IJPRP

Indian Journal of Pathology: Research and Practice
Volume 7 Number 8, August 2018
DOI: http://dx.doi.org/10.21088/ijprp.2278.148X.7818.3

Original Research Article

Comparison Study of Platelet Count Estimation by Two Methodologies: an Automated Hematologyanalyzer and Peripheral Blood Smear Examination

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(**Received on** 21.05.2018, **Accepted on** 24.05.2018)

Abstract

Introduction: Many diseases such as malaria, dengue, pyrexia of unknown origin, pregnancy-induced hypertension are associated with low platelet counts. Automated method is the most reliable method. It is simple, fast, and most widely used. But automated cell counters are not available at underresourced laboratories, especially in rural settings. Hence, platelet estimation by peripheral blood smear is more easy and cost-effective.

Aim: To compare platelet count estimation performed by the peripheral blood smear method and the automated cell counter method.

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

Materials and Methods: Present study was carried out in the Department of Pathology at a tertiary care centre in rural Haryana. Study included 95 random blood samples collected into ethylenediaminetetraacetic acid. These were examined by both peripheral blood smear and automated cell counter for platelet estimation.

Results: In the present study there was no significant (p = 0.866) difference of values between manual peripheral blood smear (PBS) method (platelets average per 100x, multiplied by 15.0x109/L) of platelet estimation (1.90±0.97 lacs/mm3) when compared with that of automated cell counter platelet value (1.88 lacs/mm3±0.98). Significant positive correlation between the result of both methods (r=0.996, p=0.0001) was observed when samples were analysed by Pearson correlation test.

Conclusion: Although the necessity of automated cell counters for rapid generation of results of vast number of blood samples is undeniable, yet the results of peripheral blood smear platelet estimation are comparable with them. Hence manual smear examination serves as a quality control tool in assessing the results of the automated cell counters.

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

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Introduction

Many diseases such as malaria, dengue, pyrexia of unknown origin, pregnancy-induced hypertension is associated with low platelet counts. Thrombocytopenia is one of the critical parameters in management of these patients. Automated method is considered as the most reliable method. It is simple, fast, and most widely used. But automated cell counters are not available at under resourced laboratories, especially in rural settings. In such scenario, platelet estimation by peripheral blood smear is easier. Peripheral Blood smear (PBS) is a useful haematological test and can be used for the screening, diagnosis and monitoring of disease progression, and also for therapeutic response [1].

Nowadays, in routine clinical practice, there is an increase in request for accurate platelet count by the physicians. This need is being attributed to the presence of increase in number of cases with thrombocytopenia. Thrombocytopenia is one of the critical parameters in patient management. It is observed to be associated with various conditions like malaria, dengue, pyrexia of unknown origin, pregnancy-induced hypertension, and leukemia chemotherapy for various malignancies. Few clinicians ask for repeat estimation of platelet count manually if the result of electronic count does not correlate with clinical condition of patient, and some prefer the automated count since they lost credibility in the results of manual counting method.

More difficult is to estimate plateletsthan the red or white cells [2]. In 1950 Brecher and Cronkite method [3] was described and was considered to represent the optimum compromise between accuracy, time and cost.

Laboratories should assess platelet counts with utmost accuracy. The normal range of platelet count in a healthy individual is 150000-400000/ µL. Methods commonly performed for platelet estimation aremanual counting with the counting chamber, manual peripheral blood smear method, and automated cell counters.

Peripheral blood smear examination evaluates the results of automated cell counters that are prone to interferences from particles of similar size and/or light scatter properties such as red cell fragments, apoptotic white blood cell fragments, platelet clumps amongst the other cells [4,5,6]. PBS examination can also serve as a quality control tool in confirming the results derived from automated analyzers [7].

This is a cross sectional study performed to compare the platelet count estimation by the manual Leishman stained thin blood film peripheral blood smear method and automated cell counter method.

