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Original article

Determination of suitable multiplication factor for estimation of total platelet count from peripheral blood smear

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Abstract
Introduction: Automated haematology analyzers are commonly used for accurate and precise platelet estimation. Still false low or false high results are common with flagging on analyzers which needs to be reviewed. So commonly we perform peripheral blood smear (PBS) evaluation for cross checking the platelet count. For estimation of total platelet count on peripheral blood smear a multiplication factor is required. Different laboratories use different multiplication factors including 10,000, 15,000 or 20,000 for platelet count estimation on peripheral smear.
Methods: This was an observational study included total 300 cases including 100 cases each for low, normal and high platelet counts estimated by Sysmex XN 3100 haematology 6 part analysers using K3 ethylene diamine tetra-acetic acid blood samples. Peripheral smears were prepared. After blinding of analyser results, manual peripheral smear examination was carried out to calculate average number of platelets for per oil immersion field (OIF). Platelet counts were derived by multiplying average number of platelets for per OIF by multiplication factors of 10,000, 15,000 and 20,000. Comparison of these values was done with actual automated analysers counts.
Results: We found multiplication factor of 20,000 as most suitable which correlated well with actual platelet count.
Conclusion: A standard multiplication factor determination is necessary for accurate manual platelet count on peripheral blood smear examination. This method is easy, simple, less time consuming and can be used in platelet count discrepancies, mandatory reviews and in resource poor settings.
Keywords- Platelet count, Automated, Manual, Peripheral blood smear, Multiplication factor

Introduction-

Platelet count is an integral and very important component of complete blood count examination. It is essential to know accurate count for proper management of critical patients, especially in low count cases and haemorrhagic disorders. There are various methods used for evaluation of platelet counts which include automated as well as manual methods. Automated methods include Immuno-platelet counting method, Impedance platelet counting method, Optical platelet counting method and Fluorescence platelet counting method. While manual or microscopic methods include Counting using Improved Neubauers chamber and using Peripheral blood smear. Some of the authors have tried to evaluate platelet counts using unstained peripheral blood smears also with satisfactory correlation. Though automated analysers give precise and correct results, sometimes there can be an overestimation or underestimation of count can occur. False high platelet counts can be seen in cases of fragments of erythrocytes, severe microcytosis, fragments of other nucleated cells, fungi, bacteria, lipids as well as cryoglobulins.

While false low platelet count can be observed in cases of large platelets, platelet aggregates, fibrin and platelet satellitism.[1,3] Also manual blood smear review is mandatory when an adult patients show platelet count less than 100 x 10^9/L in the initial investigation or >3 months after the initial result. For children, the cut off used is counts less than 150 x 10^9/L in the initial investigation or >1 month after the initial results[4]. Thus in such situations cross checking of platelet count is crucial for giving correct results. So routinely a simple peripheral blood smear examination is carried out to count number of platelets using various multiplication factors like 10,000, 15,000 or 20,000.[5-7] The current study is carried out to evaluate suitable multiplication factor for estimating near correct platelet count using peripheral blood smear counting of platelets.

Material and Methods-

The study was conducted after approval of Institutional ethical committee Of Pravara Institute of Medical Sciences, deemed university vide approval number PIMS/DR/RMC/IEC-UG-PG/2023/333 dated on 06.12.2022.

This was a descriptive cross sectional observational study in which total 300 cases were selected including 100 cases each for low, normal and high platelet counts as obtained on 6 part automated haematology analysers. We included consecutive cases coming to haematology section of central clinical laboratory of a tertiary care hospital. Total 100 cases each were selected for high platelet count (PC>4.5 lac/cmm), normal platelet count (PC 1.5 to 4.5 lac/cmm) and low platelet count (PC<1.5 lac/cmm) and they were divided into three groups Group 1, Group 2 and Group 3 respectively. Platelet count estimation was done using Sysmex XN 3100 haematology autoanalysers using fluorescent based detection. Two ml blood samples in tubes containing K3 ethylene diamine tetra-acetic acid (K3EDTA) anticoagulant were used for analysis. Simultaneously peripheral blood smears were prepared for all selected samples using SP 50 fully automated slide maker and stainer system (Sysmex) using Wright-Giemsa stain. Samples with platelet aggregates flags on automated analyser or platelet clumps or giant platelets on smear were excluded. Blinding of automated analyser results was done to avoid any bias at the time of peripheral evaluation.

