Comparison of Two Platelet Count Estimation Methodologies for Peripheral Blood Smears

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OBJECTIVE: To compare two manual methods for estimating platelet counts from Wright's stained peripheral blood smears regarding their correlation with each other and with automated platelet counts. This correlation was examined in relation to whether the platelet count was high, low, or normal and in relation to whether the hemoglobin value was low versus normal or high.

DESIGN: Peripheral blood smears were Wright's stained and both platelet count estimation methodologies were performed on each slide. The traditional estimation method was the average number of platelets per oil immersion field (OIF) multiplied by 20,000 to yield a platelet count estimate per uL. The alternate estimation method was the average number of platelets per OIF multiplied by the patient's hemoglobin value in g/dL and then multiplied by 1,000 to yield a platelet count estimation per uL. The platelet count estimates were performed without the technologists having prior knowledge of the automated platelet counts which were produced on a Coulter LH750 analyzer. The agreement between the two manual methodologies with each other and each method with the automated count was assessed using the paired T-test and correlation coefficient analyses. These analyses were performed for the whole dataset as well as for subsets based on the automated platelet count and the hemoglobin value.

SETTING: East Carolina University's Clinical Laboratory Science program in collaboration with the Clinical Pathology/Laboratory at Pitt County Memorial Hospital (PCMH) in Greenville NC.

PARTICIPANTS: One hundred eighty-four blood samples in EDTA-anticoagulant Vacutainer® tubes were used to conduct this study. Each blood sample had two peripheral blood smears made and stained on an automatic slide stainer. The blood samples were obtained from the Clinical Pathology/Laboratory of Pitt County Memorial Hospital in October and November of 2004. Each sample was given a unique numeric identifier with no personal identifying information from any sample being recorded.

MAIN OUTCOME MEASURE: Platelet counts by two slide estimation methods and by an automated reference method.

RESULTS: The traditional platelet count estimation method had a mean for the sample of 269,000/uL, while the alternate estimation method had a mean of 155,000/uL. The mean for the automated platelet counts was 268,000/uL. The traditional estimation method showed no statistically significant difference in mean from the automated platelet counts based on the paired T-test ($p = 0.87$). The traditional estimation method counts and automated counts had a high Pearson Product Moment correlation coefficient of r = .90 and a minimally dispersed scatterplot, thus showing strong agreement. The alternate platelet count estimation method had a mean for the sample of 155,000/uL which, based on the paired T-test, was highly significantly different from the automated count mean ($p < 0.0001$) and the traditional estimation method mean ($p < 0.0001$). The alternate estimation method and automated counts had a lower r value of .81 and greater dispersion in the scatterplot. In comparing the estimation methods with each other and with the automated method, the differences and similarities in agreement observed for the whole dataset were also observed with each platelet count and hemoglobin subset of data.

CONCLUSIONS: Though the alternate platelet count estimation method has been recommended for use particularly with patients with low hemoglobin values, this study found that the traditional estimation method provided more agreement with automated counts than did the alternate estimation method for all samples as well as for the subset

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of samples with low hemoglobin values. For the present, the traditional method of estimating platelet counts from blood smears to evaluate automated results appears to provide adequate quality assurance.

ABBREVIATIONS: ANP = average number of platelets; ECU = East Carolina University; g/dL = grams per deciliter; PCMH = Pitt County Memorial Hospital; OIF = oil immersion field; uL = microliter.

INDEX TERMS: peripheral blood smears; platelet counts; platelet estimates.

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PURPOSE OF STUDY

This study was conducted as a senior student research project while the first four authors were enrolled in the Clinical Laboratory Science baccalaureate degree program at East Carolina University in Greenville NC, with the fifth author being the supervising faculty for this project. Consequently, constraints in time and budget necessitated a project that was feasible to conduct in about a six month period and would require minimal funding from the program.

Manual smear evaluations serve to evaluate abnormal patient samples and provide quality control for automated results. An important part of the manual smear evaluation is the platelet count estimate. The purpose of this study was to evaluate the accuracy of platelet count estimates obtained by two different slide methods. The more commonly used methodology, referred to as the traditional estimation method, entails multiplying the average number of platelets per oil immersion field (OIF) by a factor of 20,000/µL.[1] The second estimation method, referred to as the alternate estimation method, is multiplying the average number of platelets per OIF by the patient's hemoglobin (in g/dL) by 1,000/µL.[1] The two count estimation methods were evaluated by comparison to an automated platelet count provided by a Beckman Coulter LH750 as well as by comparison of the two methods to each other.

