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Comparison of Automated Platelet Count with Multiple Manual Platelet Count Methodologies in Subjects with Normal Mean Platelet Volume

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Abstract

Objective: The objective of the present study is to compare values of platelet count on automated coulter with different manual platelet counts and also to ascertain the better methodology to do the same, particularly when coulters are not available in remote areas. *Materials &Methods*: Platelet count was estimated by four different methods in 75 blood samples(25 each with low, high and normal platelet count) received in Department of Pathology, Dayanand Medical College & Hospital, Ludhiana and compared with platelet count obtained from automated analyzer Beckman coulter LH750. Methods A and B were based on counting the average number of platelets per oil immersion field (OIF) in 10 fields multiplied by factor of 2 and 3 respectively and to yield a platelet count estimate per 10^9/l. Methods C and D were based on multiplying the total number of platelets counted under 2 high power fields by factor of 2 and 3 respectively. All cases had a normal mean platelet volume(MPV). *Statistical Analysis:* The agreement between the manual methodologies with each other and each method with the automated count was assessed using the unpaired T-test and correlation coefficient analysis done. *Results:* Method A and Method C showed no significant differences in platelet values in all the three groups and were comparable to those obtained by automated analyser. *Conclusion:* Manual methods give reliable and accurate results similar to automated analyser and can be used in rural settings and also automated counts should be counter checked by manual method especially in cases of thrombocytopenia.

Keywords: Platelet; Manual Count; Automated Analyser.

Introduction

Platelets are anucleate cell fragments derived from bone marrow megakaryocytes measuring 1-3μm in Giemsa stained peripheral blood preparation.They appear as small pink purple coloured bodies with irregular borders. The normal platelet count ranges from 150 to 450 $x10^9$ /l.The mean platelet volume (MPV) in health ranges from 7.7 to 11.2 fl. Platelets have proved more difficult to count than either red blood cells or white blood cells because of wide normal range. Thus, a person with platelet count of $150x10^9$/l is considered normal as is person with a count of $450x10^9$/L which is 3 times of this value.Manual counting using phase contrast microscopy, impedance analysis, optical light scatter/fluorescence analysis and immuno-platelet counting by flow cytometry are the main procedures that are used for platelet counting [1]. The platelet count estimation is an important and routinely requested laboratory investigation and its assessment form an essential part in both clinical haematology and platelet research laboratories. Both numerical and functional alterations in platelets may lead to bleeding. The best method for estimation of platelet count is done by manually counting the platelets under a phase contrast microscope. However, it is not easily available and is a tedious procedure [2]. The automated blood cell counters are now more commonly used owing to faster and accurate results in a short time however they have certain limitations in having stringent quality assurance, not cost effective and unavailability in small peripheral centres.

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Manual methods, though suffer from drawbacks such as suboptimal staining, stain deposits leading to false high or low counts, but still are considered as the best reference method and are also used in calibrating the automated cell counters and doing quality control [2,3]. Many researchers in past have used different methodologies to ascertain the best and most accurate manual platelet count method and the goal of this study was to find the best manual method that correlates well with the results of automated analyser and can also be trusted in a basic laboratory set up where automated coulter facilities and oil immersion lens are not available.

Materials and Methods

The study was conducted in the Department of Pathology in which 75 peripheral blood smears were analysed. The samples were randomly selected of patients who attended outpatient department or were admitted in the wards between January to February 2017 and samples were received in the clinical laboratory. Data of age and sex of patients were taken from the requisition forms sent along with the samples in the laboratory.

Inclusion Criteria:

The samples with normal mean platelet volume were included in the study, thereby ruling out marked variation in platelet size.

Exclusion criteria:

However, the samples which had an abnormal MPV or giant platelets were observed on peripheral smears were excluded from the study.

Thus, total of 75 EDTA samples were taken from patients with low, normal and high platelet counts (25 each). The samples were processed by trained hematology technicians in pathology laboratory ruling out any hemolysed or clotted sample. The samples were run on Beckman LH750 automated analyser. The values were blinded from the pathologist who did the manual count without knowing the count given by coulter. For manual platelet count, the peripheral smears were made, fixed and stained with Giemsa stain. The slides were given to the 2 pathologists for examination. The platelets were counted by 4 different manual methods [Table 1].