Materials and Methods

The present study was carried out in the Department of Pathology at a tertiary care centre in rural Haryana. Study included 95 random blood samples collected into ethylenediaminetetraacetic acid. These were examined by both peripheral blood smear and automated cell counter. Each blood sample was used for the determination of complete blood count using Mindray BC 5000 5 part hematology analyser. Preparation of Leishman's stained blood films was done using standard laboratory methods. The calculation was done by; performing platelet count in 10 an oil immersion fields, and the average was multiplied by using the multiplication factor of $15.0x10^9/L$.

Statistical Analysis: Statistical analysis was done by Student's t-test by using SPSS 20.

Results

In the present study there was no significant ($p = 0.866$) difference of values between manual PBS method (platelets average per 100x, multiplied by $15.0 \times 10^9/L$) of platelet estimation (1.90±0.97 lacs/ mm^3) when compared with that of automated cell counter platelet value (1.88 lacs/mm^3 ±0.98). (Table 1) (Figures 1 & 2)

When all the samples were analyzed by the Pearson correlation test, we observed significant positive correlation between the result of both methods ($r=0.996$, $p=0.0001$).

Discussion

Peripheral blood smear examination remains a very important diagnostic test to the haematologist regardless of the remarkable advances in haematology automation and molecular techniques [1].

Momodu I [8] study, observed significantly lower value of platelet count on PBS (with multiplication factor of $15.0 \times 10^9/L$) compared to

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

Variable	No. of samples (n)	Mean (lakhs/mm^3)	Standard deviation
Manual method	95	1.90	0.97
Automated method	95	1.88	0.98

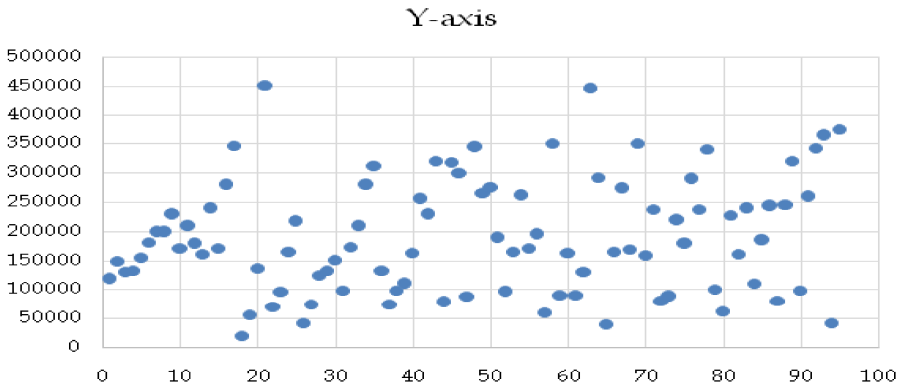


Fig. 1: Scatter diagram depicting platelet counts using manual peripheral blood smear examination

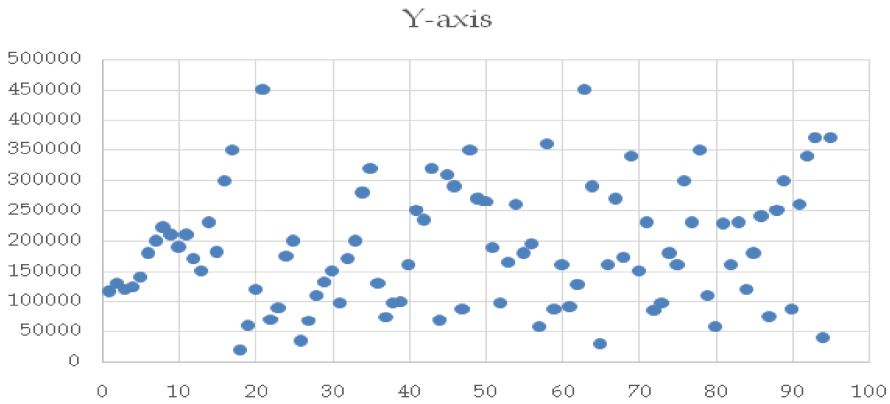


Fig. 2: Scatter diagram depicting platelet counts using automated cell counter method

automated platelet count as against the findings of Webb et al. [9] and Bajpai et al.[10] that reported slightly better results with 15.0 $x10^9$/L multiplier than the multiplication factor of 20.0$x10^9$/L. However, Momodu I [8] study had a strong positive correlation between platelet counts from automation and PBS (platelets average per 100x, multiplied by 15.0 $x10^9$/L).

