

White Blood Cell and Platelet Counting Performance by Hematology Analyzers: A Critical Evaluation

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ABSTRACT

The platelet and leukocyte counts obtained from 3 clinical hematology analyzers (Coulter Gen•S, Bayer ADVIA 120, and Abbott CellDyn 4000) and from 1 instrument in the final phases of development (Coulter LH 750) were compared with counts obtained using flow cytometric methods. We studied samples from 3 subject populations: a platelet control group of 30 subjects with normal or near-normal hematological values, a group of 31 patients with platelet counts less than $30 \times 10^9/L$, and a group of patients with conditions known to affect leukocyte counts. This group included 10 patients with sickle cell disease, 10 with presumed thalassemia, and 10 with renal and/or liver disease. In the platelet control group, the differences between counts obtained using the flow cytometric method and clinical analyzers were of little clinical significance. According to results of a paired *t* test, the samples from patients with low platelet counts showed that 3 of the 4 analyzers had a positive bias for platelet counts. This bias can be clinically important because it may lead to withholding platelet transfusions from thrombocytopenic patients. All 4 analyzers counted low numbers of platelets with good accuracy but with different flagging patterns. Our limited data suggest that for routine analyzers a platelet count of $15 \times 10^9/L$ or lower may be the most suitable value to correctly identify patients who need transfusions (assuming a reference platelet count of $\leq 10 \times 10^9/L$ as

a true cutoff). The third sample set, from patients with conditions that potentially cause interference in the white blood cell count, showed different patterns, with the analyzers flagging 0% to 60% of the samples from sickle cell patients, 0% to 30% of the samples from thalassemia patients, and 0% to 20% of the samples from patients with liver/renal disease. *Lab. Hematol.* 2001;7:255–266

KEY WORDS: Platelets · White blood cell · Hematology analyzers

INTRODUCTION

Hematology instruments are expected to give accurate and reproducible results for a wide variety of clinical conditions and to provide these results without unnecessary delay. With their ability to measure large numbers of cells in a highly automated manner, modern analyzers have, in general, adequately addressed precision. However, there have been issues concerning accuracy. For example, it is well known that platelet results may be elevated due to cellular fragments or microcytes [1-6]. Also, there may be difficulties in obtaining valid white blood cell (WBC) counts due to incomplete lysis of erythrocytes, the presence of nucleated red blood cells (NRBCs), or other interference. These difficulties can occur in neonates; in patients with certain hemoglobinopathies, such as sickle cell disease, and with thalassemia syndromes [7,8]; and in patients with liver and/or renal disease [7]. Whenever these conditions are present, there is the potential for inaccurate results and/or the need for operator intervention, leading to delays in releasing results.

To overcome or minimize these problems, manufacturers specifically design their instrument and reagent systems to prevent or correct for interference. Furthermore, they develop algorithms to minimize these problems or at least to alert the

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TABLE 1. Formulas for Screening for Thalassemia Trait*

Investigators	Formula
England and Fraser [14]	$MCV - [RBC(5 \times Hb)] - 3.4$
Ricerca et al [15]	RDW/RBC
Eldibany et al [16]	Fisher linear discriminant equations

*MCV indicates mean corpuscular volume; RBC, red blood cells; Hb, hemoglobin; RDW, red cell distribution width.

user to potentially incorrect results. These algorithms are developed and verified by comparing results to manual, flow cytometric, or other reference methods. Manual methods, however, do not use a sample size as large as that of most automated methods and thus cannot be as accurate and precise. Flow cytometric methods using specific antibodies and sampling large numbers of cells can be both accurate and precise.

Recently, we had the opportunity to use flow cytometric methods for platelet and WBC counting and to compare these results with those obtained from 3 hematology clinical analyzers, the Coulter Gen•S (Beckman Coulter, Miami, FL), the CellDyn 4000 (Abbott Laboratories, Abbott Park, IL), and the ADVIA 120 (Bayer Diagnostics, Tarrytown, NY). In addition, we evaluated a clinical instrument in development, the Coulter LH 750 (Beckman Coulter). This analyzer uses new algorithms for its impedance platelet count and, according to the manufacturer, is also less sensitive to interference in WBC counts. The LH 750 analyzer enumerates NRBCs on each sample and corrects the WBC count automatically. Unfortunately, the analyzer we tested was a prototype model and was not yet equipped with software to supply us with NRBC counts, although WBC count corrections were carried out.

