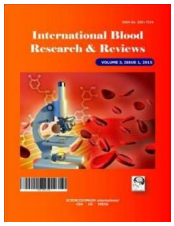

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Determination of Platelet and White Blood Cell Counts from Peripheral Blood Smear: An Indispensable Method in Under-resourced Laboratories

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Objective: Blood smear examination serves as a quality control tool in verifying the results generated by the automated analyzer and identification of abnormal or immature cells amongst other functions. This study was undertaken to estimate platelet and White Blood Cell (WBC) counts from Peripheral Blood Smear (PBS) and to correlate them with the results from automated method.
Materials and Methods: Fifty blood samples collected into K_3 EDTA from 30 males and 20 females whose ages were from 2 to 50 years, and attended General Out-Patient Department (GOPD) of Aminu Kano Teaching Hospital, Kano between May and November, 2015 were considered for the study. Each blood sample was used for the determination of full blood count using Swelab Alfa hematology analyzer, and preparation of stained blood films using standard laboratory methods.
Results: There were significantly lower values of platelet count (using multiplication factor of 15.0 $x10^9$/L) and white blood cell count (using multiplication factor of 2.0 $x10^9$/L) to derive (22.42±60.77) $x10^9$/L and (4.49±1.04) $x10^9$/L by manual (PBS) method as compared to (267.86±77.28) x 10^9/L and (5.86±1.36) $x10^9$/L respectively, for automated method (P<0.001). However, there was no

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significant difference in estimated platelet count by manual (PBS) method using multiplication factor of 20.0 x10^9/L compared to automated method (P>0.05). Fairly strong positive correlations were observed for platelet and white blood cell counts when manual method was compared to automated method (r= 0.6828 - 0.7321, P<0.05).
Conclusion: It can be concluded that multiplications factors of 20.0 x10^9/L per 100X, objective lens and 2.7 x10^9/L per 40X, objective lens can be used for average numbers of platelets and white blood cells respectively to estimate platelet and white blood cell counts from PBS as the results are comparable to that of the hematology analyzer.

Keywords: Platelet and WBC counts; peripheral blood smear method.

1. INTRODUCTION

Peripheral Blood Film (PBF) is a highly informative haematological tool that can be used for the screening, diagnosis and monitoring of disease progression, and for therapeutic response [1]. The microscopic blood smear examination may be limited to a blood smear scan or may include complete blood smear examination with manual differential count and/or a blood smear review [2].

Blood smear examination serves as a quality control tool in verifying the results generated by the automated analyzers, identification of abnormal or immature or atypical cells and recognition of clinically significant morphological abnormalities, which the analyzers are incapable of either flagging or detecting and identifying [2].

It has been reported that the common clinical indications for peripheral blood film analysis include unexplained cytopenia such as anemia, leucopenia or thrombocytopenia; unexplained leucocytosis such as lymphocytosis, monocytosis; suspected chronic or acute myelo-proliferative disease such as chronic myeloid leukaemia; suspected cases of nutritional anemia; suspected chronic lympho-proliferative disease such as chronic lymphocytic leukemia; suspected organ failure such as liver disease, renal disease; several bacterial sepsis and parasitic infections and evaluation of therapeutic response amongst other conditions [3-5].

It has been observed that the platelet counts derived from the average numbers of platelets from Peripheral Blood Smear (PBS) using 100x, oil-immersion objective, multiplied by 20.0 x10^9/L yielded no significant differences when compared to the results of hematology analyzers [6-9] while Webb et al. [10] reported that a multiplication factor of 15.0 x10^9/L for an average number of platelets from PBS per High Power Field (HPF) gave slightly better result than multiplication factor of 20.0 x10^9/L when compared to automated method.

However, this study was undertaken to determine the White Blood Cell (WBC) count estimation factor as scanty information is available, and platelet count from PBSs since the findings can be useful in the estimation of platelet and WBC counts in under-resourced laboratories apart from verifying results of automated cell counters that are prone to interferences from particles of similar size and/or light scatter properties such red cell fragments, apoptotic white blood cell fragments, platelet clumps amongst other cells [11-13].

