

LeukoChek Validation Study—WBC & Platelets

The BMP LeukoChek is used to measure and dilute whole blood for manual counting of leukocytes (WBC) and platelets. A validation study was performed to compare WBC and platelet counts obtained with the LeukoChek and an automated hematology analyzer. Since automated hematology instruments are commonly used in clinical laboratories, the method comparison study used automation hematology counts as the reference method. Results were analyzed using existing performance limits for professional use according to CLIA regulations.

Specifications

Method comparisons for clinical laboratory validation often use CLIA proficiency testing criteria in setting acceptance values. These values can be found in the code of federal regulations (42 CFR Part 493). Listed acceptance criteria are 15% for WBCs and 25% for platelets. This means that the average of two manual readings on the hemocytometer must be within 15% of the average reading of the comparator method for WBC counts and within 25% for platelet counts for at least 95% of the test samples.

Materials and Methods

Twenty-three (23) citrated whole blood samples collected for routine hematological testing were used for this study. Three samples were excluded from data analysis because they did not meet inclusion criteria, therefore a total of twenty (20) samples were included in the analysis. An attempt was made to include samples with low, normal, and high values for WBC and platelet counts.

Each sample was tested on a Sysmex XE 2100 automated hematology analyzer in duplicate and white blood cell (WBC) and platelet readings were recorded. Each sample was then prepared for testing using LeukoChek vials.

Because manual counting methods are subject to user error, clinical protocols require duplicate manual counts. The average is then reported to the clinician. To further address user error, many laboratories also impose acceptance criteria for duplicate counts. If counts are vastly different, even an average value might be suspect. Therefore, if duplicate counts vary by more than 10%, the entire process is repeated. By imposing these acceptance criteria, the laboratory is able to reduce the impact of user error on the final result and has greater confidence in the result that is reported to the clinician.

To assess whether LeukoChek is comparable to an automated hematology instrument for WBC and platelet counts, it is also important to avoid potential user error. Therefore, Biomedical Polymers also used the 10% acceptance criteria for inclusion of data points in the LeukoChek validation study. Three (3) samples of twenty-three (23) did not meet inclusion criteria for this study because duplicate counts differed by more than 10%. Therefore, a total of twenty (20) samples were included in data analysis.

Calculations for WBC and Platelets

Automated WBC and platelet counts were chosen for the reference method because hematology results are commonly obtained using automated instruments. To validate that the LeukoChek method is comparable to the automated method for WBC and platelet counts, the average of two counts for each method was calculated.

A WBC count is performed under 100x magnification. To obtain the number of WBCs per μL , all nine large squares of the hemocytometer counting chamber are used. An average of two raw counts is calculated and 10% is added to that number. The total number is then multiplied by 100.

A platelet count is performed under 430x magnification. To obtain the number of platelets per μL , all 25 squares in the large centerpiece of the hemocytometer are counted. To get the total platelet count, the number counted is multiplied by 1000.

Calculations for Total Allowable Error

The formula for calculation of total allowable error is $(y-x)/\text{ATE}$ where y is equal to the LeukoChek count and x is equal to the automated count. For example, sample 1 WBC manual count less automated count = $(1,127.5 - 1,116.50) = 11$. 15% of the automated count = $1,116.50 \times .15 = 167$. This means that the difference would have to be less than 167 to be acceptable. Since 11 is less than 167, the WBC count for sample #1 is within the Allowable Total Error of 15% for WBCs.

Results are plotted using a comparison plot where the automated WBC or platelet results are on the x axis and the LeukoChek results are plotted on the y axis. A regression line shows the relationship between the methods and helps identify discrepant results. This graph also enables one to see the analytical range of data points, and the linearity of results over that range.

An error index chart is also shown where the automated results are on the x axis and the ATE value plotted on the y axis. The scatter plot displays values around the line of zero differences. Because the ATE value plotted is based on acceptable range of 15% for WBCs and 25% platelets, only those values plotted between 1 and -1 are within acceptable range.

Results

Twenty-three total samples were evaluated in this study. Three samples did not meet inclusion criteria; therefore twenty samples were available. Total allowable error was calculated using acceptance criteria of 15% for WBCs and 25% for platelets. Samples included in the study represented high (10,720/ μL) and low (504/ μL) WBC counts and high (312,000/ μL) and low (39,500/ μL) platelet counts. The ATE range for WBCs was -0.70 to 0.94 and for platelets the ATE range was -0.83 to 0.97. All twenty samples for both WBCs and platelets were within acceptable total error ranges.

Average calculated WBC and platelet counts per μL of blood as well as ATE values are shown in Table 2.

