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Pathology

COMPARISON OF THREE MANUAL PLATELET COUNT ESTIMATION METHODOLOGIES IN PERIPHERAL BLOOD SMEAR AND THEIR CORRELATION WITH AUTOMATED PLATELET COUNT VALUES

Dr. B. Saranya*	Assistant professor, Department of pathology,Vinayaka Mission's Kiriupanandha Variyar Medical College and Hospitals,Vinayaka Missions Research Foundation – (Deemed to be University) , Salem , Tamilnadu - 636308. *Corresponding Author
Dr. Thamilselvi Ramachandran	Professor & Head, Department of pathology, Vinayaka Mission's Kiriupanandha Variyar Medical College and Hospitals, Vinayaka Missions Research Foundation – (Deemed to be University) , Salem , Tamilnadu - 636308.
Dr. M. Roopmala	Associate professor, Department of pathology, Vinayaka Mission's Kiriupanandha Variyar Medical College and Hospitals, Vinayaka Missions Research Foundation – (Deemed to be University) , Salem , Tamilnadu - 636308.
Dr. T. Sreneela	CRRI, Vinayaka Mission's Kiriupanandha Variyar Medical College and Hospitals, Vinayaka Missions Research Foundation – (Deemed to be University) , Salem , Tamilnadu - 636308.

ABSTRACT **INTRODUCTION:** Platelets play a key role in hemostasis and thrombosis. The methods commonly used for counting are manually by using a counting chamber, manually by examination of peripheral smear and using a automated hematology analyser. Though automated analyser has replaced the manual methods, Abnormal values generated by automated analyser has to be verified by manual methods. Estimation of platelet counts from peripheral blood smear based on average number of platelets in an oil immersion fluid is approximate and doesn't give the real number of platelets. Hence other methods for estimation of platelet counts by manual method were advocated for accurate estimation. The alternate estimation method used by Malok.M et al is the average number of platelets per oil immersion field multiplied by patients haemoglobin value in g/dl and then multiplied by 1000 to yield a platelet count estimation in microlitre. Umarani.MK et al estimated the platelet count from peripheral blood smear based on platelet:RBC count. So,the aim of this study is to compare the traditional counting methods done by Malok.M et al and umarani.Mk et al and to correlate the values obtained by these methods with the platelet value of automated platelet counter.

MATERIALS AND METHODS: EDTA sample was obtained from 100 patients visiting OPD of Vinayaka missions kirupanandha variyar medical college and Hospitals, Salem and smear is prepared and the platelet values were estimated by using the three manual methods of our study. Method 1: Platelet count in microlitre =average number of platelet in OIF X 15000 Method 2: Platelet count in microlitre =average number of platelets in OIF X Hb in g/dl x 1000 Method 3: Platelet count in 103 /microlitre =number of platelets per 1000 RBC X RBC count in 106/microlitre . The values obtained from the three methods were correlated with the automated analyser value and the correlation coefficient for the three methods were calculated.

RESULTS:

- Platelet count ranged from Method A: 115000 to 430000, method B: 930000 to 402000, method C: 120000 to 450000 , Automated analyser : 110000 to 432000
- Mean values of the methods Method A: 240782 method B : 218310 method C 225000 , Automated analyser: 240161
- Correlation coefficient Method A: 0.967 Method B: 0.879 Method c: 0.973

CONCLUSION: Method C has a high correlation with the automated analyser value followed by method A. Method B had only a medium level of correlation with the automated analyser value. Method C and A can be used to verify and crosscheck the values of the platelet count given by the automated analyser

KEYWORDS : Platelets, Manual Method, Automated Analyser

INTRODUCTION:

Platelets play a key role in hemostasis and thrombosis. Platelet count is one of the critical parameters in the patients care. Normal range of platelet counts in healthy individual is 150-400*10 ^{3} microlitre. The methods commonly used are

1) Manual method using counting chamber
2) Examination of peripheral blood smear
3) Using automated hematology analyser

Accurate and reproducible platelet count is essential for patient management. Manual method is time consuming, subjective, tedious with high levels of imprecision[1]. Automated hematology analyser has largely replaced the manual method of platelet counting as it is simple and fast but has limitation such as cost, stringent quality assurance and their unavailability in small peripheral centres. Automated hematology analyser produce erroneous results in presence of particles of similar sizes, fragmented platelets and platelet clumps[2,3]. Moreover it is the standard procedure that all the abnormal platelet values generated by the cell counters should be confirmed by manual examination of leishman stained peripheral smear. Estimation of platelet counts from peripheral blood smear based on average number of platelets in an oil immersion fluid is approximate and doesn't give the real number of platelets. Hence other methods for estimation of platelet counts by manual method were advocated for accurate estimation. The alternate estimation method used by Malok.M et al[4] was the average number of platelets per oil immersion field multiplied by patients haemoglobin value in g/dl and then multiplied by 1000 to yield a platelet count estimation in microlitre. Umarani. MK et al[5] estimated the platelet count from peripheral blood smear based on platelet: RBC count. So, the aim of this study is to compare the traditional counting methods done by Malok. M et al and umarani.Mk et al and to correlate the values obtained by these methods with the platelet value of automated platelet counter.