The chosen investigation is a continuation of an earlier study performed by Torres and Velez.[2] The results of this previous study suggest that the "old method is less useful and specific than multiplying by the factor of patient hemoglobin."[2] The study was performed because the researchers had noted a variation between patient platelet estimates using the traditional method and instrument counts, particularly for patients with low hemoglobin values and/or low platelet counts.[2] Though there have not been many studies made on this alternate estimation method, the traditional estimation method has been evaluated for accuracy and is the platelet count estimation method usually recommended for use in clinical laboratories.[3-6]

MATERIALS AND METHODS

Sample

Pitt County Memorial Hospital (PCMH) is the primary clinical education site for health professional programs at ECU. After approval by the ECU Institutional Review Board, 184 blood samples in EDTA-anticoagulant Vacutainer® tubes were obtained from the Clinical Pathology/Laboratory of PCMH in October and November of 2004. All samples were processed by a Coulter LH750 which provided the automated platelet count (i.e., reference count). Only blood samples that did not produce any platelet flags (i.e., cautions such as platelet clumps, platelet fragments, giant platelets, platelet satellitism, and bizarre platelets) were included in the study. Each sample in the study had two peripheral blood smears made manually using the push-slide technique by the

same researcher to eliminate smear variation from technique. All slides were Wright's stained on a Sukara slide stainer. Each blood sample was coded by a unique numerical identifier with no personal identifying information recorded on any blood sample or any data records to maintain patient anonymity.

Platelet estimation methods

In agreement with previous studies, the platelet count estimation methods on the smears were performed as follows. The number of platelets were counted in an area of the stained smear that was thin where red blood cells did not overlap and there was a fairly even distribution of white blood cells and platelets, and where there were not large numbers of broken cells or precipitated stain.[2-5] The average number of platelets (ANP) per oil immersion field (OIF), based on ten fields, was determined for both slides on each blood sample by the same two researchers for all blood samples. One researcher determined the ANP per OIF on one slide and the second researcher determined the ANP per OIF on the second slide for each blood sample. If the two ANPs per OIF agreed within ten percent, the two ANPs were averaged. The ANP per OIF for each sample was then multiplied by 20,000 to obtain the sample's platelet count estimation/uL for the traditional method. The ANP per OIF was multiplied by the patient's hemoglobin in g/dL and then by 1000 to obtain the platelet count estimation/uL for the alternate method. The ANPs per OIF for all samples were performed using the same two microscopes, both AO binocular light microscopes, throughout the study. In summary, the platelets counts by traditional method, alternative method, and automated method, and the hemoglobin value were recorded for each blood sample on a master data sheet.

Group designation

The original study upon which this study is based examined the two platelet count estimation methods in relation to whether the hemoglobin value was normal or low and whether the automated platelet count was low, normal, or high. This study employed this group evaluation as well. Though reference ranges vary among clinical laboratories, an accepted normal range for platelet counts is 150,000 to 400,000/uL (or 150-400 x 10^3/uL).[5] The gender of the patients from whom the blood samples were obtained was unknown; therefore, to accommodate normal ranges for both males and females a hemoglobin value below 13 g/dL was designated as low. These reference ranges were used to designate groups for the blood samples. The automated hemoglobin value and platelet count were used for each sample to determine placement into the appropriate group. The number of blood samples in each of the six groups is displayed in Table 1.

Data analysis

Following collection of data, statistical analysis was performed using SPSS-PC+ version 10.0. Descriptive statistics included mean and standard deviation as well as the absolute value of the differences between each estimation method and the automated counts and between the two estimation methods. Descriptive statistics were calculated for each variable for the whole dataset as well as for the six groups based on automated hemoglobin and platelet count values.

Paired samples student T-tests were performed comparing the means for the two estimation methods and for each estimation method with the automated count. These T-tests were performed on the whole dataset and on the subgroups. Pearson Product Moment correlation coefficients (r) were calculated for each estimation method with the reference count and for the two estimation methods to each other, using the whole dataset. Scatterplots comparing each estimation method with the automated count method were gener-

Table 1. Sample sizes (N) of all groups used for analysis.