The following lots of LeukoChek vials were used:

Table 1. Reagents and Equipment

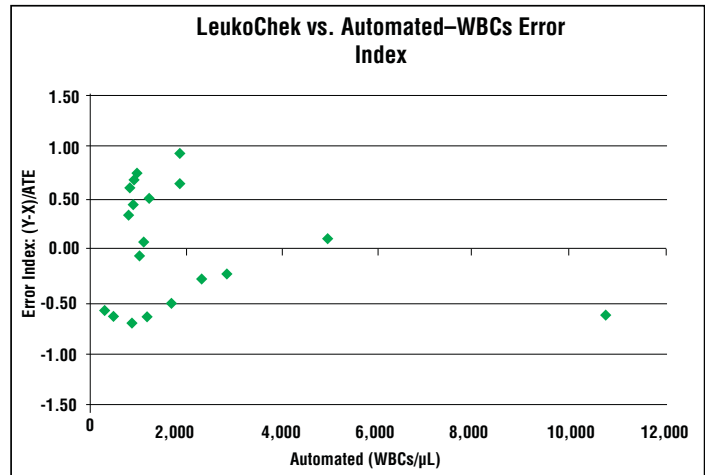
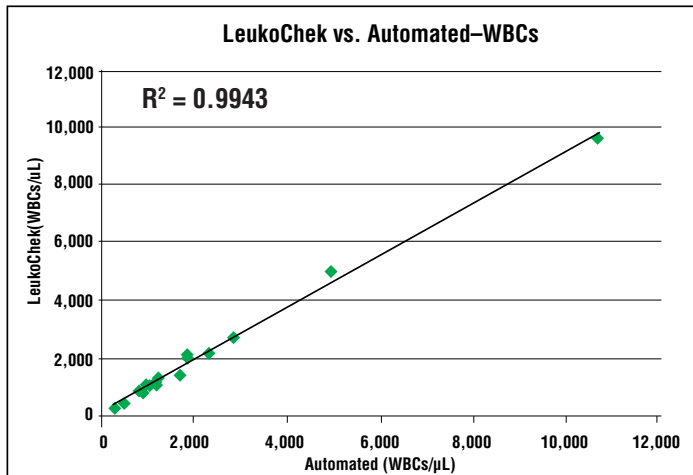
Materials	Lot #	Expiration Date
LeukoChek	2T30C354	5/20/11
LeukoChek	2T30C355	5/20/11
LeukoChek	2T30C356	5/20/11

Table 2. Average Calculated Counts WBC and Platelets

Sample Number	Automated		LeukoChek		Allowable Total Error ¹	
	WBC	Platelet	WBC	Platelet	WBC	Platelet
1	1,116.50	173,000	1,127.50	187,000	0.07	0.32
2	925.10	257,500	1,017.50	281,500	0.67	0.37
3	903.65	39,500	808.50	42,500	-0.70	0.30
4	984.50	196,500	1,094.50	195,000	0.74	-0.03
5	307.45	163,000	280.50	129,000	-0.58	-0.83
6	924.55	277,000	1,017.50	258,500	0.67	-0.27
7	1,859.00	156,000	2,040.50	182,000	0.65	0.67
8	891.55	312,500	951.50	320,500	0.44	0.10
9	1,237.50	185,000	1,331.00	207,500	0.50	0.49
10	852.50	118,500	929.50	127,000	0.60	0.29
11	504.35	232,000	456.50	262,000	-0.63	0.52
12	816.20	223,000	858.00	240,500	0.34	0.31
13	1,196.80	239,500	1,083.50	276,500	-0.63	0.62
14	4,955.50	97,500	5,032.50	104,500	0.10	0.29
15	1,875.50	45,500	2,139.50	56,500	0.94	0.97
16	1,710.50	93,500	1,430.00	89,000	-0.52	-0.19
17	10,720.00	185,500	9,700.00	186,500	-0.63	0.02
18	2,321.00	228,500	2,227.50	240,500	-0.27	0.21
19	2,860.00	90,000	2,755.50	92,000	-0.24	0.09
20	1,042.30	249,500	1,034.00	268,000	-0.05	0.29
Average					0.07	0.23
Max					0.94	0.97
Min					-0.70	-0.83