2. MATERIALS AND METHODS

The study was conducted on 50 blood samples collected from the patients that attended the General Out-Patient Department (GOPD) of Aminu Kano Teaching Hospital, Kano between May and November, 2015. These samples with the normal haematocrits, platelet counts and WBC counts were collected from 30 males and 20 females whose ages were from 2 to 50 years.

Each blood sample collected into K_3EDTA container was used for the preparation of thin blood film or PBS and for full blood count determination. The thin blood films were stained by Leishman`s method as described by Bajpai et al. [14] for the determination of estimated platelet and WBC counts while a quality-controlled Swelab Alfa 3-part hematology analyzer, manufactured by Boule medical AB in Sweden with impedance and spectophometry methods, was used for the determination of platelet and WBC counts.

PBS was examined where the red cells were not overlapping or showing platelet clumps and platelet counts were determined by multiplying the average numbers of platelets per 10, 100X oil immersion fields by 20.0 x10^9/L and 15.0 x10^9/L respectively [6,7,10,14,15] while WBC count was determined by multiplying the average number of WBCs counted in 10, 40X high power fields by 2.0 x10^9/L (WBC count multiplication factor) [16].

2.1 Statistical Analysis

Data were analysed using Statistical Package for the Social Sciences (SPSS) 20 statistical software (version 20 SPSS Inc., Chicago) and microsoft word excel 2010. Data were expressed as mean ± standard deviation, and one-way analysis of variance (ANOVA) and Student's t-test were used to compare manual (PBS) platelet and WBC counts to that of automated counts. Correlation and linear regression analyses were employed to determine the relationship between manual (PBS) and automated methods for platelet and WBC counts. P-values of ≤ 0.05 were considered as statistically significant.

3. RESULTS

Table 1 shows platelet counts using automated and manual (PBS) methods. There was significantly lower value of platelet count by manual using PBS method (average number of platelets on PBS/ 100x objective lens, multiplied by 15.0 $x10^9$/L) of (220.42±60.77) $x10^9$/L compared to (267.86±77.28) $x10^9$/L of automated method (P= 0.001) while the values of platelet count using PBS (average number of platelets on PBS multiplied by 20.0 $x10^9$/L) of (293.54±81.03) $x10^9$/L compared to (267.86±77.28) $x10^9$/L of automated methods, showed no statistically significant difference (P= 0.051).

Comparison of WBC counts using automated and manual (PBS) methods is displayed in Table 2. There was significantly lower value of white blood cell counts of (4.49±1.04) $x10^9$/L by PBS (average number of WBCs per 40X, HPF multiplied by 2.0 $x10^9$/L) compared to (5.86±1.36) $x10^9$/L of automated cell counter (P= 0.000).

Table 3 reveals the determination of white blood cell count estimation factor using automated count and average number of WBCs on PBS. The value of WBC count estimation factor of 2.7 $x10^9$/L was observed in this study.

Fig. 1 shows correlation and regression analysis between platelet counts from automation and PBS (platelets average/HPF x 20.0 $x10^9$/L) with the equation of y= 0.7675x + 87.957 (r= 0.7321, P<0.05).

