

## Original Article

# Platelet estimation by peripheral smear: Reliable, rapid, cost-effective method to assess degree of thrombocytopenia

Ritu Bajpai<sup>1</sup>, Chanda Rajak<sup>2</sup>, Meghna Poonia<sup>1</sup>

<sup>1</sup>Department of Physiology, Shyam Shah Medical College, Rewa, Madhya Pradesh, India, <sup>2</sup>Department of Physiology, Shyam Shah Medical College, Rewa, Madhya Pradesh, India

## ABSTRACT

**Aim:** To compare the estimation of platelet count done by peripheral smear method and by automated cell counter. **Background:** Thrombocytopenia is associated with many diseases such as malaria, dengue, pregnancy-induced hypertension, etc., and is one of the critical parameters in patient management. The automated method is considered as the most reliable method. It is simple, fast, and most widely used, but the accurate count of platelets by automated cell counters is not available for all patients, especially in rural areas. In such settings, platelet estimation by peripheral smear is more feasible, than by automated cell counter in thrombocytopenia patients. **Materials and Methods:** A total of 92 ethylenediaminetetraacetic acid samples of patients were received in the laboratory and platelet count was evaluated by two techniques: (1) Automated platelet count, (2) Assessment of platelet count on Leishman's stained smear. **Results:** There is no significant ( $P = 0.69$ ) difference of values between our method of platelet estimation ( $0.94 \pm 0.29$  lacs/mm<sup>3</sup>) when compared with that of automated cell counter platelet value ( $0.91$  lacs/mm<sup>3</sup>  $\pm 0.27$ ). **Conclusion:** The method of platelet estimation by peripheral smear is useful as a rapid, cheap method to assess platelet count and can be done in rural hospital settings.

**Keywords:** Peripheral smear, platelet, thrombocytopenia

## INTRODUCTION

Platelets are subcellular fragments derived from megakaryocytes in the bone marrow, circulating in the blood as small discs having a precise and reproducible structure.<sup>1</sup> A single megakaryocyte can give rise to 1000-3000 platelets.<sup>2</sup> Megakaryocytes are rare myeloid cells (constituting <1% of these cells) that reside primarily in the bone marrow.<sup>3</sup> The platelets are very small, non-nucleated, about 3  $\mu$ m in diameter, and consist of cytoplasm enclosed within a cell membrane. The life span of a normal platelet is about 7-12 days, and they are destroyed by the macrophages in the spleen. The platelet in peripheral blood is heterogenous with respect to size, density, and staining characteristics.<sup>4</sup> Their morphology also varies greatly depending on the methods by which they are examined, and the anticoagulant used.<sup>5</sup> In wet preparations, they are colorless, moderately refractile bodies that are discoid or elliptical. In Romanowsky stained smears, they appear round, oval or rod-shaped. Azurophilic granules are seen in hyaline, light blue cytoplasm. These granules may be so tight in the central

portion of the platelet that may give the appearance of the nucleus.<sup>6</sup> Platelets are multifunctional and play a key role in many physiological processes (e.g. wound repair, immune response) apart from their well-known roles in hemostasis and thrombosis.<sup>7</sup>

It is well-known that thrombocytopenia is one of the critical parameters in patient management. Therefore, it is very important that laboratories assess platelet counts with utmost accuracy. The normal range of platelet count in a healthy individual is 150000 - 400000/ $\mu$ L.<sup>8</sup>

The common methods of platelet estimation are:

1. Manual counting using counting chamber
2. Evaluation on the peripheral smear
3. Assessment using the automated cell counters.

## MATERIALS AND METHODS

The present study was done in Department of Physiology of SS Medical College, Rewa, Madhya Pradesh from October 2012 to December 2013.

### Corresponding Author:

Ritu Bajpai, Shyam Shah Medical College, Rewa, Madhya Pradesh, India. E-mail: drritubajpai@gmail.com

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### Source of Data

Samples were received for platelet count evaluation in the central diagnostic laboratory of Sanjay Gandhi Hospital associated with SS Medical College, Rewa, Madhya Pradesh. Samples and readings of automated cell counter were collected after taking permission from a laboratory in charge of hospital and estimation of platelet count by peripheral smear was done in the Department of Physiology.

### Inclusion Criteria

Ethylenediaminetetraacetic acid (EDTA) anticoagulated samples of patients were received in the laboratory for platelet count evaluation.

### Exclusion Criteria

Hemolyzed samples, clotted samples.

### Sample Size

A total of 92 samples with low platelet counts. The EDTA samples of patients received in the laboratory were evaluated by 2 techniques.

### Automated Platelet Count

Platelets were 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 min 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. MINDRAY BC 3600 (3 PART). The platelet count values found are shown in (Figure 2).

### Assessment of Platelet Count on Leishman's Stained Smear

- Place the air-dried smear film side up on the staining rack
- Cover the smear with Leishman stain and leave for 3 min
- Dilute with the phosphate buffer (pH - 6.9) volume of the buffer to one volume of stain until a metallic scum appears. Allow this to stand for 7 min
- Wash the smear with tap water
- Air dry the smear
- Count the platelets under oil immersion objectives in an area where the red blood count (RBC) morphology is well made out (RBC's are separated without overlapping)
- The platelets are counted in the ideal zone of a smear stained with Leishman's stain where blood cells did not overlap, and there is fairly even distribution of white blood cells and platelets (Figure 3).