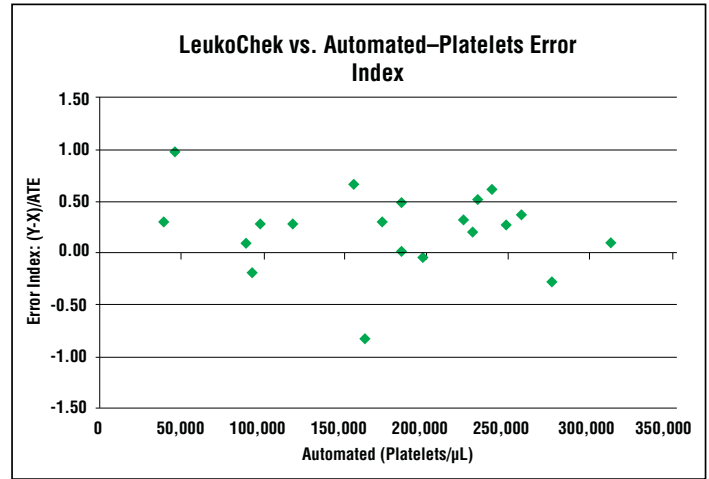
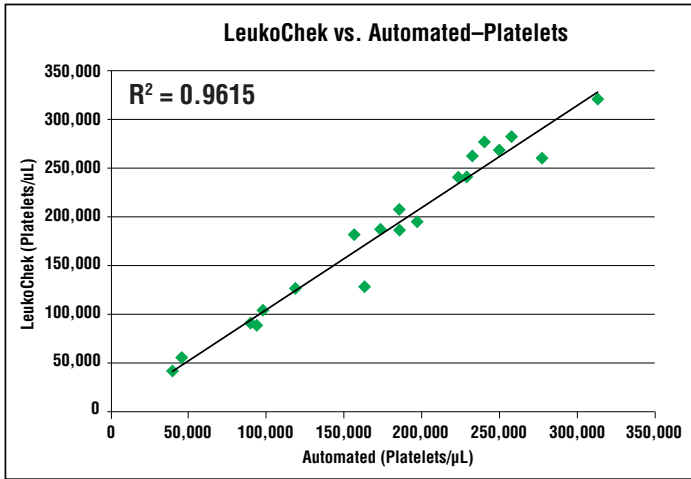
¹ (y-x)/ATE: ATE=15% WBC, ATE=25% Platelet; y = LeukoChek; x = Automated

Comparison and Index Plots for WBCs





Comparison and Index Plots for Platelets



Conclusions

All 20 samples were within the allowable total error acceptance criteria of 15% for white blood cell counts and 25% for platelet counts when comparing the manual LeukoChek method to an automated hematology counter—the Sysmex.

An R^2 value of 1 represents a perfect linear relationship between two variables. The R^2 value for WBC is 99.4% and for platelets the R^2 value is 96.1%. Both tests demonstrate a strong correlation between the LeukoChek and automated methods.

Regression Analysis For Platelet and WBC Values

Linear Regression

Regression Statistics

R	0.98056
R Square	0.96149
Adjusted R Square	0.95935
S	16,587.09376
Total number of observations	20

$$A = 1315.3107 + 1.0441 * B$$

ANOVA

	<i>d.f.</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>p-level</i>
Regression	1.	1.2365E+11	1.2365E+11	449.42214	3.51941E-14
Residual	18.	4,952,370,231.34052	275,131,679.51892		
Total	19.	1.28603E+11			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>LCL</i>	<i>UCL</i>	<i>t Stat</i>	<i>p-level</i>	<i>HO (2%) rejected?</i>
Intercept	1,315.31067	9,525.93597	-22,998.49425	25,629.11559	0.13808	0.89171	No
B	1.04412	0.04925	0.91841	1.16983	21.19958	3.51941E-14	Yes
T (2%)	2.55238						

LCL - Lower value of a reliable interval (LCL)

UCL - Upper value of a reliable interval (UCL)

Residuals

<i>Observation</i>	<i>Predicted Y</i>	<i>Residual</i>	<i>Standard Residuals</i>
1	181,947.79034	5,052.20966	0.31293
2	270,175.79342	11,324.20658	0.70142
3	42,557.98667	-57.98667	-0.00359
4	206,484.57227	-11,484.57227	-0.71135
5	171,506.60655	-42,506.60655	-2.63285
6	290,536.10183	-32,036.10183	-1.98431
7	164,197.77789	17,802.22211	1.10267
8	327,602.3043	-7,102.3043	-0.43992
9	194,477.2109	13,022.7891	0.80663
10	125,043.33866	1,956.66134	0.1212
11	243,550.77474	18,449.22526	1.14274
12	234,153.70933	6,346.29067	0.39309
13	251,381.66259	25,118.33741	1.55583
14	103,116.85268	1,383.14732	0.08567
15	48,822.69694	7,677.30306	0.47553
16	98,940.37917	-9,940.37917	-0.61571
17	194,999.27009	-8,499.27009	-0.52644
18	239,896.36041	603.63959	0.03739
19	95,285.96484	-3,285.96484	-0.20353
20	261,822.84639	6,177.15361	0.38261