Research Article

A comparative study of platelet counts by manual and automated methods in platelet poor plasma

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Abstract

Introduction: Reliable platelet count is crucial for minimizing errors in coagulation studies. Platelet poor plasma is routinely used for coagulation studies as platelets interfere with coagulation. In this study randomly collected 30 samples of platelet poor plasma for routine coagulation studies received in haematology laboratory of Father Muller Medical College Hospital, Mangalore for a period of 3 months were analysed and counts were done by both manual method and automated methods. Platelets were analyzed by using Neubauer chamber and were compared with automated method. The study analysis showed that there was no significant difference between manual and automated platelet counts in platelet poor plasma. The intraclass correlation between automated and manual method showed significance with p value 0.00, and also showed an excellent agreement (ICC: 0.868) with a positive correlation between the two methods. Hence this study shows that both methods can be used to asses platelets in platelet poor plasma as a part of quality control procedure. Manual method is thus recommended even at places where automation is unavailable to improve quality of coagulation studies.

Keywords: Coagulation studies, platelet poor plasma, quality control, automated and manual methods

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INTRODUCTION

Prothrombin time and activated partial thromboplastin time are the two most commonly requested routine coagulation tests for the screening of acquired and inherited coagulation disorders and monitoring of anticoagulation therapy. Reliable results can be achieved by carefully controlling preanalytical variables, which are responsible for 64% of all errors in testing [1]. The shortest recommended centrifugation time to produce the recommended platelet poor plasma with platelet count less than 10,000 per micro litre in 10 minutes at 2,000g using regular centrifuges. High speed centrifuge have been used for rapid preparation of PPP in 2-3 minutes[2, 3] Automation has afforded high precision and accuracy for platelet counting in normal individuals. Automated method is less useful in thrombocytopenic or other patients in which other small particles could generate electrical or optical signals that are similar to platelets, eg: debris and red cell fragments [4,5,6,7]. Presence of large platelets beyond the upper threshold may leads to under estimation of platelet count. Manual platelet counting in the Neubauer chamber by means of a phase- contrast microscope, has been recommended as the reference method for assessing the platelet number by the international committee for standardization in haematology (ICSH)for low count [8-11]. PT and APTT are not a single measurement of a single quantitative component but a result of a coagulation cascade system. There are several factors that can influence the process from clinical finding to the diagnosis, monitoring and treatment of patients, this process is called brain to brain loop because the loop start and ends in clinician brain. The pre –analytical phase includes patient identification, sample collection, sample stability, centrifugation, handling of sample preparation of reagent followed by the analytical phase (eg: instrumentation, analytical principle,

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lot number of reagent, calibration, maintenance),and the post analytical phase (data entry, reporting and communication of result). The preanalytical and post analytical phase takes place inside the laboratories and it is the laboratories responsibility to assure that the quality in the different phases is good.[12] The preanalytical phase of testing offers the greatest opportunity for introducing result error in the haemostasis lab and it is imperative that sample are properly collected, transported and stored. Laboratory data must be accurate and reliable as erroneous result may lead to patient misdiagnosis and therefore therapeutic misadventures.[13] Hence the study aims at finding the variation in the platelet counts by automated method and manual methods to assess the accuracy and reproducibility.

MATERIAL AND METHODS

A prospective study was carried out for a period of three months. Following ethical clearance, 30 samples of platelet poor plasma received for coagulation studies in haematology laboratory of Father Muller Medical College Hospital was included in the study.

- Samples received for PT/APTT irrespective of gender or specific age were included in the study. Clotted samples were rejected.
- Platelet poor plasma separated from whole blood collected in sodium citrate vacutainer by centrifugation.
- Platelets were analyzed by electrical impedance using coulter LH 750 analyzer
- Manual platelet count was done using neubauer counting chamber[25]

Procedure

1. Dilution: 1:20 dilution was made by adding 50µl sample(platelet poor plasma) in to 950µl of lysing fluid (1% aqueous ammonium oxalate)and kept for 10 to 15 minutes at room temperature with intermittent mixing.
2. The counting chamber were cleaned thoroughly and placed in flat position. The special coverglass was pressed down.
3. The sample diluting mixture was taken in to a micropipette and both side of the chamber were filled with diluted sample to flow under the upper edge of the cover glass in one smooth action. Care was taken to prevent overflowing, under filling, air bubbles and debris.
4. Charged chamber was left in moist petridish (petridish with moisted cotton) for 20 minute for the platelet to settle down in the chamber.
5. Counting: Chamber was placed on microscope stage, and using 40X objective platelet in 5 small squares of the central square of the chamber were counted. Platelet appear as small refractile particle.