AIMS AND OBJECTIVE:

1. Estimation of platelet count from peripheral blood smear by using three manual methods.
2. Compare three manual method for estimating platelet count from peripheral blood smear regarding their correlation with each other and with automated platelet count.
3. To verify the reliability of these three methods by comparing the platelet count obtained by these methods with the automated platelet count.

MATERIALS AND METHODOLOGY:

DESIGN OF STUDY-Prospective study

SOURCE OF DATA

Samples received for complete blood count and platelet count estimation in central laboratory of Vinayaka Missions kirupanada

variyar medical college, Salem.

DURATION OF STUDY: 2 months
Sample size: 100 samples received in the period of two months with varying platelet counts.

INCLUSION CRITERIA:
EDTA anticoagulated samples sent to laboratory for complete blood count and platelet count estimation from subjects of any age and gender and with any diagnosis during the study period.

EXCLUSION CRITERIA:
Hemolysed and clotted samples.

METHOD:
EDTA sample of patient is analyzed by automated hematology analyzer and 3 manual methods.

AUTOMATED PLATELET COUNT:
The mixed sample received is rotated with rotator for 3-5 minutes and is fed into automated hematology analyzer Merilyzer celQuant 3. 100 micro litre of blood is sucked into the analyzer with the help of a needle provided by manufacturer. RBC, WBC and platelet count is obtained based on volumetric and rate measurement

PRINCIPLE OF AUTOMATED HAEMTOLOGY ANALYSER:
The method for counting cells in automated analyzer method is electrical impedence method. Here the whole blood is passed between two electrodes through an aperture so narrow that only one cell can pass at a time. The impedance changes as the cell passes through. The change in impedance is proportional to cell volume resulting in cell count and measure of volume. Counting rate of upto 10000 cell/sec can be achieved and it can be carried out in less than a minute.

MANUAL METHOD:
Peripheral smear is prepared from the same blood used for automated hematology analyzer. Smear is air dried and stained with leishmann.

PROCEDURE OF LEISMANN STAINING:
Place the air dried film on the staining rack.

Put 8 drops of leishmann stain so that it covers the smear.

Leave for 2 minutes.

Dilute with 16 drops of distilled water until a metallic scum appears.

Allow this to stand for 10 minutes.

Wash the smear with tap water and dry the smear.

Count the platelet under oil immersion filled in an area were the RBC morphology is well made out (RBCs are separated without overlapping)

MANUAL METHOD 1:
Average number of platelet in oil immersion field is calculated and the number is multiplied by 15000 to get approximate platelet count in microlitre.

Platelet count in microlitre =average number of platelet in OIF X 15000

MANUAL METHOD 2:
Average number of platelet per oil immersion field is calculated which is multiplied by patients hemoglobin value in g/dl and then multiplied by 1000 to yield a platelet count estimation per microlitre Platelet count in microlitre =average number of platelets in OIF X Hb in g/dl x 1000

MANUAL METHOD 3:
In a monolayered zone of the smear ,platelets are counted simultaneously with RBC till 1000 RBCs are counted.

Number of platelet per 1000 RBCs thus obtained is multiplied by automated RBC count in 106 /microlitre to get an estimation of platelet count in 103 /microlitre

Platelet count in 103 /microlitre =number of platelets per 1000 RBC X RBC count in 106/microlitre

The platelet count by manual method is assessed without the prior knowledge of automated analyzer value.

The platelet count obtained by three manual methods and the automated hematology analyzer is tabulated. The agreement between the three manual methodologies with each other and each method with the automated count is assessed using correlation coefficient analyses. The best manual method for assessing the platelet count is analyzed with automated analyzer value as gold standard.(automated analyzer is properly caliberated).

Table 1: Methods Of Platelet Estimation Used In Our Study

Method	Formula used to calculate the platelet count of the sample per microlitre
Method A	Number of platelets in 10 OIF/10 X 15000
Method B	Number of platelets in 10 OIF/10 X Hb in grams/dl X 1000
Method C	Number of platelets in 10 OIF/Number of RBC's in 10 OIF X RBC count/microlitre

OBSERVATION AND RESULTS:
Haemoglobin values ranges from 4gm/dl to 15gm/dl while the RBC count ranges from 1.5 to 5.5 million/microlitre. Platelet count from method A ranged from 115000 to 430000 and the mean platelet count is 240161. Platelet count from method B ranged from 93000 to 402000 and the mean count is 218310. Platelet count from method C ranged from 120000 to 450000 and the mean count is 225000. Platelet count from automated analyser ranged from 110000 to 432000 and the mean count is240161. Platelet count and the mean value , correlation coefficient of all the three methods are given in Table 2.