The calculation is done by; the average number of platelet in an oil immersion field multiplied by 15 thousand.<sup>9,10</sup> Count at

least 10 fields of each platelet in an average oil immersion field represents 15000 platelets/cu mm (Figure 1).

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.<sup>10</sup>

### Statistical Analysis

Statistical analysis was done by Student's *t*-test by using Office Excel 2007.

## RESULTS

There is no significant ( $P = 0.69$ ) difference of values between our method of platelet estimation ( $0.94 \pm 0.29$  lacs/mm<sup>3</sup>) when compared with that of automated cell counter platelet value ( $0.91$  lacs/mm<sup>3</sup>  $\pm 0.27$ ).

## DISCUSSION

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 ( $n = 92$ ) did not show significant statistical difference ( $P = 0.69$ ) in the results. The mean platelet count was  $<1$  lac by both methods. The standard deviations of platelet count in the whole blood by automated and manual method are 28579.84 and 27163.94, respectively (Table 1).

In their study Webb *et al.*<sup>10</sup> 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 cross-sectional study conducted in National Centre for Public Health Laboratories of Aden yemen, Bakhubaira<sup>11</sup> found that the mean platelet count estimated by manual method was not significantly different from that estimated by the electronic method, which is similar to our study. In another study by Oliveira *et al.*<sup>12</sup> suggested, a platelet count below 30,000/ $\mu$ l 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.<sup>13</sup> 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.<sup>14</sup> This method and platelet count by the automated cell counter are though more sensitive but expensive; time-consuming and require well-equipped hospital and, therefore, cannot be affordable in many rural settings as in India. On the other hand, platelet estimation method is rapid, cheaper and easier, and does not need any expensive materials. It takes about 30 min. In centers where advanced methods are unavailable, platelet estimate is an important step in the assessment of platelet count, especially when the count is low.

**Table 1: Showing mean and standard deviation values**

Parameter	Mean in lacs/mm <sup>3</sup>	Standard deviation
Platelet count by peripheral smear	0.94	0.29
Platelet count by automated cell counter	0.91	0.27
Difference between the two methods	0.0214	0.0265

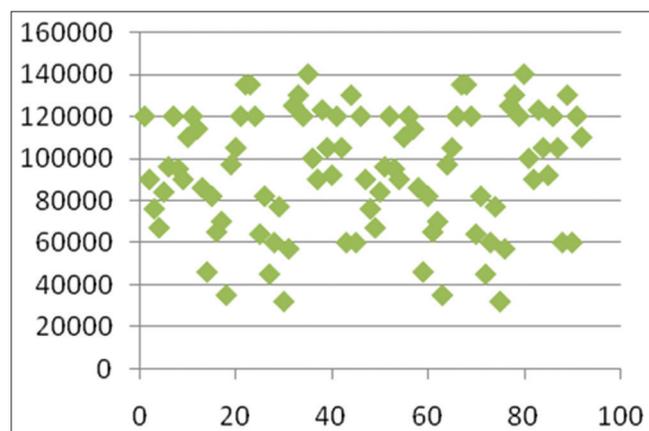


Figure 1: Platelet count values by peripheral smear method

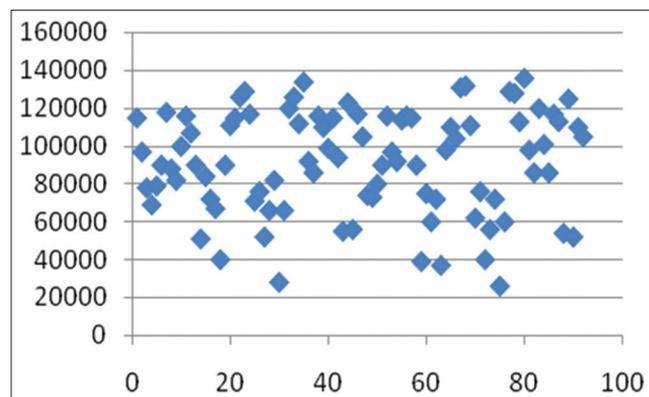


Figure 2: Platelet count values by automated cell counter

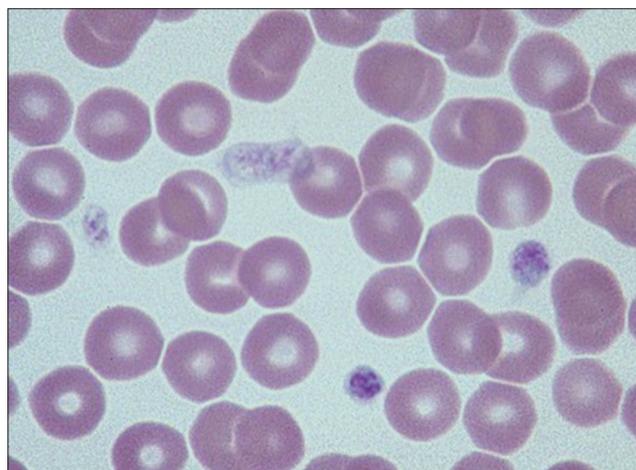


Figure 3: Platelet under ×100 (oil immersion)

## CONCLUSION

The platelet count estimated by the manual method was not significantly different from that estimated by the electronic method. Platelet estimation method can be taken as an early and rapid procedure for platelet assessment in cases where low platelet count needs an early intervention such as in pregnancy-induced hypertension, dengue, and malaria, etc., for their management. This method is not only rapid but also cheaper. It can be done even in rural hospital settings where automated facility is not available.

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## PEER REVIEW

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## CONFLICTS OF INTEREST

Nil

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