	N
Total number of samples	184
Group 1: Low platelet (<150 x 10^3/uL) Low hemoglobin (<13 g/dL)	40
Group 2: Low platelet (<150 x 10^3/uL) Normal hemoglobin (≥13 g/dL)	18
Group 3: Normal platelet (150-400 x 10^3/uL) Low hemoglobin (<13 g/dL)	40
Group 4: Normal platelet (150-400 x 10^3/uL) Normal hemoglobin (≥13 g/dL)	40
Group 5: High platelet (>400 x 10^3/uL) Low hemoglobin (<13 g/dL)	39
Group 6: High platelet (>400 x 10^3/uL) Normal hemoglobin (≥13 g/dL)	7

ated, and as appropriate, prediction equations were determined by linear regression. A significance level of $p < 0.05$ was used for interpretation of all statistical analyses.

RESULTS

The mean and standard deviation values for the entire dataset are shown in Table 2. The differences and absolute differences are used to indicate the accuracy of the methods. The absolute value of the difference was calculated to prevent directional error. If the difference was positive for one slide and negative for the next slide they would cancel out the overall difference calculated. The paired student T test p-values are shown in Table 2. The statistics are evaluated for significance at $p < 0.05$. These three basic comparisons can also be seen in Figures 1 through 3 as scatterplots, r values (noted as R), and linear regression equation for each estimation method compared to the automated counts and to each other. The correlation coefficients (R) shown on the scatterplots support the results of the T-tests.

The traditional platelet count estimation method gave a mean platelet count of 269 x10^3/μL, with a mean difference from the automated count of -1 x10^3/μL and a mean absolute difference of 36 x10^3/μL. The T-test ($p = 0.87$) shows no significant difference between the automated counts using the traditional platelet count estimation method. This is further supported by the scatterplot in Figure 1. The correlation coefficient for this linear regression is 0.90. This value shows there is a fairly strong linear relationship between the automated counts and estimates by the traditional method.

Table 2. Descriptive statistics for whole dataset (N = 184)

	Mean*	Standard deviation*
Automated platelet count	268	166
Traditional platelet count estimate	269	167
Differences	-1	76
Absolute value of the differences	36	67
Alternate platelet count estimate	155	95
Differences	113	105
Absolute value of the differences	119	98
	T-test	
Traditional estimate to automated count	$p = 0.87$	
Alternate estimate to automated count	$p < 0.0001$	
Traditional estimate to alternate estimate	$p < 0.0001$	

* All platelet count values are x 10^3/μL
"Differences" are the differences between automated count and the specific platelet count estimation method

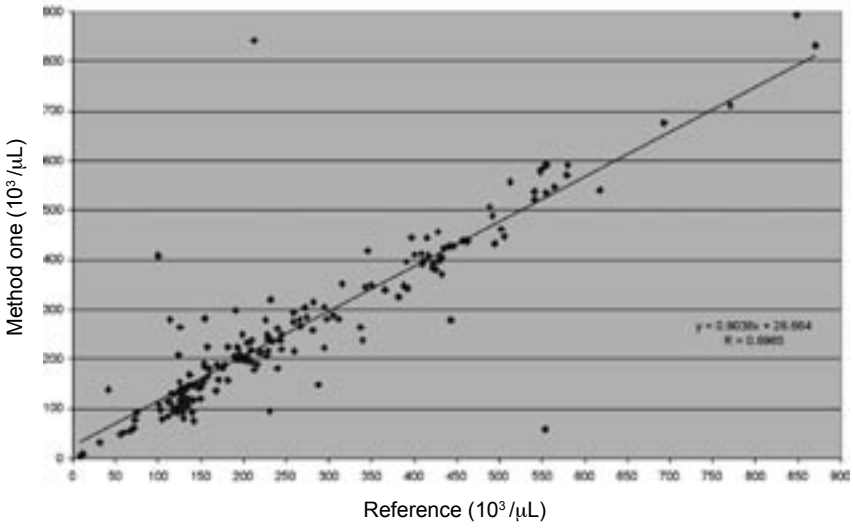
The alternate platelet count estimation method gave a mean platelet count of 155 x10^3/μL, with a mean difference of 113 x10^3/μL, and a mean absolute difference of 119 x10^3/μL. The large average absolute difference shows that the alternate platelet count estimation method tends to give values that are very different from the automated counts. There is a significant difference in means between the alternate estimates and automated counts by the T-test ($p < 0.0001$). The scatterplot for this comparison gives a lower correlation coefficient of 0.81 with greater scatter of data, showing the relationship between alternate estimates and automated counts is weaker and less linear than the relationship between the traditional estimates and automated counts.