Table 2: Statistical Data Of The Results

	Method A	Method B	Method C	Automated Analyser
Platelet Count Range(per Micro Litre)	115000 TO 430000	93000 TO 402000	120000 to 450000	110000 TO 432000
Mean Of The Platelet Count	240782	218310	225000	240161
Correlation Coefficeient	0.967	0.879	0.973	

Method C had a high Pearson correlation coefficient of 0.973 with the results of automated analyser. Method A had a correlation coefficient of 0.967 with the results of automated analyser.Method B had a correlation coefficient of 0.879 with the results of automated analyser.

DISCUSSION:
Platelets are subcellular fragments derived from megakaryocytes in the bone marrow,circulating in blood as small discs having an precise and reproducible structure. A single megakaryocyte can give rise to about thousand platelets. The platelets are very small non nucleated about 3micrometre in diameter and consist of cytoplasm enclosed within a cell membrane. The life span of normal platelet is about 7-12 days and they are destroyed by the macrophages in the spleen. The platelet in peripheral blood are heterogenous with respect to size,density and staining characteristics. Their morphology also varies greatly depending on the methods by which they are examined and the anticoagulant employed. In wet preparations,they are colorless,moderately refractile bodies that are discoid or elliptical. In romonowsky stained smears they appear round,oval or rod shaped. azurophilic granules are seen in hyaline ,light blue cytoplasm.these granules may be so tightly in the central portion of the platelet that may give appearance of nucleus. Platelet are multifunctional and play a key role in many physiological processes(Eg.wound repair,immune response)apart from their well known roles in haemostasis and thrombosis.

The normal range of platelet count in a healthy individual is 150000-400000/microlitre.

Accurate measurement of the platelet counts is essential particularly in the management and monitoring of patients with thrombocytopenia. Automated hematology analyzers in spite of various advances have the limitation of inability to produce precise and reproducible platelet counts particularly in patients with thrombocytopenia.Presence of

background debris,micro organisms,fragmented RBCs can all hinder an accurate measurement of platelets in automated hematology analyzers by mimicking the platelets due to their small size.On the other hand,platelet clumps and giant platelets owing to their large size are not counted as platelet in automated analysers. All these lead to to inaccurate platelet counts in an automated hematology analysers.Hence platelet counts values obtained from automated analysers should be crosschecked by some other method particularly in cases of thrombocytopenia[6].

The four main procedures for platelet counting are: manual phase contrast microscopy, impedance, optical light scatter/fluorescence and flow cytometry. Early methods to enumerate platelets were inaccurate and irreproducible. The manual count is still recognized as the gold standard or reference method, and until very recently the calibration of platelet counts by the manufacturers of automated cell counters and quality control material was performed by this method. However, it is time-consuming and results in high levels of imprecision. The introduction of automated full blood counters using impedance technology resulted in a dramatic improvement in precision. However, impedance counts still have limitations as cell size analysis cannot discriminate platelets from other similar-sized particles. More recently, light scatter or fluorescence methods have been introduced for automated platelet counting, but there are still occasional cases where an accurate platelet count remains a challenge. Thus, there has been interest in the development of an improved reference procedure to enable optimization of automated platelet counting. This method utilizes monoclonal antibodies to platelet cell surface antigens conjugated to a suitable fluorophore. This permits the possible implementation of a new reference method to calibrate cell counters, assign values to calibrators, and to obtain a direct platelet count on a variety of pathological samples. In future, analysers may introduce additional platelet parameters; a reliable method to quantify immature or reticulated platelets would be useful [1]. Recently the assessment of platelet count has been done by flow cytometry, using antibodies specific to platelets like CD41, CD61

The methods used for counting platelet count with their advantages and disadvantages are discussed below

1. By using peripheral smear:
The number platelets are counted in the ideal zone of a smear stained with Romonowsky stain where blood cells did not overlap and there is fairly even distribution of white blood cells and platelets.[7]
The calculation is done by ;

The average number of platelet in an oil immersion field multiplied by 15 to 20 thousand[4,8].

