INTRODUCTION

Platelets are non-nucleated discoid 1-3µ cells, produced in bone marrow megakaryocytes by fragmentation of cytoplasm. Platelets serve both structural and molecular functions in blood clotting. Platelet count is frequently advised recently, especially in dengue fever season. Almost all pathology labs are overloaded with requests for platelet counts during outbreak of dengue fever every year, because of risk of bleeding if count goes very low (<10,000/mm³). Apart from this, regular platelet count is needed in patients on chemotherapy and in pregnancy induced hypertension, malaria, bacterial sepsis, leukemia.

Platelet being common investigation in laboratory, we require economical and accurate method. Manual method by Neubauer chamber needs 1% ammonium oxalate as diluting fluid while automated method requires costly equipment (4-5 lakhs) as well as maintenance whereas manual slide method is simple, cheap, feasible, reliable if done properly. Results are comparable to automated method except if count is very low. Normal platelet count in healthy person is 1.5-4.0 lakh/mm³ of blood. Imoru in his study found multiplying by 20,000 to the average of 10 oil field platelet count yielded better results comparable to hematology analysers than multiplying by 15000 as advocated by some other authors.

Occasionally platelet satellitism may give wrong results.
by automated cell counter in ethylenediaminetetraacetic acid (EDTA) samples. Results of automated counters can’t be totally relied in severe thrombocytopenia also.

Aims and objectives

To evaluate accuracy of manual slide method in comparison to automated method by processing same sample at the same time by both methods in same laboratory.

METHODS

We took 532 patients’ (460 adults and 72 children) platelet count into consideration between May to August 2019 including indoor and outdoor patients, attending Hind institute of medical sciences (HIMS), Safedab, Barabanki. 2 ml blood samples were collected in tubes containing K3EDTA anticoagulant in central laboratory of HIMS. After proper mixing on blood shaker for 10 minutes, a CBC (complete blood count) including platelet count was done by Mindray cell counter (BC-5150). Simultaneously peripheral blood smears were made from freshly collected EDTA blood after proper mixing on shaker and stained with Leishman stain. Automated method on Mindray cell counter is based on principle of electronic impedance for cell counting. Automated hematology analyzer was regularly maintained and calibrated as per company guidelines. In slide method, we counted platelets under oil immersion lens (100X) in 10 fields, where RBCs are just touching each other in monolayer sheet, and then took average of ten fields multiplied it by 20,000. Those slides showing platelet aggregates or giant platelets were excluded from study.

Estimated platelet count/cu mm is equal to average count in 10 fields multiplied by 20,000 (thousand/mm³). The results were grouped as follows, a total of 532 samples were processed and platelet counts done by both methods. Out of these 238 were males and 294 females, 460 adults, 72 children. The processing of the data was performed using R statistical software.

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Platelet count</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>93</td>
<td>&lt;1.5 lakh/mm³</td>
<td>Low, group 1</td>
</tr>
<tr>
<td>426</td>
<td>1.5 - 4.0 lakh/mm³</td>
<td>Normal, group 2</td>
</tr>
<tr>
<td>13</td>
<td>&gt;4.0 lakh/mm³</td>
<td>High, group 3</td>
</tr>
</tbody>
</table>

Table 1: Categorisation of patients into different group based on their platelet count.

Simple linear regression analysis and coefficient of determination (R²) for correlation analysis between the two methods were used. All tests were applied at a 99% level of significance. Mean platelet count by manual method was 2.02 lakh/mm³, while with automated method was 1.78 lakh/mm³.

RESULTS

Platelet counts by manual slide method are comparable to results by automated method done on Mindray (BC5150) 5part blood cell counter. The platelet count by manual method was slightly higher than automated method, but is quite accurate (p<0.01).

Figure 1: Platelets seen in clump (Leishman stain 10×100).

A linear regression analysis was run for group 1 (using R statistical software) keeping manual platelet count as the dependent variable and automated platelet count as the independent variable. The results obtained were:

Coefficients:

*Estimate std. error: t value Pr(>|t|) with (intercept) 0.43075 0.04871 8.843 6.74e-14, ceosal1$Automated 0.77730 0.05423 14.335 < 2e-16.
Residual standard error: 0.1785 on 91 degrees of freedom
Multiple R-squared: 0.6931, Adjusted R-squared: 0.6897
F-statistic: 205.5 on 1 and 91 DF, p value: <2.2e-16

The generated equation was:

\[ Y = 0.43075 + 0.77730 \times X \]

Where \( Y \) = manual platelet count and \( X \) = automated platelet count.
Table 2: Group 1 statistics (central tendencies).

