ORIGINAL RESEARCH PAPER

Volume-7 | Issue-9 | September-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ACE (ACCURATE, CHEAP, TIME EFFICIENT) METHOD FOR PLATELET ESTIMATION USING STAINED AND UNSTAINED PERIPHERAL SMEARS

Pathology

Major Felice Faizal Medical Officer (pathology), Indian Armed Forces

Dr Pankti Haria* Medical Director, Regenerative Medical Services, Lonavala *Corresponding Author

Major Vandana Dahake Medical Officer (pathology), Indian Armed Forces

Dr Archana Khade Asst Professor, RNCH, Mumbai

Dr Manisha Khare Professor and Head, RNCH, Mumbai

KEYWORDS

INTRODUCTION

In a tropical country like India where acute febrile illness with thrombocytopenia is common, platelet count is one of the most commonly ordered laboratory investigations. With the advent of sophisticated automated cell counters, the gold standard manual method of platelet estimation using counting chambers and diluting fluid is fast becoming obsolete as it is time consuming and requires the use of phase contrast microscope which is not available everywhere[1].

However, common laboratory protocols advocate the use of peripheral blood smears to confirm the platelet count generated by the automated cell counters especially in cases of low counts as platelet count is an important clinical parameter affecting the patients management and formation of platelet clumps in the sample can give a falsely low automated count[2].

Apart from this drawback, cell counters are expensive machines and require technical maintenance, calibration and proper power supply which are not available in all parts of a developing nation but invariably have a high patient case load. Therefore the need to find a economical , efficient and effective method to determine the platelet count in areas where an automated count may not be available or to confirm the platelet count generated by an automated cell counter

In literature various methods have been used to estimate platelet counts using the peripheral smear. The most commonly used method recommends calculating the average number of platelets counted in 10 immersion fields and then multiplying by a factor of 20,000 for wedge preparations to obtain and estimate the platelet count per micro liter[3].

An alternate estimation method suggests multiplying the average number of platelets per oil immersion field by the patient's hemoglobin value in g/dL and then multiplying by 1,000 to yield platelet count estimation per microliter[4].

A study conducted by Brahimi et al compares platelet estimation by using platelet to red cell ratio by counting average number of platelets per 1000 RBCs and multiplying by RBC count and finds excellent agreement with automated counts[5].

All the above studies have conducted the platelet estimation on stained smears whereas a recent study in India has highlighted the usefulness of unstained smears for the same. Before this, use of unstained smears was limited to determining adequacy of cytology specimens[6]

The current study aims to compare all these scattered methods and determine the best possible method for platelet estimation in terms of cost, efficiency and accuracy.

MATERIALS AND METHODS

Prospective study over a period of one month – January 2016.

1) Any 5 consecutive samples chosen randomly from the samples received daily in the haematology laboratory in RNCH for complete blood count.
2) Samples processed by laboratory technician in ERMA cell counter to generate automated platelet count , haemoglobin(Hb) and RBC count and result withheld from investigator
3) Wedge smears prepared on clean glass slides from each sample and allowed to dry
4) Unstained smears observed under scanner to identify area with uniformly spread RBCs with minimum overlapping and marked with pencil
5) Marked area observed under 100 X magnification WITHOUT oil with condenser lowered and diaphragm allowing minimum light and average number of platelets per high power field noted after observing ten fields
6) Number of platelets per 1000 RBCs also noted . 1000 RBCs are counted by counting number of RBCs in a quarter of the field and multiplying by 4. All the platelets in the field were counted. Repeated till 1000 RBCs counted
7) The same smear was stained using fields stain
8) Marked area observed under 100 X magnification WITH oil and average number of platelets per high power field noted after observing ten fields
9) Number of platelets per 1000 RBCs also noted . 1000 RBCs are counted by counting number of RBCs in a quarter of the field and multiplying by 4. All the platelets in the field were counted. Repeated till 1000 RBCs counted
10) mathematical methods labelled as Method 1 , 2 and 3 applied on the readings derived from both unstained (U) and stained (S) smears

a. **METHOD 1:** Platelet count=average number of platelets per high power field x 20000
b. **METHOD 2 :** Platelet count = average number of platelets per high power field x Hb x 1000
c. **METHOD 3 :** Platelet count = average number of platelets per 1000 RBCs x RBC count

11) Note time taken for each step from steps 5 to 10
12) Results analysed using medcalc version 16.1 software to test agreeability between each method on stained and unstained smears to the automated platelet count using Bland and Altman plots.

RESULTS

A total of 100 random samples were analysed as described. The readings were noted as U 1, U2, U3 for unstained smears using methods 1, 2 and 3 respectively and S1, S2, S3 for readings obtained using stained smears.

