Platelet Estimation from Peripheral Blood Smear: Does it Really Work?

Estimation des plaquettes à partir de frottis sanguins périphériques : ça marche vraiment ?

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Dear Editor,

Assessment of platelet count is an important diagnostic parameter in haematology. However, despite of the performing techniques used by automated analysers, platelet count is sometimes challenging due to their small size. Therefore, manual methods are still used to verify any questionable platelet count and to avoid any interferences that can make the automated counting erroneous (macro thrombocytes, aggregates...) [1].

In this study we used three manual methods proposed by many researchers [2-6] and compared our results to the automated method, which we considered as a reference one.

220 blood samples were collected, 131 from blood donors with a healthy physical condition, and 89 from patients admitted in our hospital. The tubes used to collect blood samples contained ethylenediaminetetraacetic acid (K2 or K3EDTA) to avoid any in vitro clotting, blood samples containing clots were systematically excluded from the study.

Complete blood count was performed on the Sysmex XT4000i counter, which uses both impedance and optical numeration techniques for the platelet count.

Two blood smears were performed for every blood sample, then stained with an automatic May Grunwald Giemsa stain (Hemateck), and examined under light microscopy first with a 40X lens to search any aggregates or macrothrombocytes, if any are found that exclude the blood sample from the study. Platelet estimation was then done on a 100X oil immersion fields as follow:

1st method: this method was proposed by Brahimi and other researchers [2] and consists of calculating the red cell: platelet ratio: The number of erythrocytes observed in a quarter of the oil-immersion field was multiplied by four. Then all the platelets in the same field were counted. Other fields were examined until we reached a number of 1000 erythrocytes. The number of platelets per 1000 erythrocytes was multiplied by the automated Red Blood Count to give an approximate count.

2nd method: we counted the number of platelets per 10 oil immersion fields and divided by 10 to find the average number of platelets per field then multiply by the field factor that must be determined for each brand of as follow:

First, we perform 30 consecutive automated patient samples and ensure the PLT QC is within two Standard Deviations, and then we prepare and stain a smear on each specimen. For each smear, we count the number of PLTs in 10 consecutive OIFs and divide by 10 to get the average number per field, after we divide the automated PLT count by the average number of PLTs per field for each specimen, we add the numbers obtained in step four and divide by number of specimens (30) to get the average ratio of automated PLTs to PLTs per OIF. Round ratio value to the nearest whole number. For example, if the ratio value is 15.0, one platelet per oil field 15.0 x 10^9/L. Finally, we calculated the average number of platelets per OIF and multiplied by the platelet factor. Therefore, for an average of 15 PLTs per OIF, the calculation would be: 15 x 15 = 225 x 10^9/L [3-5]. In our study the field factor was equal to 14.

3rd method: proposed by Sheila Torres *et al.* [6], we multiplied the average number of platelets per 10 fields by the patient's haemoglobin and then by 1,000 mm^3 to obtain the number of platelets/mm^3.

Data were analyzed by statistical software (Excel, EpiData and MedCalc) and expressed in mean ± SD. Comparison between the methods was done by calculating the intraclass correlation ICC . An ICC ≥ 0.75 refers to an excellent correlation (Cicchetti 1994) [7]. A paired t-test was performed in order to assess the match between platelet count results by both methods. In this evaluation, a statistically significant difference in platelet level was set at a level of p=0.05 [2]. We also analysed the ROC curves (receiver operating characteristic curves), which allowed us to compare both sensitivity and specificity of the four methods, and to identify the most performing method and thus the reference one.

Three populations were obtained based on the automated platelet count: low, normal and high platelet count (table 1):

Table 1. Populations based on platelet count.

Populations	Total N = 220	Male N = 123	Female N = 97
Low plat acc < 120 G/l	57	34	23
High plat acc > 450 G/l	32	18	14
Normal (120-450 G/l)	131	71	60

The results showed positive correlation between the four methods (automated and manual). In our study, the ICC was equal to 0.946, 0.937, 0.888 for method 1, 2 and 3 respectively. The paired t-test showed no significant difference between the four methods (p>0.05).

Table 2. The mean platelet count by manual versus automated methods.

Platelet count (G/L)	N	Automated count	Method 1	p	Method 2	p	Method 3	p
< 120	57	81 ± 30	70 ± 36	0,158	94±45	0,266	65 ± 35	0,228
120- 450	131	250 ± 55	274 ± 68	0,126	271 ± 58	0,236	273 ± 60	0,387
> 450	32	570 ± 119	562 ± 151	0,244	600±183	0,170	450 ± 162	0,244

The mean platelets count did not show significant difference between the four methods (p≥0.05).

Table 3. Correlation of samples with low/normal and high platelet count by manual vs. automated methods.

	Platelet count (G/L)						
Sex	automate	M1	Correlation	M2	C	M3	C
Male	244±175	257±180	r=0,952 p=0,260	266±187	r=0,942 p=0,280	247± 152	r=0,898 p=0,170
Female	259±144	268±163	r=0,934 p=0,262	282±163	r=0,930 p=0,300	241± 137	r=0,874 p=0,188

After analyzing ROC curves which allowed us to compare both specificity and sensitivity of the three manual methods, we have concluded to the following results:

We defined two reference methods for manual platelet count based on the highest sensitivity and specificity of each method. Method 1 for patients with low platelet count (sensitivity: 89 %, specificity: 100 %) and method 2 for patients with high platelet count (sensitivity: 84 %, specificity: 99%) with a cut-off of 130 and 327 G/L respectively.

The platelet count is possible without any automated help thanks to the manual methods using peripheral blood smears. These methods allow an easy and simple count using only a slide and an optical microscope.

This is particularly interesting when automated platelet count may be affected by some sample characteristic such as blood clots or by a particular physical condition especially in disseminated intravascular coagulation (DIC) where schizocytes found in the blood can be confused with platelet due to their small size [1].

Up to date, many researchers attempted to verify the effectiveness of these manual methods under the name of indirect platelet count [2,6]. Our results join the majority of the studies done in this context [2,6,8,9]. However, even though the easiness these methods provide, several limits have been noticed while performing the platelet count, for example for method 1 is still an automated dependent method as well as method 3.

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