Average of platelet count of both pathologists were taken into consideration. All the 4 methods were done on all 75 blood samples and then the comparison between the values given by automated cell coulter was done with the manual count values and the agreement between these values and also among the different methodologies for manual count of platelet was done by applying unpaired t-Test. The data were analyzed using Microsoft Excel and SPSS version 20.0 (IBM SPSS, Chicago, Illinois, USA).

Results

The age of patients included in the study ranged from as young as a 6 days old male child to as old as an 85 year old female. The automated platelet count estimation method had a mean for the sample of $255.6 \times 10^9/l$ (SD =85.94) in the normal range of platelet count while the mean of the manual count done by method A was $248.8 \times 10^9/$ L (SD=79.55) which, according to the unpaired T-test, was significantly not different from that of automated method ($p > 0.05$). The traditional estimation method counts and automated counts had a high Pearson Product Moment correlation coefficient of $r = 0.76$ [Table 2] and a minimally dispersed scatter plot, thus showing strong agreement [Figure 1]. The platelet count done by method B, C, D had a mean of $371.4 \times 10^9/l$ (SD=101.5), 233.1x109/L (SD= 95.83) and 349.68/ $10^9/l$ (SD=143.74) respectively.

In the subset of patients having a high platelet count, the mean of platelet count done by automated method was $725.42 \times 10^9/l$ (SD=35.89) while that done by traditional manual method was $676.48 \times 10^9/L$ (SD=244.48) which, according to the unpaired T-test, was significantly not different from that of automated method ($p > 0.05$). With method B, this subset had a mean of $1014.72 \times 10^9/L$ (SD =366.73) and that done by methods C and D respectively was $560.24 \times 10^9/L$ (SD = 233.14) and $840.3 \times 10^9/L$ (SD =349.72) respectively.

A minimally dispersed scatter plot, showing strong agreement was similarly noted in high platelet count group showing strong agreement between the findings of automated analyser and method A having a high pearson coefficient ($r=0.95$) [Table 3 & Figure 2].

Lastly, the patients with a low platelet count had a mean platelet count of $81.24 \times 10^9/L$ (SD =22.86) by the automated method while that done by the method A showed a mean value of $78.8 \times 10^9/L$ (SD=22.93), which according to the unpaired T-test, was significantly not different from that of automated method ($p>0.05$) and had high correlation ($r=0.71$) [Table 4 & Figure 3]. The mean platelet count done by methods B, C and D were $117.6 \times 10^9/L$ (SD=34.2), $82.4 \times 10^9/L$ (SD=29.85) and $123.4 \times 10^9/L$ (SD=44.46) respectively.

Hence in conclusion, the Pearson correlation test

showed significant positive correlation between the automated and method A. This correlation remained significant when the samples of normal count by the two methods were correlated (r=0.77). Similarly, positive correlation was also found when the samples of low or high counts by the two methods were correlated (r equal to 0.71 and 0.95) respectively. No significant difference was found on unpaired t-test done between counts done by Method A and C and also with automated counts.

Table 1: Methods and formulas used to calculate platelet count

Method	Formula used to calculate platelet count (Unit)
Method A (traditional)	Average no. of platelets in 10 OIF X 2 (10^9/l)
Method B	Average no. of platelets in 10 OIF X 3 (10^9/l)
Method C	Total Number of platelets counted in 2 Hpf (40X) x 2 (10^9/l)
Method D	Total Number of platelets counted in 2 Hpf (40X) x 3 (10^9/l)

Table 2: Statistical Analysis in group of patients having normal platelet count range

Type of Manual Count			Gold Standard (100X x 2)		Statistical Analysis	
	Mean	SD	Mean	SD	'r'	p
Automated Count	255.6	85.94	248.08	79.55	0.77	<0.01
100Xx3	371.4	101.5			**0.891**	<0.01
40Xx2	233.12	95.83			0.526	<0.01
40Xx3	349.68	143.74			0.526	<0.01