In unstained smears, platelets appear as small refractile bodies as compared to small round purple bodies on stained smears (figure 1)

Turnaround time (TAT) for each method was as follows (Table 1)

TABLE 1: Turnaround time of each method

METHOD	TURN AROUND TIME (TAT)
U1	3 to 3.5 MINUTES
U2	3 to 4 MINUTES
U3	7 to 8 MINUTES
S1	15 to 17 MINUTES
S2	16 to 18 MINUTES
S3	20 to 23 MINUTES

The automated platelet counts (APC) were analyzed for distribution using medcalc software and data was found to be normally distributed with an average count of 238000/uL.Readings from all the methods were compared to the automated platelet count by drawing Bland and Altman plots and the results can be interpreted as follows (table 2)

TABLE 2: comparison of various methods according to Bland and Altman plots (refer figure 2-7)

METHOD	AVERAGE PLATELET COUNT	MEAN DIFFERENCE FROM APC IN % (bias)	% OF DIFFERENCES LESS THAN 2 SD
U1	223000	+7.9%	95%
U2	136000	+59.4%	96%
U3	236000	+2.4%	96%
S1	246000	-2.9%	93%
S2	151000	+48.3%	95%
S3	240000	-0.8%	97%

Stained methods cost more than the unstained methods owing to the cost of the staining reagents and apparatus required.

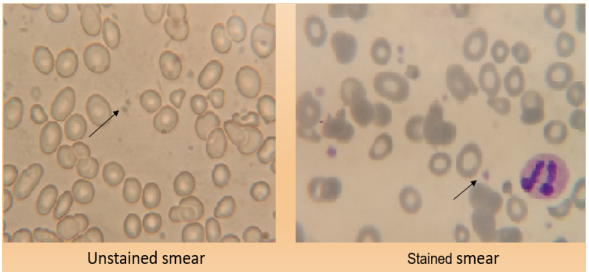

FIGURE-1 HERE

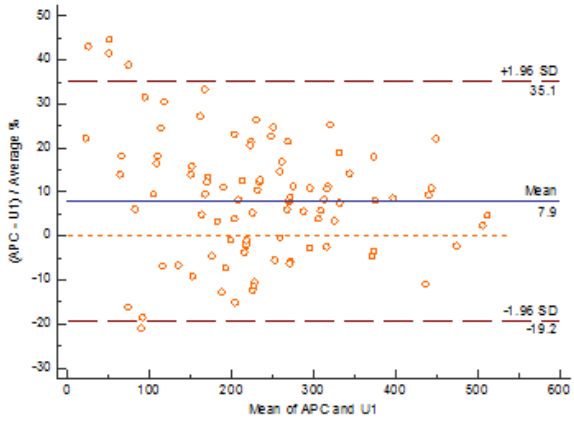

FIGURE 2: APC vs U1. Mean difference is 7.9%. 95% of values are within +/- 2SD.

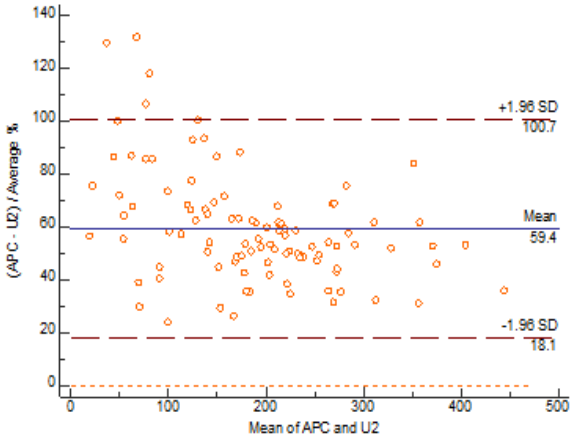

FIGURE 3: APC vs U2. Mean difference is 59.4%. 96% of values are within +/- 2SD.

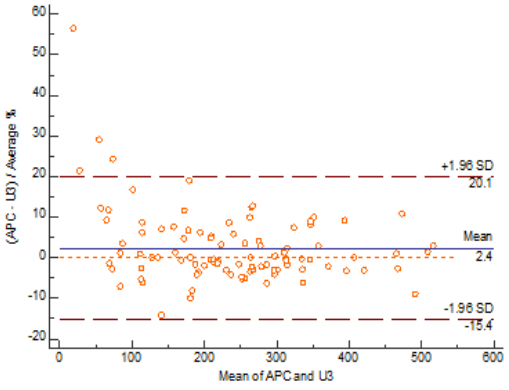

FIGURE 4: APC vs U3. Mean difference is 2.4%. 96% of values are within +/- 2SD.

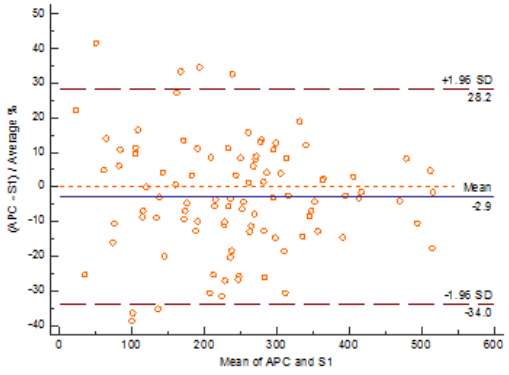

FIGURE 5: APC vs S1. Mean difference is 2.9%. 93% of values are within +/- 2SD.