Advantages
Characteristic morphology is seen

Disadvantages
Unfiltered stain containing artifacts may interfere

Reusing of slides –scratches present in the slides may containing unwashed cells

2. By using counting chamber:
Platelet count assessment is done with EDTA anticoagulation of venous blood. The Improved neubauer counting chamber is most commonly used. The Fuchs Rosenthal counting chamber may also be used

Advantages
Reagents are cheap
Easy to perform

Disadvantages
Technical and inherent errors
Technical-
Poor technique in obtaining blood specimen
Insufficient mixing of blood specimen
Inaccurate pipetting or badly calibrated pipettes or counting chamber
Faulty filling of counting chambers
Unfiltered diluting fluid

3. Automated methods:
Platelets are analyzed in automated counters by (Electrical impedance) DC detection methods. The principle is that the blood sample is aspirated and measured to predetermined volume, diluted at a specific ratio and fed into each transducer. The transducer chamber has 2 minute holes called aperture. Blood cells suspended in the diluted sample are passed through an aperture causing a change in the direct current resist between electrodes. The size of the blood cell is detected as electric pulses. The number of blood cells is calculated by counting the pulses.

Advantages
Highly reliable
Greater precision

Disadvantages
Giant platelet counted as RBC
Small RBC counted as platelet

In their study Webb et al[8] reviewed 35 samples with normal , low , high platelet counts. They compared the smear assessment with the automated counter results.

There was fair concordance in 27 specimens. In three specimens underestimation was found, overestimation in five. A 15,000 multiplier gave slightly better results than 20,000. Average in 10 high-power fields was as good as 25. Abnormal counts could be assessed as well as normal[8].

The clinical decision to proceed with prophylactic platelet transfusions is widely based on trigger points for platelet counts being equal to 20, 10, or even 5 x 10(9)/L. But an increasing number of publications show evidence that the conventional automated platelet counting methods are unable to provide consistently accurate results in this lower thrombocytopenic range. These measurement errors are mainly associated with the most commonly used impedance principle; optical methods seem to be more precise. The problems of counting imprecision in the low thrombocytopenic range can be avoided with direct or indirect immunological counting methods using monoclonal antibodies or by time-consuming manual procedures. But how should new counting procedures be evaluated? Which method should be used as the "gold standard" for platelet counting? As a way out of this apparent dilemma it is suggested that, the application of a statistical procedure as proposed by Gautschi et al which uses a mathematical model. Using this evaluation procedure, it can be shown that immunological automated counting methods can provide reliable, sufficient, and prompt platelet counts, especially in the thrombocytopenic range [9].

Various studies have been conducted, comparing the counts assessed by analyzers using different principles. Platelet counts estimated by analyzers using optical, impedance and immunological methods were compared with the International Reference Method (IRM)[10] for platelet counting. The results demonstrated variation in platelet counting between different analyzers and even the same type of analyzer at different sites. Finally they re-emphasize the need for external quality control to improve analyzer calibration for samples with low platelet counts, and suggests that the optimal thresholds for prophylactic platelet transfusions should be re-evaluated [11].

In our study, Method C had a correlation coefficient of 0.973 which is high agreement with the values of values of automated analyser which is similar to the studies of muthu sudalaimuthu et al.[6], Brahmi et al.[2], Abid et al.[12], umarani et al.[5],.This method resembled the International reference method recommended by ICSH and ILH but done manually by peripheral smear. This method was taken to check the acuuracy of this method.

Method A had a correlation coefficient of 0.967 which is also high agreement with the automated analyser value but is less than that of Method C. This is similar to studies done by Webb et al.[8], and Malok M et al.[4],. Malok M et al had a correlation coefficient of 0.9 similar to our study.A multiplication factor of 20000 is based on a study done by Nosanchuk et al.[13], but Webb et al[8]., in 2004 proved that a multiplication factor of 15000 provided better correlation than 20000, so, 15000 was used as multiplication factor in our study. But, muthu sudalaimuthu et al.[6], had a poor correlation.

Method B had a correlation coefficient of 0.879 which is medium correlation with the automated analyser value. This medium correlation is similar to studies done by Malok M et al.[4], and muthu

sudalaimuthu et al.[6],. But, Torres et al.[14], who described this method in 2004 said that this method is more specific than the traditional counting method.

Our study show that the estimation of platelets based on method C yields results comparable to that of the automated analyser. A strong correlation coefficient (0.973) and minimal dispersion in scatterplot shows that this method is an ideal method to crosscheck the results of the automated analyser. But, the major disadvantage is that it may be difficult for the beginners. The method A in contrast to the study done the muthu sudalaimani et al.[6], has a better correlation coefficient with the automated analyser but less than that of method A. Hence, in places of difficulty with method C, this can be used as an alternative method for platelet count estimation by manual method. Method B values differed significantly from that of the automated analyser value and wider range of dispersion in scatterplot in contrast to Torres et al.[14], and similar to Malok M et al.[4],

CONCLUSION:

Thus our study results show that Method A and C can be used as a method of choice for estimating platelet counts in a peripheral smear manually. This method can be used to verify and crosscheck the values obtained from the automated analyser. However these findings need to be validated in large scale studies and by correlating with the International reference method recommended by ICSH and ILSH.

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