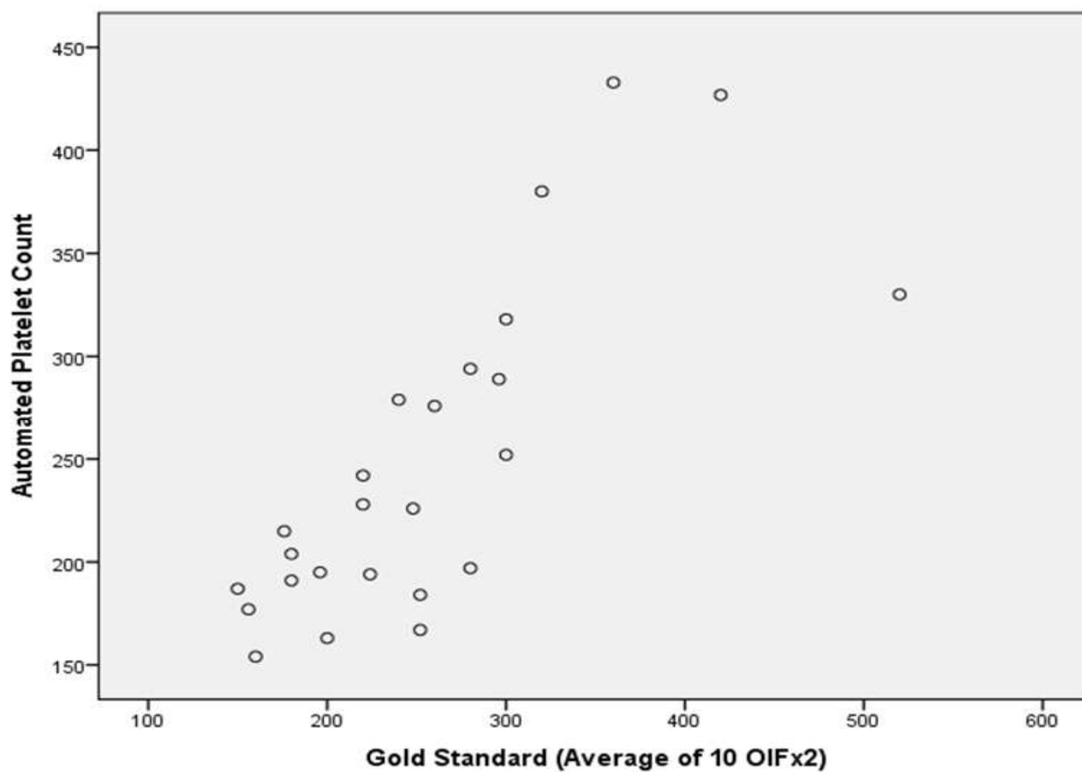

Fig 1: Scatter Diagram showing traditional manual method Vs automated count in group having normal platelet count

Table 3: Statistical Analysis in group of patients having low platelet count range

Type of Manual Count			Gold Standard (100X x 2)		Statistical Analysis	
	Mean	SD	Mean	SD	'r'	p
Automated Count	725.4	235.89	676.48	244.48	0.95	<0.001
100Xx3	1014.72	366.73			**1.0**	<0.001
40Xx2	560.24	233.14			0.87	<0.001
40Xx3	840.36	349.72			0.87	<0.001