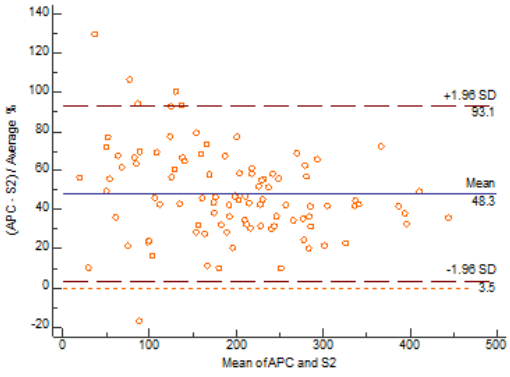

FIGURE 6: APC vs S2. Mean difference is 48.3%. 95% of values are within +/- 2SD.

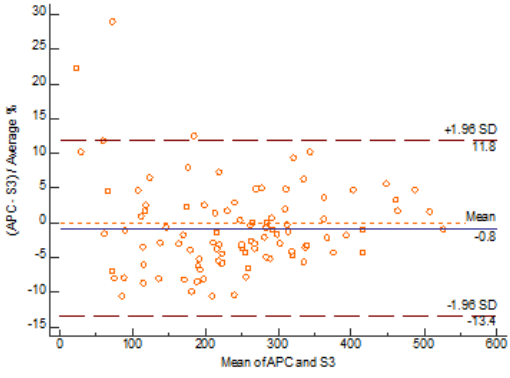

FIGURE 7: APC vs S3. Mean difference is 0.8%. 97% of values are within +/- 2SD.

DISCUSSION

Even though automated cell counters have become widely available, the need for platelet estimation on peripheral smear still exists because many factors like red cell fragments, microcytic red cells, apoptotic bodies may overestimate the platelet count owing to similar size and presence of giant platelets or platelet clumps may underestimate the same. Many different methods have been described in literature using stained peripheral smears and recently unstained smears have also been used with satisfactory results. Here we assess these methods using proper statistical tests to determine the best method in terms of accuracy, cost and time efficiency.

Quite obviously, methods using the unstained smears are cheaper and faster as compared to those using stained smears. Amongst those method U1 and U2 are fastest as compared to U3 as the latter involved counting of 1000 RBCs which is time consuming.

Coming to the accuracy, the mean platelet count of 100 samples by automated method was 238000/u L and barring methods U2 and S2, remaining methods show similar mean values.

Many studies have used paired t test and intraclass correlation coefficient to prove statistical agreement between two different methods to measure the same parameter, but Bland and Altman state that these methods are unreliable and in 1983 they re-proposed an alternative analysis, firstly presented by Eksborg in 1981[7], based on the quantification of the agreement between two quantitative measurements by studying the mean difference and constructing limits of agreement.[8]

In this graphical method the differences between the two techniques are plotted against the averages of the two techniques. Differences can also be expressed as percentage of the averages when there is an increase in variability of the differences as the magnitude of the measurement increases as is the case here.

The plot should include a line depicting mean difference (thick blue line in figures), a line of equality (broken orange line) and two lines depicting the +/- 2SD (broken red lines). The interval between the line of mean difference and line of equality is the bias

This method requires to define acceptable difference between the two methods prior to analyzing and then determining if the differences between the two methods lie in the interval.

We defined accepted limits of difference as +/- 20% of the APC as difference of this magnitude would not alter the clinical decision significantly.

Comparing the Bland and Altman plots for the various methods, methods U1, U3, S1 and S3 satisfy the predefined limit with the highest difference of 7.9% in U1 and minimum difference of 0.8% in S3. Methods U2 and S2 show differences of 59.4% AND 48.3 % respectively which is way outside the limit of 20%.This shows that the most reliable method in terms of accuracy is S3 with only 0.8% variation in the readings as compared to APC. However, methods U1, U3, and S1 also show considerably accurate results with the differences not altering the clinical outcome significantly.

Repeatability of the test can also be determined by this plot if more than 95 % of the values lie within the +/- 2SD of mean differences. Here all methods show more than 95% of the values within the +/- 2SD except S1 in which 93% of the values lie so. Therefore almost all methods show good repeatability

Unstained smears are not conventionally used for estimating platelet count on peripheral smear as identifying platelets require slightly more skill than in stained smears. However if the condenser is lowered and the diaphragm is open only to allow minimum light ,on 100 X objective without oil, the platelets appear as small refractile round bodies which can be readily identified. Results of platelet estimation using unstained smear are comparable to those of stained smears.

CONCLUSION

Of the various methods available for platelet count estimation on peripheral smear, the method using percentage of platelets to the RBCs appears to be the best in terms of accuracy with negligible difference from automated counts. However the method involving multiplying average platelet count per field by 20000 also yields satisfactory results. The methods using hemoglobin as a multiplication factor do not provide reliable results. Unstained smears show great utility and cost effectiveness for the purpose as the time taken for a test is minimal and cost is considerably cut due to non requirement of expensive stains and staining apparatus. Unstained smears should be routinely used in places with high patient load and limited resources using the RBC percentage method as this would provide the best combination in terms of accuracy, cost effectiveness and time efficiency.

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