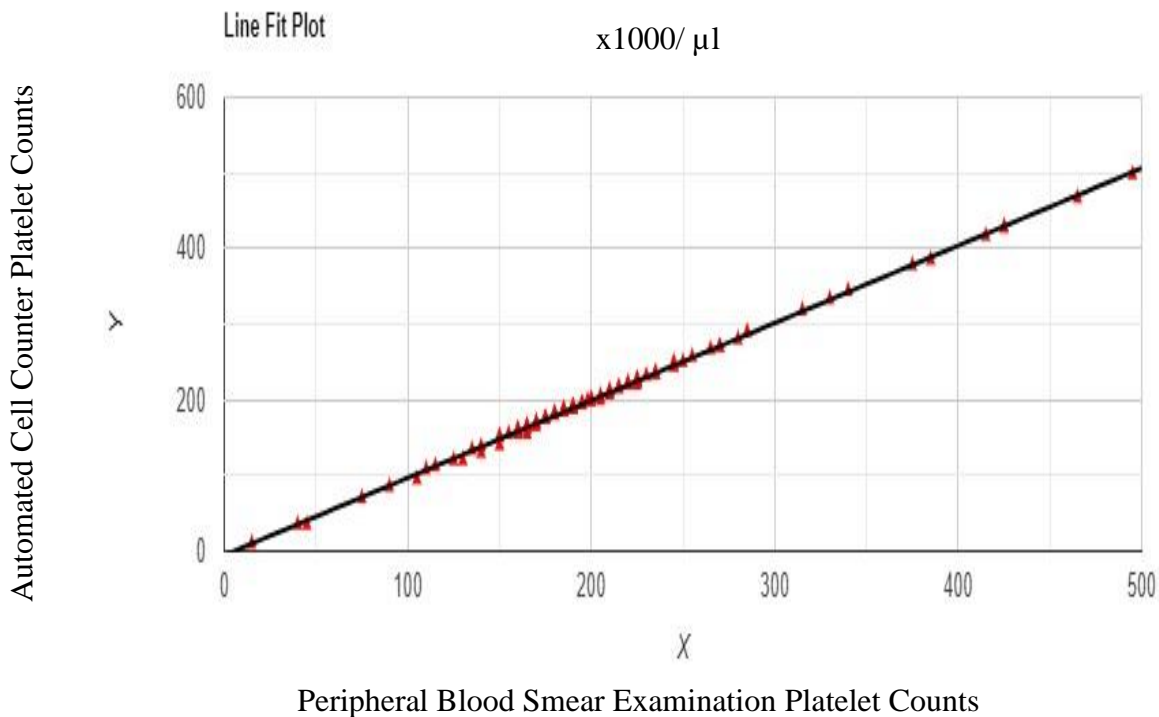
**Fig. 1: Scatter diagram depicting platelet counts using manual peripheral blood smear examination.**



**Fig. 2: Scatter diagram depicting platelet counts using automated cell counter method.**



**Fig. 3: Line fit plot scatter graph for between platelet count values using peripheral blood smear and automated cell counter method.**



Regardless of the remarkable advances in haematology automation and related molecular techniques, peripheral blood smear examination remains a very important diagnostic test in day to day

laboratory set up for the pathologists. The precise, accurate and reliable assessment of platelet count is warranted for the proper and correct diagnosis of platelets related medical conditions and to avoid unnecessary platelet transfusion in the management of thrombocytopenic patients. The accuracy is also required for monitoring the therapeutic response after platelet transfusion.

The platelets are small disc shaped blood components, 2 to 4  $\mu$  in diameter and 7 to 8  $\mu$  in volume, are derived from megakaryocytes, giant cells in the bone marrow. Megakaryocytes constitutes of less than 1% of myeloid cells in the bone marrow. One megakaryocyte can give rise to 1000 to 3000 platelets, by pinching off and extruding pieces of cytoplasm. The platelets measures are non-nucleated with the life span of about 7-12 days. They are destroyed by spleen macrophages. In Leishman's stained PBS, under microscope, platelets appear as light blue to purple coloured, round, oval or rod shaped structures with irregular margins. Platelets play key role in hemostasis, thrombosis and wound repair. The normal range of platelets count in healthy human being is  $150 \times 10^3$  to  $450 \times 10^3$  platelets per  $\mu$ l.[1,4,5]

The hematology analyzer platelet count estimation accuracy is observed to be compromised while processing blood samples with low platelet counts or with abnormal platelets morphology like giant platelets, platelet clumps or in the presence of non platelet particles like RBC, WBC fragments. Inadequate calibrations and lack of adequate quality control material also compromise the platelet count accuracy in automated analyzer. [6,7] Thus, PBS has its own importance for validating results of other methods for platelet counting. PBS evaluation cannot replaced by even the accurate and best quality hematology analyzer.[1]

The present study documented no significant difference ( $p = 0.06635$ ) between manual peripheral blood smear (PBS) method (platelets average per 100x, multiplied by 15000/  $\mu$ l) of platelet estimation ( $207.13 \pm 81.11 \times 1000/ \mu$ l) and that of automated cell counter platelet value ( $206.53 \pm 83.05 \times 1000/ \mu$ l).