In addition, a T-test was performed to compare the platelet counts for the traditional versus alternate estimation methods. The T-test shows a significant difference between the two methods with a $p < 0.0001$. The scatterplot between the two estimation methods gives a correlation coefficient of 0.92. These values show that there is a strong relationship between the two methods, but the linear regression equation indicates that the values the two methods produced on the samples are considerably different.

In the analyses by group it was found that for all six groups (Table 3), the T-tests performed comparing the traditional estimation method with the automated counts were not significant at $p < 0.05$, while the T-tests comparing the alternate estimation method with the automated count and comparing the two estimation methods with each other were all significant at $p < 0.05$. As with the whole dataset, the alternate platelet count estimate was lower than

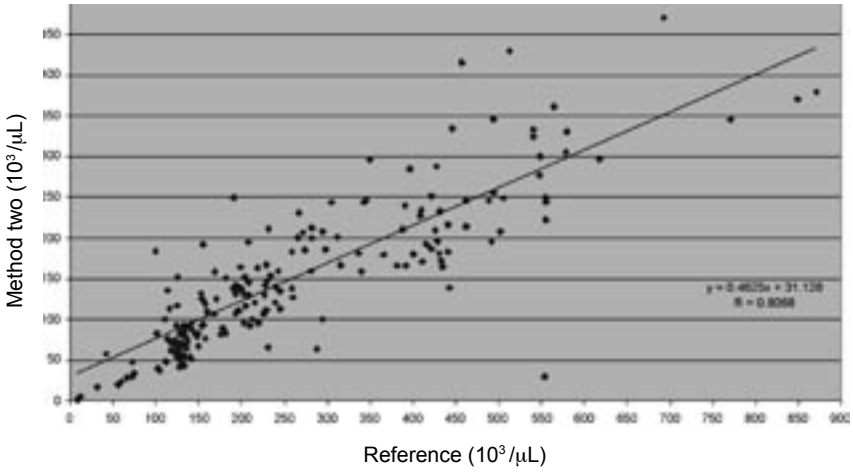
either the traditional platelet count estimate or the automated count, while the traditional platelet count estimate was considerably closer to the automated count. The difference in means between the traditional estimation method and the automated count varied from -7 to +28 for the six groups, while the difference in means between the alternate estimation method and the automated count varied from +14 to +268. Interestingly, the group with high platelet count and low hemoglobin value (group 5) had the greatest difference in mean from the automated count, as compared to the other five groups, for both the traditional and alternate estimation methods. In looking at the groups with low hemoglobin (groups 1, 3, 5) versus normal hemoglobin (groups 2, 4, 6), there were no consistent findings to indicate low hemoglobin groups having consistently lower or higher differences from the automated count mean for either platelet estimation method. The same held true in comparing the groups with low, normal and high platelet counts.

Figure 1. Reference vs. method one



All values are x $10^3/\mu L$
Method one is traditional platelet count estimate
Reference is automated platelet count

Figure 2. Reference vs. method two



All values are x $10^3/\mu L$
Method two is alternate platelet count estimate
Reference is automated platelet count

DISCUSSION

Based on the study results, the traditional method for performing platelet count estimates on blood smears gives estimates that are not significantly different from the counts by the automated method on the Coulter LH750 based on paired T-test analysis at $p < 0.05$. The platelet count estimations with the traditional method in general are slightly higher than the automated count, but are accurate enough to provide platelet count estimates from peripheral blood smears to use in quality assurance. The alternate platelet count estimation method gives estimates that are significantly lower than the automated counts on the Coulter LH750 and significantly lower than the traditional platelet count estimates, based on paired T-test analyses at $p < 0.05$. These results hold true when the data are compared by hemoglobin value as low or normal and by platelet count as low, normal, or high.

The results from this study are different from those found in an earlier study by Torres and Velez[2] upon which this study design was based. The Torres and Velez study found automated counts to be more in agreement with the alternate platelet count estimation method than with the traditional method, particularly in patients with low hemoglobin values or high platelet counts. The difference in findings in these two studies may be related to different models of Coulter instrumentation used in the two studies to produce the automated counts or differences in cut-off values used for designating samples with a low hemoglobin value, as this information was not included in the Torres and Velez study publication.[2]

The research performed by the authors of this study attempted to limit extraneous factors while increasing the accuracy of the data collected and analysis performed.