<table>
<thead>
<tr>
<th>Methods</th>
<th>Mean</th>
<th>Median</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual</td>
<td>1.08</td>
<td>1.2</td>
<td>0.32</td>
</tr>
<tr>
<td>Automated</td>
<td>0.83</td>
<td>0.88</td>
<td>0.34</td>
</tr>
</tbody>
</table>

The above-mentioned results are statistically significant at 99.99% (p<0.0001) level of significance. Thus, we can reject null hypothesis at 99% level of significance. The same has been graphically depicted (Figure 3).

A linear regression analysis was run for group 2 (using R statistical software) keeping manual platelet count as the dependent variable and automated platelet count as the independent variable. The results obtained were:

**Coefficients:**

Estimate std. error: t value Pr(>|t|) (Intercept): 0.82855
0.03834 21.61 <2e-16, ceosal1$ Automated 0.70749
0.01909 37.07 <2e-16
Residual standard error: 0.2873 on 424 degrees of freedom
Multiple R-squared: 0.7642,
Adjusted R-squared: 0.7636
F-statistic: 1374 on 1 and 424 DF, p-value: <2.2e-16

The generated equation was:

\[ Y = 0.56386 + 0.80911 \times X \]

Where \( Y \) = manual platelet count and \( X \) = Automated platelet count.

Table 3: Group 2 statistics (central tendencies).

<table>
<thead>
<tr>
<th>Methods</th>
<th>Mean</th>
<th>Median</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual</td>
<td>2.15</td>
<td>2</td>
<td>0.59</td>
</tr>
<tr>
<td>Automated</td>
<td>1.87</td>
<td>1.79</td>
<td>0.73</td>
</tr>
</tbody>
</table>

The above-mentioned results are statistically significant at 99.99% (p<0.0001) level of significance. Thus, we can reject null hypothesis at 99% level of significance. The same has been graphically depicted (Figure 4).

Figure 3: Regression analysis scatterplot of group 1 comparing manual and automatic platelet counts showing moderate to wide dispersion.

A linear regression analysis was run for group 3 (using R statistical software) keeping Manual Platelet count as the dependent variable and Automated Platelet count as the independent variable. The results obtained were:

**Coefficients:**

Estimate std. error: t value Pr(>|t|) (Intercept): 0.56386
0.37257 1.513 0.158, ceosal1$ Automated 0.80911
0.07525 10.752 3.57e-07
Residual standard error: 0.3341 on 11 degrees of freedom
Multiple R-squared: 0.9131,
Adjusted R-squared: 0.9052
F-statistic: 115.6 on 1 and 11 DF, p-value: 3.566e-07

The generated equation was:

\[ Y = 0.56386 + 0.80911 \times X \]

Where \( Y \) = manual platelet count and \( X \) = Automated platelet count.

Table 4: Group 3 statistics (central tendencies).

<table>
<thead>
<tr>
<th>Methods</th>
<th>Mean</th>
<th>Median</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual</td>
<td>4.44</td>
<td>4.50</td>
<td>1.09</td>
</tr>
<tr>
<td>Automated</td>
<td>4.84</td>
<td>4.91</td>
<td>1.28</td>
</tr>
</tbody>
</table>

The above-mentioned results are statistically significant at 99.99% (p<0.0001) level of significance. Thus, we can reject null hypothesis at 99% level of significance. The same has been graphically depicted (Figure 5).
platelet count results with p value <0.05. Balakrishnan et al also found significant correlation between manual and automated platelet count (p=0.50). 13 Webb et al found quiet close results in comparison to automated method by multiplying 15000 to average number of platelets in 10 oil fields. 19 Anchinmane et al found very strong correlation by multiplying with 20,000 (r=0.9789). 3 Malok et al also found strong correlation with automated count by multiplying with 20,000 (r=0.90). 17 Lazreg et al in their study found Brahmi’s method that derives platelet count in stained blood smears by RBC: platelet ratio show better correlation with automated count (r=0.834) than Anitha’s method (r=0.596) where RBC count is not required , better suited for rural areas. 20, 21 Zainab et al found excellent agreement between different raters using manual platelet estimation. Intraclass correlation coefficient (ICC) across the four raters was 0.840 in patients with platelet count less than 1.0 lakh per cubic millimeter. 22 Lawrence et al compared triplicate automated and manual platelet counts on thrombocytopenic patients with platelet counts from 4-30×10^9/l. The triplicate automated platelet counts differed by no more than 5×10^9/l among themselves, whereas the manual counts varied by as much as 30×10^9/l. 23

**CONCLUSION**

A significant positive correlation is present between the manual slide and the automated method though correlation is slightly low in group 1 (<1.5 lakh/cubic millimetre). Thus, manual method can be used in small labs where patient load is less, who can’t afford blood cell counter as it is costly to operate and maintain, especially for a country like India.

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**Conflict of interest:** None declared

**Ethical approval:** Not required

**REFERENCES**