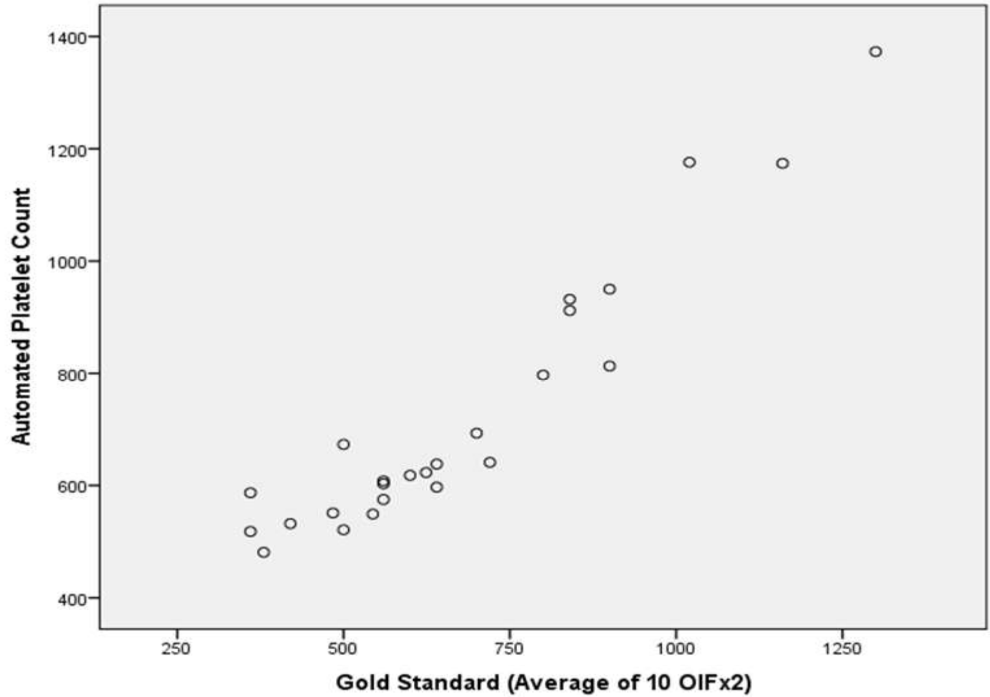

Fig 2: Scatter Diagram showing Method AVs Automated Count in group having high platelet count

Table 4: Statistical Analysis in group of patients having low platelet count range

Type of Manual Count	Mean	SD	Gold Standard (100X x 2) Mean	SD	Statistical Analysis 'r'	p
Automated Count	81.24	22.86	78.8	22.93	0.71	<0.01
100Xx3	117.6	34.2			**0.996**	<0.001
40Xx2	82.4	29.85			0.628	<0.01
40Xx3	123.4	44.46			0.632	<0.01

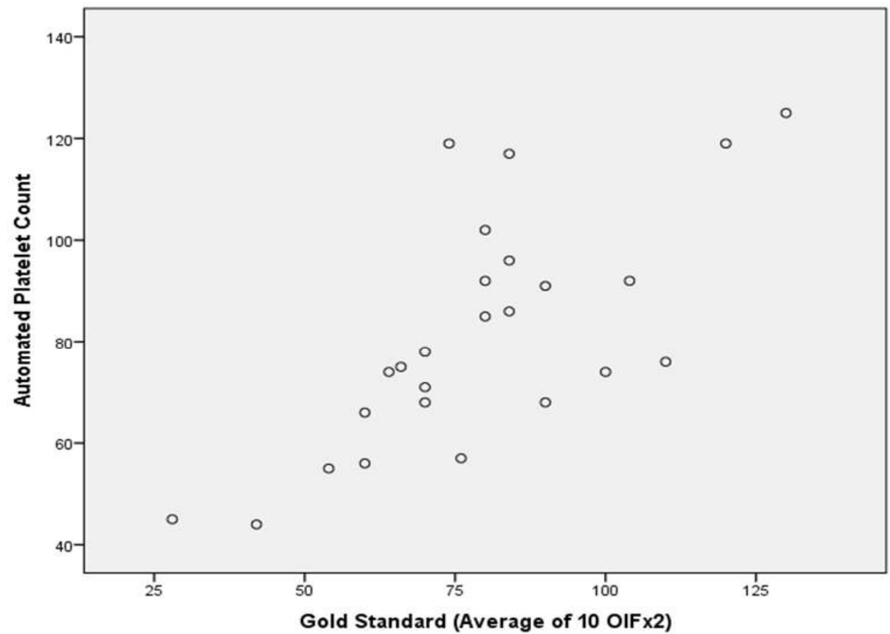

Fig 3: Scatter Diagram showing Method AVs Automated Count in group having low platelet count

So, traditional Method 'A' Vs Automated Vs Method 'C', all came non-significant by comparison of means by unpaired 't' test in normal, high as well as low platelet counts. Hence, they stand equal and can be used alternatively in required settings and can guide consultants where accurate monitoring of platelets is required.

Discussion

Automated blood cell counters are nowan important component of a modern laboratory set up which use impedance for platelet estimation according to which the blood cells are regarded as nonconductive resistivity particles which when pass through an aperture (sensing zone) suspended in electrolyte solution lead to a change in electric impedance, which is then detected. The impedance signal is proportional to the volume of the cell detected, and so this method can be used to size and count individual cells [1,4]. However, its reliability in counting low platelet counts has been hindered owing to the presence of background debris, microorganisms, fragmented RBCs, apoptotic bodies and markedly microcytic RBCs that mimic as platelets. In addition, platelet clumps and giant platelets are not counted by automated analyzers. All these lead to inaccurate platelet counts in an automated hematology analyser [4]. Therfore, most of the laboratories use a standard protocol of manual checking the platelets on peripheral smear in cases of thrombocytopenia or if any flag sign is showed by the analyser. In addition, high cost, maintenance, stringent quality assurance which is more cumbersome for platelet counts than other counts hamper their use in peripheral set ups where they rely more on the manual counting methods [1-4].