In the present study there was no significant (p = 0.866) difference of values between manual PBS method (platelets average per 100x, multiplied by 15.0 $x10^9$/L) of platelet estimation (1.90±0.97 lacs/mm^3) when compared with that of automated cell counter platelet value (1.88 lacs/mm^3 ± 0.98) (Table 1).

Bajpai R et al. [10]. study, observed that the mean platelet count estimated by the manual method (platelets average per 100x, multiplied by 15.0 $x10^9$/L) and the automated method for all the 92 samples studied did not show significant statistical difference (p = 0.69) in the results.

Momodu I [8] study showed that there was no significant difference between platelet count estimate using PBS (with multiplication factor of 20.0 $x10^9$/L) and that of automated cell counters. There was fairly strong positive correlation of manual platelet count on PBS (multiplication factor of 20.0 $x10^9$/L) with automated method.

Study done by Webb et al. [9]. reviewed 35 samples with normal, low, high platelet counts. They compared PBS examination with the automated counter results. Their study showed fair concordance in 27 specimens. In three specimens' underestimation was seen, and overestimation in five.

Bakhubaira S [11] studied 190 random samples and the mean platelet count estimated by the manual method and the automated method did not show significant statistical difference (p=0.44). This was similar to our study wherein there was no significant (P = 0.866) difference of values between manual PBS method when compared with that of automated cell counter method.

In the present study when all the samples were analyzed by the Pearson correlation test, we observed significant positive correlation between the result of both methods (r=0.996, p=0.0001).

In the study done by Bakhubaira S [11] when all the samples were analyzed by the Pearson correlation test, there was significant positive correlation between the result of both methods (r=0.563, p=0.000).

Study done by Oliveira et al. [12]. suggested, a platelet count below 30,000/µl obtained in automated counters, should be confirmed by manual method. Manual platelet counting in Neubauer chamber by means of phase contrast microscope has been recommended as the reference method [13].

The gold standard for platelet counting to assess any degree of accuracy of the automated count has been the manual phase contrast microscopic method [14]. This method and automated cell counter methods are highly sensitive but the drawback is that these are expensive; and time-consuming and hence are not cost feasible in many rural set ups in our developing country.

Manual method has significant limitations of performance, especially of imprecision. It is considered as an arbitrary method of assurance [11]. Regardless of these drawbacks, PBS platelet estimation is rapid, cheaper and easier, and does not need any expensive equipment and consumables.

Various observations from previous studies suggests that both the methods are equally efficient and can be used to count platelets without producing a significant difference in results.

Conclusion

Peripheral blood smear examination is a reliable, rapid, easy and cost-effective method that can be used for estimation of platelet counts in the haematology laboratory and especially, in the underresourced medical laboratories and in rural hospital settings, in a developing country. Platelet peripheral blood smear estimation method can be taken as an early and rapid procedure for platelet assessment in cases where low platelet count needs an early intervention such as in pregnancy-induced hypertension, dengue, and malaria, etc., for their management and for fast referral if required from the underresourced hospitals to higher centres. Although the necessity of automated cell counters for rapid generation of results of vast number of blood samples is undeniable, yet the results of peripheral blood smear platelet estimation are comparable with them. Mean platelets count estimated by the manual method do not significantly differ from that estimated by the electronic method. A significant positive correlation is present between the two counting methods of platelets. It can be concluded that blood smear examination serves as a quality control tool in assessing the results of the automated cell counters.

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