Anchinmane VT et al.,(2019) blood samples of the 100 patients and observed no significant difference between the platelets values estimated by PBS method and automated analyzer( $p > 0.05$ ). [8]

Gole et al.,(2018) observed the similar findings in a study with 95 blood samples and documented no significant difference ( $p = 0.866$ ) of the values between manual peripheral blood smear method (platelets average per 100x, multiplied by  $15.0 \times 10^9 /L$ ) of platelet estimation ( $1.90 \pm 0.97$  lacs/  $\text{mm}^3$ ) and that of automated hematoanalyzer ( $1.88$  lacs/ $\text{mm}^3 \pm 0.98$ ).[2]

Momodu I et al., (2016) studied 30 males and 20 females patient's blood samples and evidenced no significant difference in estimation of platelet count by peripheral blood smear method (PBS) using multiplication factor of  $20.0 \times 10^9/L$  compared to automated method ( $p > 0.05$ ). [9]

Bajpai R et al.. (2015) study, observed that the mean platelet count estimated by the PBS examination method (platelets average per 100x, multiplied by  $15.0 \times 10^9 /L$ ) and the automated hematoanalyzer method didn't show significant difference ( $p = 0.69$ ) in results for all the 92 blood samples studied.[10]

Bakhubaira S et al., (2013) studied 190 random samples and the mean platelet count estimated by the PBS method and the automated method did not show significant difference ( $p = 0.44$ ). [11]

In the present study, we documented significant positive correlation between the two methods ( $r=0.9995$ ,  $p < 0.001$ ), when analysed by Pearson correlation test.

Anchinmane VT et al.,(2019) also showed excellent positive correlation between the two methods with the coefficient of correlation of the linear regression for analysis of platelet count estimation of  $r = 0.9789$ . [8]

Gole et al.,(2018) observed significant positive correlation by Pearson correlation test between the two methods ( $r=0.996$ ,  $p < 0.0001$ ).[2]

Bakhubaira S et al., (2013) analyzed the samples by the Pearson correlation and evidenced significant positive correlation between the results of both PBS and automated platelets counts ( $r=0.563$ ,  $p=0.000$ ). [11]

The manual phase contrast microscopic method has been the gold standard for platelet estimation to assess any degree of accuracy of the automated cell counter platelet count. [12] Though both these methods are highly sensitive but they are expensive as well as timeconsuming methods and hence are not cost effective in many rural set ups in developing country. Manual method has significant limitations of precision and considered arbitrary method of assurance. [11] Regardless of all these limitations and drawbacks, by peripheral blood smear method platelet estimation is easier, rapid and cheaper, and does not require any expensive equipment and consumables. Also, PBS examination aid to detect the giant platelets which were not counted by automated analyzer, hence correcting the estimated false low platelet counts by automated cell counter.

Various previous studies have shown and suggested that both the methodologies are equally effective and efficient without any significant difference.

## CONCLUSION

Platelet estimation by PBS examination is effective, efficient, reliable, rapid, easy as well as a cost effective method. It can be used in under resourced hematology laboratories and suitable for rural areas and developing countries. It can be used for early and rapid platelet estimation needed for early interventions in patients with thrombocytopenic conditions or platelet transfusion and its monitoring. This method is also important for verifying the platelet count obtained from automated cell counter, especially in samples with abnormal platelets morphology or presence of non platelets particles mimicking platelets in hematoanalyzers. Hence, it can be concluded that peripheral blood smear (PBS) examination can be used as a quality control tool in assessing the platelet count results of the automated cell counters.

## References:

1. Adewoyin AS and Nwogh B. Peripheral Blood film A review. Ann of Ib Postgrad Med 2014;12(2):71-9.
2. Gole SG, Krishna M, Gole GN. Comparison study of platelet count estimation by two methodologies: An automated hematology analyzer and peripheral blood smear examination. Indian J. Pathol. Res. Pract. 2018;7:905-9.
3. Malok M, Titchener EH, Bridgers C, Lee BY, Bamberg R Comparison of two platelet count estimation methodologies for peripheral blood smears. Clin Lab Sci. 2007 summer; 20 (3): 154-60.
4. Ogawa M. Differentiation and proliferation of hematopoietic stem cells. Blood. 1993;81:2844-53.
5. Brecher G, Cronkite EP. Morphology and enumeration of human blood platelets. J Appl Physiol. 1950;3:365-77

6. Briggs C, Harrison P, Machin SJ. Continuing developments with the automated platelet count. *Int J Lab Hematol*. 2007;29(2):77-91.
7. Charie LA, Harrison P, Smith CU, Cobb JR, Briggs C, Machin S. Accuracy in the low platelet count range: A comparison of automated platelet counts on Beckman coulter high-volume hematology analyzers with the ISLH/ICSH platelet reference method. *Lab Hematol*. 2001;7:236-44.
8. Anchinmane VT, Sankhe SV. Utility of peripheral blood smear in platelet count estimation. (2019)
9. Momodu I. Determination of Platelet and White Blood Cell Counts from Peripheral Blood Smear: An Indispensable Method in Under-resourced Laboratories. *International Blood Research & Reviews* 2016,5(2): 1-7.
10. Bajpai R, Rajak C, Poonia M. Platelet estimation by peripheral smear: Reliable, rapid, cost effective method to assess degree of thrombocytopenia. *Inter J Medical Sci Prac* 2015;2(2):90-3.
11. Bakhubaira S. Automated versus manual platelet count in Aden. *J Clin Exp Pathol* 2013;3:3.
12. Brecher G, Schneiderman M, Cronkite EP. The reproducibility and constancy of the platelet count. *Am J Clin Pathol* 1953;23:15-26.