The peripheral blood smear examination was carried out by experienced pathologists. At first a suitable area was selected where RBCs are evenly arranged just touching to each other (junction of body and tail) avoiding overlapping and sparsely spaced areas. At 40x magnification, a suitable area with maximum number of platelets was selected. Manual examination & counting was carried out under oil immersion field (OIF). Total numbers of platelets in 10 OIF were counted and average numbers of platelets per OIF were counted by dividing number of platelets in 10 OIF divided by 10. Then we used multiplication factors of 10,000, 15,000 and 20,000 to get the approximate total platelet count by manual peripheral blood smear examination for each selected case. The results were recorded in MS Excel format. Then comparison of these values were done with actual automated analysers counts. Standard statistical analysis was carried out using regression analysis & Bland Altman Analysis.

Results-

Using standard statistical analysis by Statistical Package for Social Sciences (SPSS) version 21, mean platelet count of three groups were compared with automated analyzer and manual PBS platelet counts with multiplication factors (Table 1). The Co-efficient of Determination was also evaluated for each multiplication factor and it was highest with Pc obtained from multiplication factor 20,000. (Table 2). When PC values for multiplication factor 20000 were compared with automated analyser counts in all three groups, correlation was statistically significant for each groups ($p < 0.001$). The r values for group 1, group 2 and group 3 were 0.959, 0.981 and 0.989 respectively. The correlation was better in lower platelet counts while variation was higher in high platelet counts as shown in the regression analysis (Figure 1) and Bland Altman agreement analysis (Figure 2) along with Bland and Altman plot (Table 3)

Table 1: Comparison of Mean Platelet Count (Pc) Values

Method of Platelet count estimation	Group 1 (High Platelets) Pc (x 10^3) n=100		Group 2 (Norm. Platelets) Pc (x 10^3) n=100		Group 3 (Low Platelets) Pc (x 10^3) n=100		Total Pc (x 10^3) n=300	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Automated analyzer Pc	608.600	118.97	280.23	80.072	69.380	36.181	319.40	237.9
Manual Pc Multiplication factor x10000	297.950	606.92	130.080	40.062	32.740	17.967	153.590	117.886
Manual Pc Multiplication factor x15000	446.259	914.45	195.120	60.093	49.110	26.951	230.163	176.626
Manual Pc Multiplication factor x20000	594.600	123.560	260.160	80.124	65.480	35.935	306.746	235.615

Table 2: Co-efficient of Determination Regression Analysis

Dependent Variable	Independent Variable	Adjusted R square
Automated analyser Platelet count	Platelet count with multiplication factor X 10000	0.991
	Platelet count with multiplication factor X 15000	0.987
	Platelet count with multiplication factor X 20000	0.995

Figure 1: Regression Analysis for group 1, group2 and group 3

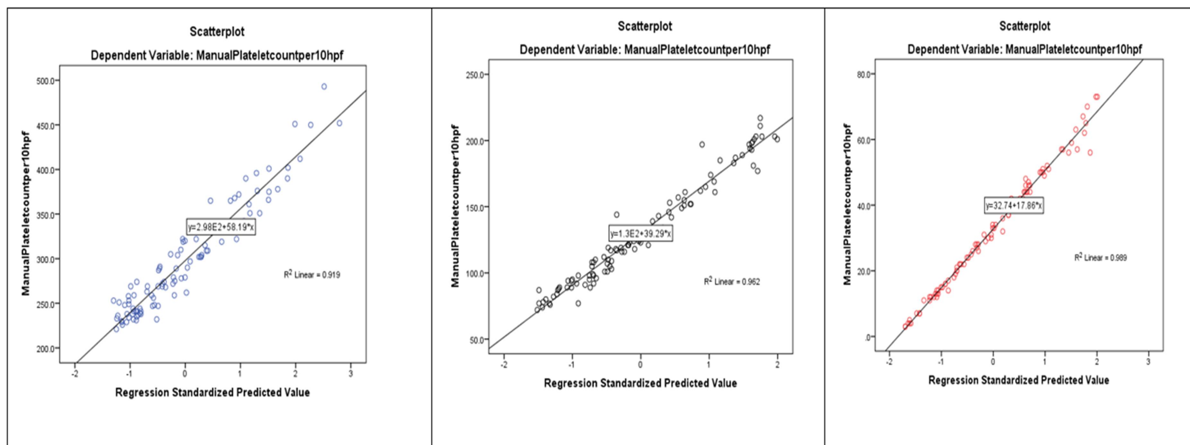

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Figure 2- Bland Altman agreement analysis for group 1, group 2 and group 3