Platelets count (per µl) = [Number of cells counted × dilution] ÷Volume of the chamber

$= [N \times 20] \div 0.02$

$= N \times 1000$

- Collected data was analyzed by mean, standard deviation and Karl pearson's correlation coefficient.

RESULTS

A total of 30 samples of platelet poor plasma were analyzed. The platelet counts were obtained by manual and automated method was compared. The analyzed data showed average age group of the patients were 41 to 60 years (Table 1andFigure1) and majority were females (Table 2). The platelet counts by the two methods showed no statistical significance. (Table 3)

Table 1: Age wise distribution of patients

	Frequency	Percent
20 - 40	6	20.0
41 - 60	16	53.3
Above 60	8	26.7
Total	**30**	**100.0**

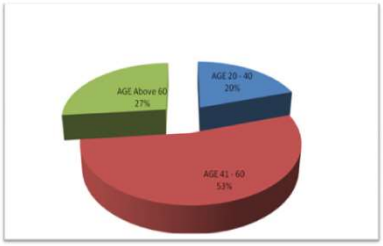

Table 2: Sex wise distribution of samples

	Frequency	Percent
F	18	60.0
M	12	40.0
Total	**30**	**100.0**

Table 3: Comparison of platelet count between manual and automated methods (p value is not significant)

	Mean	Std. Deviation	Paired t test value	P value
Automated platelet count	5333.33	2218.004	1.186	0.245
Manual platelet count	5000.00	2304.419		NS

When all the samples were analyzed by intraclass correlation, significant positive correlation with excellent agreement exists between the results of both manual and automated methods. (Table 4 and Figure 2)

Table 4: Intraclass correlation between platelet counts (excellent agreement)

Intraclass Correlation	95% Confidence Interval			
	Lower Bound	Upper Bound	p value	
0.868	0.725	0.937	0.000	Sign

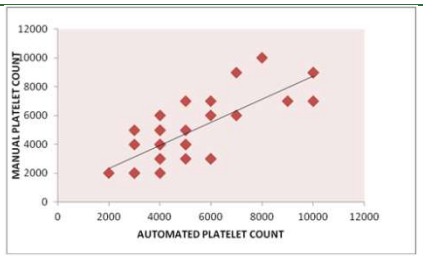

Figure 2: Intraclass correlation between manual and automated platelet counts

These findings attributed that the two methods showed excellent agreement and significant intraclass correlation when platelet counts of the two methods are compared.

DISCUSSION

The haemostasis pathway is very complex, and includes various elements of activation and regulation. The investigation of aberrant haemostasis or the monitoring of anticoagulant therapy requires the performance of multitude test procedures. Specific procedure for test collection, sample preparation, test performance and quality assurance needs to be established in every laboratory to ensure provision of accurate and reliable results to enable appropriate patient management and care. Inaccuracy in laboratory reporting can arise due to problems introduced at any point in the testing process, from sample collection to release of the reports. The pre analytical phase of testing represent the major source of inaccurate laboratory test results[14,15]. In the present study, it was observed that the mean platelet count estimated by the manual method and the automated method for all the samples studied (n=30)did not show significant statistical difference(p=0.245variation in the results. Intraclass correlation between automated and manual methods showed excellent agreement (ICC-0.868).and a significant a P value (P=0.00). When the correlation test was applied for all the 30 samples of platelet poor plasma, the platelet count was less than ten thousand by both methods, indicating there is a positive correlation between the two.. Out of 30 samples, 60% were females and 53% of patients were in an age group of 41 to 60 years. A difference in standard deviation of platelet count in platelet poor plasma by automated and manual method are 2218.004 and 2304.419 respectively. Our data showed no consistent superiority of platelet counts by two methods, emphasizing the use of both methods in platelet poor plasma obtained for coagulation studies. A cross sectional study conducted in National Centre for Public Health Laboratories of Aden yemen *et al* found that the mean platelet count estimated by manual method was not significantly different from that estimated by the electronic method[10] which is similar to our study. In another study by A G O Raimundo *et al*. A platelet count below 30,000/µl obtained in automated counters, should be confirmed by reference manual method. Manual platelet counting in neubauer chamber by means of phase contrast microscope has been recommended as the reference method.[16] Published literature, about the evaluation of routine coagulation testing relate to their application in specific disorders or to INR monitoring of anticoagulant therapy or to D-dimer testing. In our study platelet poor plasma was used for platelet count in contrast to other studies which used whole blood.[8,16,17] Up to date, the Gold standard for platelet counting available to assess any degree of accuracy of the automated count has been the manual phase contrast microscopic method.[10] In canters were advanced methods are unavailable, quality control of platelet poor plasma should be performed to maintain accurate coagulation study reports. Based on the analysed data, our study recommends the use of manual method for platelet count in platelet poor plasma to ensure test result, accuracy and precision.

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