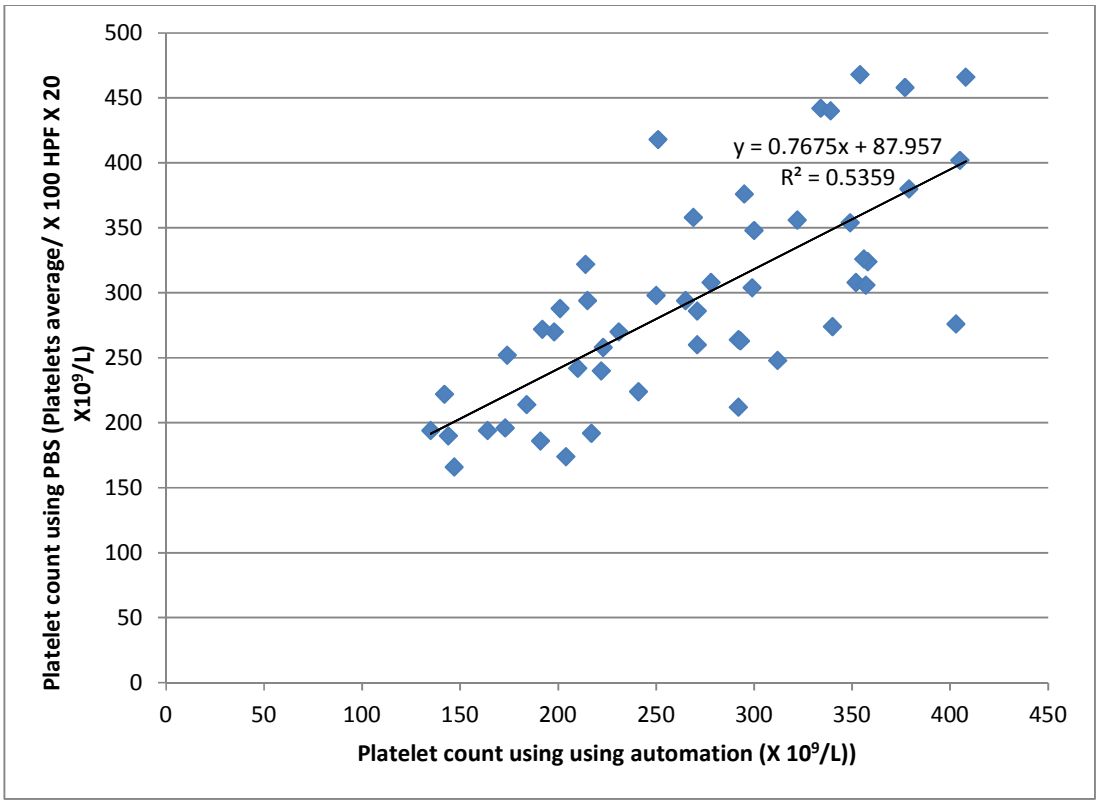

Fig. 1. Correlation between platelet counts using automation and manual (PBS) methods

Fig. 2 reveals the correlation and regression analyses between platelet counts from automation and PBS (platelets average/HPF x 15.0 x10^9/L) with the equation of y= 0.5749x + 66.421 (r= 0.7312, P<0.05).

Fig. 3 shows correlation between estimated WBC count using PBS (average number of WBCs per 40X, HPF multiplied by 2.0 x10^9/L) and WBC count using automation, and the linear equation of y= 0.5144x + 1.4744 (r= 0.6828, P<0.05).

Table 1. Platelet counts using automated and manual (PBS) methods

	Mean (x 10^9/L)	Standard deviation (x 10^9/L)
Number	50	50
Average number of platelets per HPF x 20.0	293.54	81.03
Average number of platelets per HPF x 15.0	220.42*	60.77
Platelet count using automated method	267.86	77.28

**Significantly different from automated method (P<0.001)*

Table 2. Comparison of white blood cell counts (WBCs) using automated and manual (PBS) methods

	Mean (x 10^9/L)	Standard deviation (x 10^9/L)
Number	50	50
Automated WBC count	5.86	1.36
Average number of white cells per HPF x 2.0	4.49*	1.04