Estimation of platelet count by the phase contrast microscopy was considered the traditional gold standard method, however there was a lack of precision in cases with thrombocytopenia and moreover it was time consuming [5].

The routine method used in clinical laboratories to verify the automated platelet count is by multiplying the average number of platelets counted in 10 oil immersion fields and multiplying by factor of 1.5 or 2. Nosanchuk et al in his study statistically validated a multiplication factor of 2 for estimation of platelet countin peripheral smears and since then it is used in routine practice [6]. However, Webb et al in his study reviewed 35 samples with normal, low, high platelet counts found concordance with the results of automated analyser in 27 samples and suggested 1.5 as a better multiplication factor than multiplication factor of 2. In the present study,multiplication factor of 2 was used as it is most widely used by most pathologists [7].

The mean platelet count estimated by the method A for normal, low as well as high values and the automated method for all the samples studied (n= 75) did not show any significant statistical difference (P= 0.69) in the results. The Pearson correlation test showed significant positive correlation between both methods (r: 0.76, 0.71, 0.95 respectively for normal, lowand high platelet counts)and a minimally dispersed scatterplot, thus showing strong agreement. This was in concordance with a cross- sectional study done by Bakhubaira in National Centre for Public Health Laboratories of Aden,Yemen who found that the correlation remained significant when the samples of normal count by the two methods were correlated (r: 0.359), but it was insignificant when the samples of low or high counts by the two methods were correlated [8]. Bajpai et al [9], Momodu et al [10] and Anitha K et al [11] also in their respective studies found correlation between the manual and automated methods.

However, it was in contrast to study done by Sudalaimuthu et al [12] who compared 4 methods of platelet estimation in 200 samples and found that average platelets per 10 oil immersion fields multiplied by 1.5 and 2 differed significantly from that of the automated hematology analyzer (p<0.001). He further suggested estimation of platelet counts based on platelet/ RBC ratio in ten fields as an ideal and simple method to verify the platelet count results ofautomated analyser as on comparing results he found a strong correlation coefficient (0.973) with minimal dispersion in the scatterplot when plotted against the automated analyzer values. Though in present study, we have not compared our methods with platelet/ RBC ratio.

In the present study,manual platelet counts were performed under 10 oil immersion fields, whose average were compared after multiplying them by factor of 2 and 3 respectively and also with the automated analyser. The results differed significantly (p<0.001) in normal, low and high groups suggesting that the results obtained by using multiplication factor 3 are not in correlation with those given by automated analyser. However, no study has yet been reported to support this finding.

An alternate estimation method was also used in this study in which platelets were counted under high power field (40X) and multiplied by factor of 2. No significant difference was found when the results were compared with automated counts as well as traditional manual method (p<0.001).The unpaired t-

Test done on the three methods showed no significant difference suggesting that the three methods stand equal and can be used alternatively in required settings. Our findings suggest it being a good alternative method and is the first study to report this method as a simple and faster alternative, provided the blood smear is well spread. It can be used in laboratories with basic set up, where microscopes have no oil immersion lens or sample load is very high. Thus, it saves time and facilitates faster reporting in urgent cases and health sectors where automated analysers are not there.

The method D which was adopted in our study didn't give desirable results as significant statistical difference was noted among the counts done by traditional manual method as well as done by automated analyser.

The peripheral blood film evaluation for platelet estimation cannot be substituted and forms an important part of the routine blood smear examination and should be used as a reference method for verifying results of the automated hematology analyzers .

Conclusion

The present study results showed that although the fully automated haematology analysers have made the reporting of blood samples easier and reliable however, they are known to be inaccurate at enumerating platelet counts and platelet indices in various conditions.

The manual estimation of platelet count done either as average number of platelets counted under 10 oil immersion fields and multiplying it by factor 2 or total number of platelets counted under 2 high power fields and multiplied by factor 2 (Methods A and C) also gives reliable results and comply with those of the automated analyser. The values of automated count should be counter checked by manual methods and every laboratory should set up a standard protocol especially for thrombocy-topenia in which false low platelet counts are given owing to platelet clumps or presence of giant platelets. The manual methods provide similar results and can be used under rural settings where there is dearth of automated facilities.

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