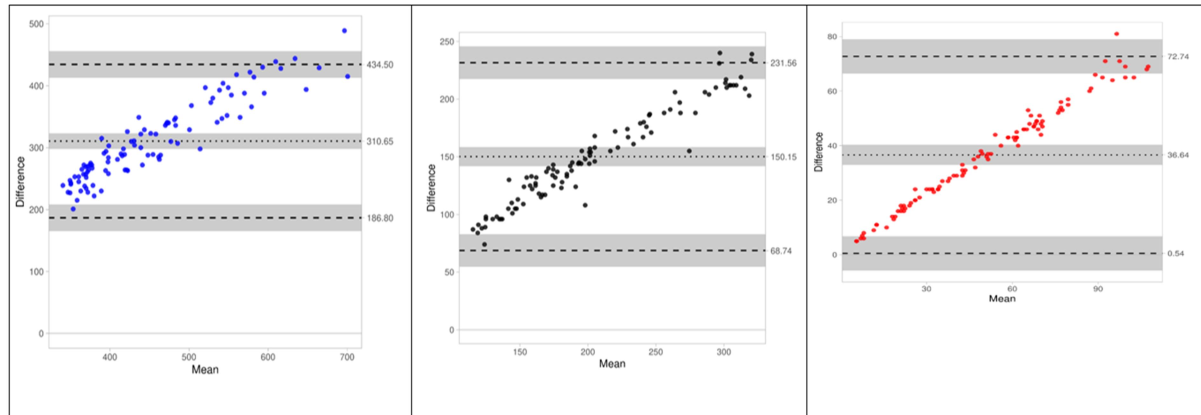

Table 3- Bland & Altman plot

Parameter	Group 1 (High platelet count)	Group 2 (normal platelet count)	Group 3 (Low platelet count)
Difference	310.65	150.15	36.64
Upper limit of agreement	434.50	231.56	72.74
Lower limit of agreement	186.80	68.74	0.54
Intercept	11.05	12.31	2.2
Slope	0.66	0.67	0.67

Discussion-

The international Council for Standardization in Haematology (ICSH) and the International Society for Laboratory Haematology (ISLH) have recommended Immuno-platelet counting method as reference method for accurate platelet count using FITC-conjugated monoclonal antibodies against epitopes of integrin Alfa IIb Beta3 (CD 41 & CD 61) analyzed with flow cytometry.[1] The other reference method as per ICSH and ILSH includes counting by RBC / Platelet ratio method.[2] Most of the automated analysers utilise the principles of impedance method, optical method and fluorescence methods. Usually, these methods give near accurate results.[1] Manual counting using improved neubaurs chamber method is still recognised as a gold standard or reference method. Though it is cheap and gives satisfactory results. However, the procedure is cumbersome, time consuming, has interobserver variability and high imprecision. Thus not suitable for laboratories with high sample load.[3] So to cross check and verify platelets count, manual peripheral blood smear examination is easy and less time consuming method. However, it requires mulitplicaion factor to get the approximate correct total platelet counts.

We found counts using multiplication factor 20,000 correlated well with actual automated analyser values among all groups i.e. high, normal and low platelet counts with statistical significance (p>0.001). Among these correlation was better for lower platelet counts while variation was higher for high platelet counts. Our results were comparable to studies conducted by Nosanchuk et al[6], Malok et al [8], Anitha et al[9], Momodu et al[10], Anchinmane et al [11] and Jain et al[12] where 20,000 was acceptable multiplication factor. Anitha et al[9] selected cases with low platelet counts only.

In contrast, Bajpai et al[13], Webb et al[7] and Tiwari et al[14] found 15000 as the most acceptable multiplication factor for correct comparable counts. Bajpai et al[13] included only pregnant females for the comparison of two methods. Moreno et al[15] suggested that suitable multiplication factor was between 15000 to 20000 without specifying any value. The comparison of findings of various selected studies are shown in table 4.