**Significantly different from automated method (P=0.000)*

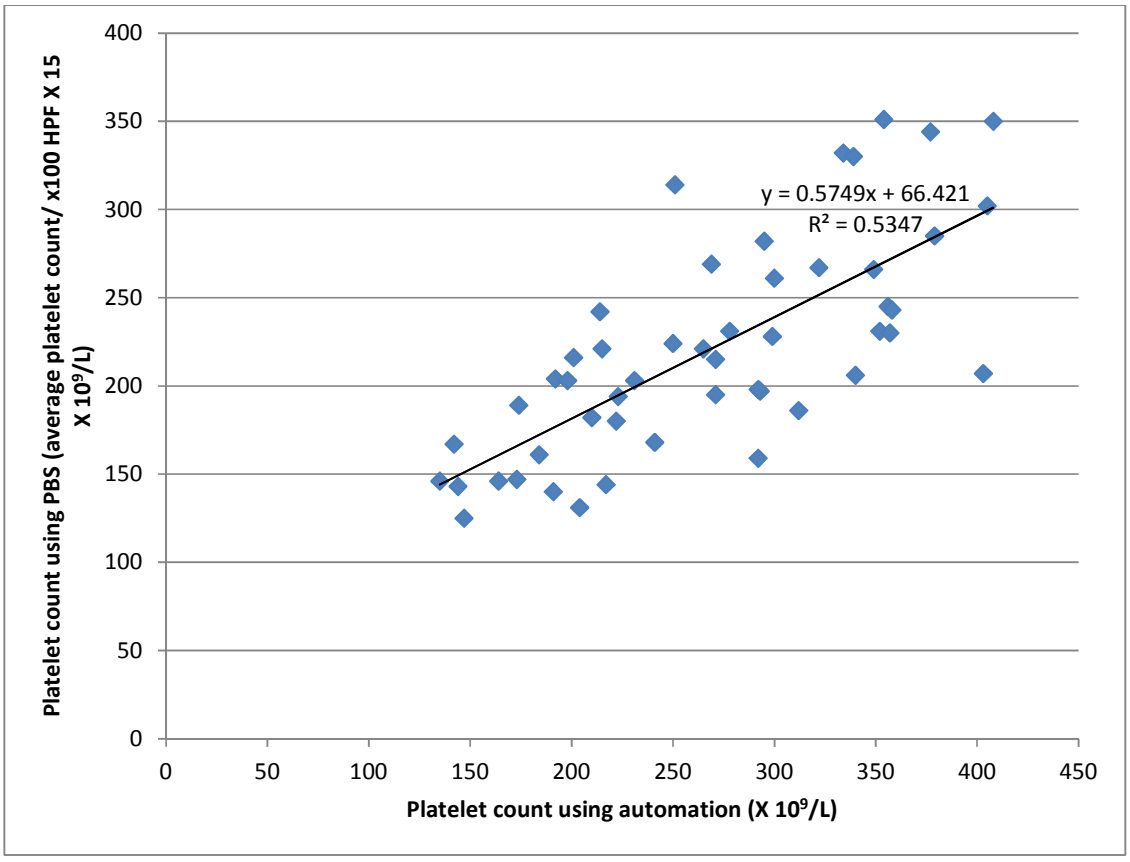

Fig. 2. Correlation between platelet counts using automation and manual (PBS) methods