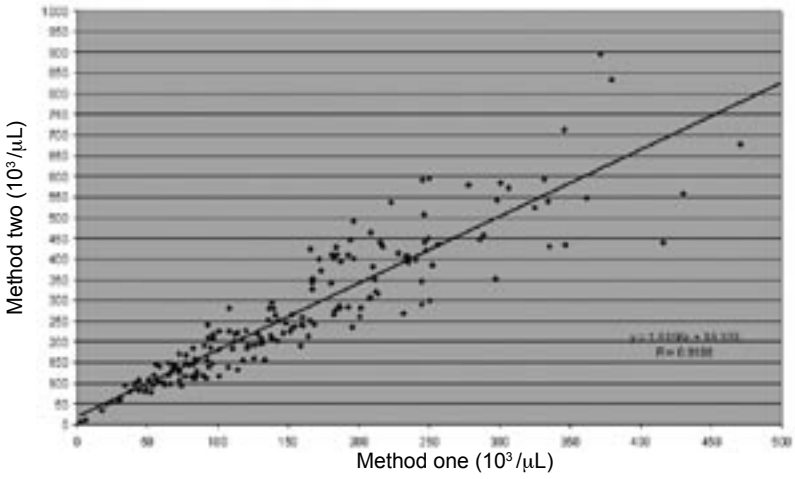
Table 3. Descriptive statistics for the subgroups based on the methodology

	Group 1 (N=40)*		Group 2 (N=18)*		Group 3 (N=40)*		Group 4 (N=40)*		Group 5 (N=39)*		Group 6 (N=7)*	
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
Automated platelet count	107	38	127	13	249	77	236	56	506	112	512	93
Traditional platelet count estimate	121	73	118	19	266	122	242	57	479	140	496	103
Differences	-14	65	9	22	-17	107	-7	47	28	86	16	32
Absolute value	33	58	18	14	45	99	31	35	43	79	29	18
Alternate platelet count estimate	61	35	86	16	144	79	173	45	238	238	373	72
Differences	46	37	41	21	106	84	63	46	268	83	139	65
Absolute value	54	23	41	21	122	58	68	38	268	83	139	65
Traditional estimate to automated count	T-test $p = 0.17$		T-test $p = 0.10$		T-test $p = 0.33$		T-test $p = 0.38$		T-test $p = 0.05$		T-test $p = 0.24$	
Alternate estimate to automated count	$p < 0.0001$		$p < 0.0001$		$p < 0.0001$		$p < 0.0001$		$p < 0.0001$		$p = 0.00136$	
Traditional estimate to alternate estimate	$p < 0.0001$		$p < 0.0001$		$p < 0.0001$		$p < 0.0001$		$p < 0.0001$		$p = 0.00192$	

* All values are x $10^3/\mu L$

"Differences" are the differences between the automated count and the specific platelet count estimation method

Figure 3. Method one vs. method two



All values are x $10^3/\mu L$
Method one is traditional platelet count estimate
Method two is alternate platelet count estimate

A few limitations of the study are worth noting. The main limitation in performing group analyses was the difference in sample sizes for the groups, which prevented more refined analyses such as by covariance. Blood samples for the groups with high and low platelet count but with normal hemoglobin value were more difficult to obtain within the time frame of the study, thus resulting in these two groups having considerably smaller sample sizes (N = 7 and N = 18) than the other groups that had 39 or 40 each. Also, this research was based on the patient population of an academic medical center that is also a tertiary referral center. Results from clinical laboratories serving non-similar patient populations could vary from those the authors found.

CONCLUSIONS

The results of this study support clinical laboratories' continued use of the fairly widespread and accepted method of performing platelet count estimates from Wright's stained peripheral blood smears by counting the number of platelets in ten oil immersion fields, determining the average number per field, and multiplying the average number by 20,000 to produce an estimate per microliter. An alternate method of multiplying the average number of platelets per oil immersion field by the patient's hemoglobin in grams per deciliter multiplied by 1000 does not appear to be warranted based on the study results. Clinical laboratory professionals should feel confident in using the traditional multiplication factor of 20,000 for their platelet estimates for comparison to automated platelet counts as one measure of quality assurance.

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