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We included cases with high, normal as well as low platelet counts similar to studies conducted by Jain et al[12] as well as Tiwari et al[14].

Sahu et al[5] estimated a multiplication factor of 9400 by calculating ratio of platelet counts by automated cell counter and total number of platelets per OIF for 100 cases and taking their mean value as multiplication factor. This factor then used to estimate platelet counts for next 100 samples which showed excellent agreement with automated analyser values. However, they mentioned that this method was not suitable for low platelet counts. In contrast to this study, our study showed good correlation with high, normal as well as low platelet groups. Also correlation was highest with low platelet counts. Low platelet count confirmation and validation is crucial for better management of patients with thrombocytopenia.

Dhakar et al[16] satisfactorily compared unstained smears with stained smears as well as automated cell counter values in 500 cases. They multiplication factor of 20000 for counting total platelet count using both stained as well as unstained smears.

Sudalaimuthu M et al[17] suggested that filed diameter and area of field viewed can affect the values.

Table 4- The comparison of findings of various selected studies

Sr No	Author (year)	Sample size (n)	Multiplication factor	Method	Mean platelet count (x 10^3)	Standard deviation (x 10^3)	Remarks
1	Malok et al [8] (2007)	184	20,000	Analyser	268	166	r= 0.90 Strong linear correlation
				Manual	269	167	
2	Anitha et al [9](2013)	50 (pregnant females)	20,000	Analyser	264	73	p=0.4 No statistical difference in two methods
				Manual	276	71	
3	Bajpai et al [13] (2015)	92 (Low platelet cases)	15,000	Analyser	91	27	No statistical difference in two methods
				Manual	94	29	
4	Webb et al [7] (2004)	35	20000 15000		-	-	Correlation with multiplication factor 15000
5	Momodu et al [10] (2016)	50	20,000 15,000	Analyser	267.86	77.28	-
				Manual (20000)	293.54	81.03	No statistical difference in two methods- Good correlation
				Manual (15000)	220.42	77.28	Statistical difference in two methods
6	Sudalaimuthu M et al [17] (2017)	200	15,000 20,000	Analyser	-	-	
				Manual (15,000)	390.03	-	r=0.944 Difference statistically significant
				Manual (20,000)	292.522	-	r=0.944 Difference statistically significant
7	Anchinmane et al [11] (2013)	100	20,000	Analyser	191.3	94.06	r=0.9789 Strong correlation
				Manual	202.5	92.08	
8	Jain et al [12] (2015)	532	20,000	Analyser	83	34	p<0.0001 No statistical difference in
				Manual	108	32	

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				(Low platelet)			two methods
				Analyser	187	73	
				Manual (normal platelet)	215	59	
				Analyser	484	128	
				Manual (high platelets	444	109	
9	Sahu et al [5] (2022)	100	9400	-	-	-	Significant agreement when platelet count >20,000
10	Current study	300	10,000 15,000 20,000	Analyser	319.40	237.9	
				Manual (20000)	306.746	235.615	R^2=0.995 Highest Coefficient of determination
				Manual (15000)	230.163	176.626	R^2=0.987
				Manual (10000)	153.590	117.886	R^2=0.991

As different researchers have found multiplications factors like 10,000, 15,000 and 20,000 as most suitable multiplication factors using different types or brands of haematology auto analyzers with different principles. Hence there might be a possibility that for different types of analysers, multiplication factors required can be different. Similarly field diameter of different microscopes might also affect the count and factor. Thus we suggest that every laboratory should standardize their own multiplication / correction factor for correct platelet count in cases of discrepancies.

To summarise the findings, use of manual platelet count using peripheral blood smear with a multiplication factor is easy, simple, less time consuming to cross check and validate count discrepancies in automated analysers. Also apart from these, in cases of resource poor settings, large sample loads or dengue outbreaks this method can be very useful to give approximate near correct platelet counts.

Conclusion-

We found that manual platelet count based on peripheral blood smear evaluation, a multiplication factor of 20,000 is most suitable to get comparable accurate results. This method is easy, simple, less time consuming and can be used in platelet count discrepancies, mandatory reviews and in resource poor settings.

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