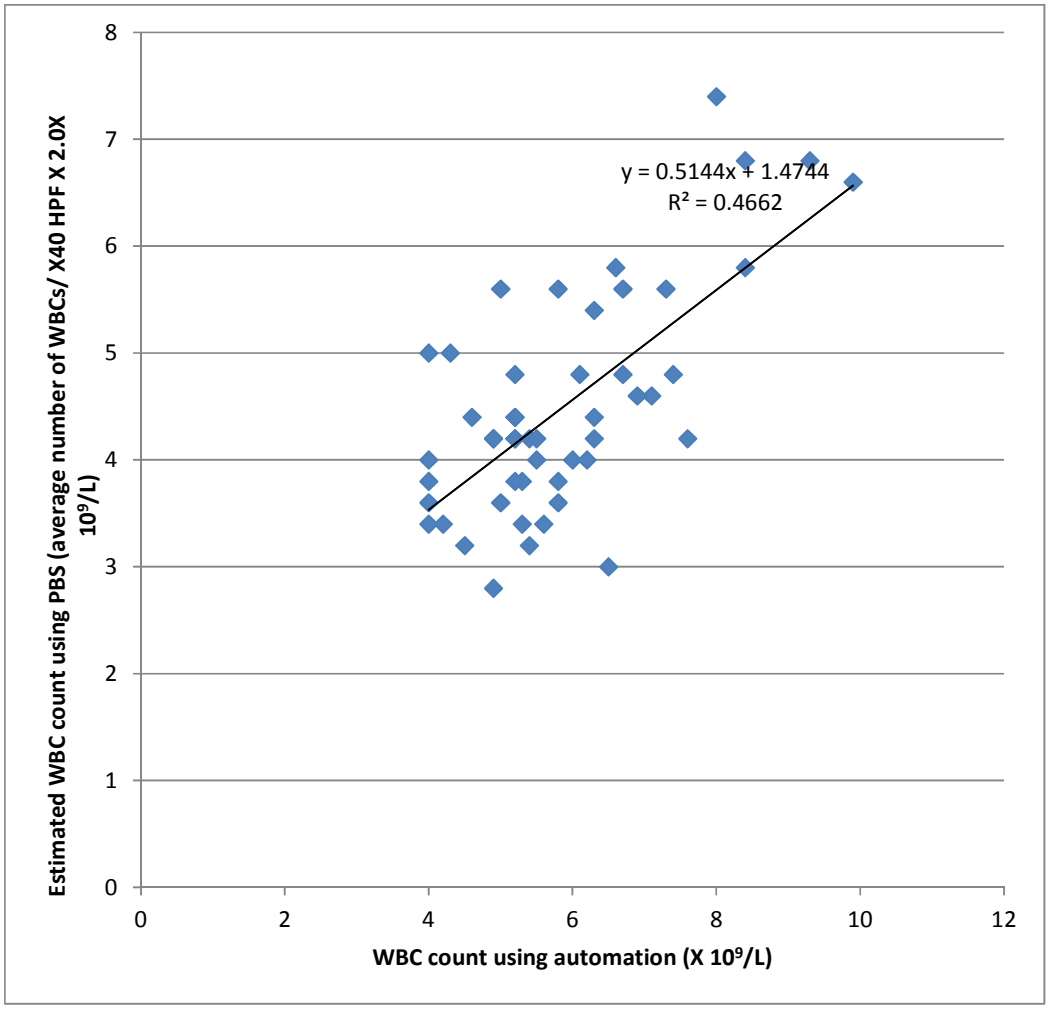

Fig. 3. Correlation between WBC counts using automated and manual (PBS) method

Table 3. Determination of white blood cell count (WBC) estimation factor using automated count and average number of WBCs on PBS

	Mean (x 10^9/L)	Standard deviation (x 10^9/L)
Number	50	50
Automated WBC count	5.86	1.36
Average number of white cells/ HPF	2.24	0.51
Average ratio of automated count/ average number of white blood cells/ HPF	2.66	0.5
WBC count estimation factor	2.7	

4. DISCUSSION

Despite the advances in haematology automation and application of molecular techniques, the PBF remains a very important diagnostic test to the haematologist [1].

In this study, significantly lower value of platelet count was observed on PBS (with multiplication factor of 15.0 x10^9/L) compared to automated platelet count as against the findings of Webb et al. [10] and Bajpai et al. [14] that reported slightly better results with 15.0 x10^9/L multiplier than the multiplication factor of 20.0 x10^9/L. However, there was fairly strong positive correlation between platelet counts from automation and PBS (platelets average per 100x, multiplied by 15.0 x10^9/L). Divergent views

expressed by the authors might be associated with the different sensitivities of the automated cell counters used, lack of quality control for the haematology analyzers and improperly prepared PBS showing uneven distribution of bloods cells.

The study has confirmed the previous reports [8,17] which showed that there was no significant difference between platelet count estimate using PBS (with multiplication factor of 20.0 $x10^9$/L) and that of automated cell counters. There was fairly strong positive correlation of manual platelet count on PBS (multiplication factor of 20.0 $x10^9$/L) with automated method and this is in line with the earlier reports [8,17]. These findings have indicated that the multiplication factor of 20.0 $x10^9$/L for platelet count by manual (PBS) method is reliable and comparable to automated result.

The study has further revealed significantly lower value of total white blood count by manual method as described by earlier authors [16,18] compared to automated method while there was fairly strong positive correlation of manual and automated methods. The significant difference in WBC count may be associated with the different automated cell counters used in the determination of WBC estimation factor of 2.0 $x10^9$/L. However, WBC count estimation factor of 2.7 $x10^9$/L has been observed in this study.

5. CONCLUSION

In conclusion, PBS is a reliable and cost-effective method that can be used for the estimation of platelet and WBC counts in the haematology laboratory and most especially, in the under-resourced medical laboratories, apart from its importance in the verification of counts from automated cell counters. However, multiplication factor of 20.0 $x10^9$/L per 100X, objective lens and 2.7 $x10^9$/L per 40X, objective lens can be used for average numbers of platelets and white blood cells, respectively to estimate the platelet and WBC counts from PBS as the results are comparable to that of hematology analyzers.

It is recommended that each hematology department or medical laboratory should determine the multiplication factors for platelet and WBCs using PBS in order to estimate platelet and WBC counts since there may be possibility of automated cell counters having different sensitivities.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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