We focused on 2 critical factors. The first was the accuracy of analyzer platelet counts from patients with platelet counts of less than $30 \times 10^9/L$. Recent recommendations that "trigger" values to initiate platelet transfusions could be lowered from 20 to $10 \times 10^9/L$ [9,10], and even to $5 \times 10^9/L$ [11], have put new demands on the accuracy and precision of clinical hematology analyzers. We compared all analyzer counts with a recently published reference method for flow

cytometric platelet counting [12,13] and compared instrument flags. The latter is of importance for a result to be reportable: a high number of flagged platelet counts requires reviews and may lead to additional testing. Such instrument flags almost certainly will result in reporting delays and higher costs. The second factor was possible interference in the leukocyte count in clinical conditions reported to have hard-to-lyse erythrocytes and other interferences, ie, sickle cell disease, thalassemia, and liver and renal disease.

METHODS AND MATERIALS

Blood Specimens

Three groups of samples were obtained over a 5-week period as residual material from the clinical hematology laboratory that services the wards and clinics of a large public hospital. The first set was of 30 samples with near-normal to normal erythrocyte ($4-6 \times 10^{12}/L$), leukocyte ($3-11 \times 10^9/L$), and platelet ($100-450 \times 10^9/L$) counts, as initially determined in the routine clinical hematology laboratory. This group was designated as the control group. A second group of 31 specimens consisted of samples reported by the clinical laboratory as having platelet counts of less than $30 \times 10^9/L$.

The final group of 30 specimens consisted of samples that were selected to test the ability of the algorithms of the various instruments to handle samples with potential red cell lysis problems that might interfere with the WBC count. These included 10 samples from patients with a history of sickle cell disease who either were admitted in crises or were receiving transfusions on a regular basis. Samples used had greater than 45% hemoglobin S as measured using the Variant hemoglobin testing system (Bio-Rad, Hercules, CA). A second subgroup of 10 samples was from patients with microcytosis (mean corpuscular volume, ≤ 79 fL) and RBC indices that met all 3 of the screening algorithms for thalassemia, as defined by the formulae of England and Fraser [14], Ricerca et al [15], and Eldibany et al [16] (Table 1). None of these patients had recently received transfusions. The final 10 specimens were samples from patients with renal and/or liver disease, conditions that have been shown in the past to cause WBC count interference problems (Berend Houwen, personal communication, 2001).

TABLE 2. Descriptive Statistics, Paired *t* Test, and Regression Analysis of the Control Group for Platelets

	No.	Group Mean (Range)	P*	Regression Analysis			S _{y x} †
				Slope	Intercept	R ²	
Flow cytometer	30	253.29 (119.23-351.75)					
LH 750	30	248.85 (123.17-338.77)	0.074	0.945	9.380	0.938	13.02
Gen•S	30	230.17 (113.30-322.50)	<0.001	0.875	8.466	0.898	15.78
ADVIA 120	30	240.80 (131.00-336.00)	0.007	0.898	13.373	0.810	23.19
CellDyn 4000/optical	30	242.93 (119.00-346.00)	0.002	0.905	13.716	0.903	15.86

*Paired *t* test; not significant, $P > .10$.

†Standard error of estimate.

TABLE 3. Descriptive Statistics, Paired *t* test, and Regression Analysis of the Control Group for Leukocytes*

	No.	Group Mean (Range)	<i>P</i> †	Regression Analysis			<i>S_{y x}</i> ‡
				Slope	Intercept	<i>R</i> ²	
Flow cytometer		7.81 (2.94-10.89)					
LH 750	30	7.88 (3.07-10.70)	NS	0.981	0.225	0.960	0.40
Gen•S	30	7.51 (3.08-10.11)	<0.001	0.926	0.286	0.958	0.39
ADVIA 120	30	7.67 (3.05-10.29)	NS	0.925	0.447	0.948	0.43
CellDyn 4000/optical	30	7.58 (3.03-10.4)	0.003	0.938	0.262	0.962	0.37

*NS indicates not significant.

†Paired *t* test; NS, *P* > 0.10.

‡Standard error of estimate.

All samples were the residuals of patient samples collected for clinical testing purposes in 4 mL Vacutainer plus tubes with 7.2 mg K₂EDTA (Becton Dickinson, Franklin Lakes, NJ). No samples were collected specifically for this study, and no subject was used twice. Every specimen was run once on all 4 hematology analyzers and on the flow cytometer within 24 hours after collection. If the sample was insuffi-

cient or if a clot was detected for any analyzer, the specimen was not included in the study.

Hematology Analyzers

Three routine clinical hematology analyzers, the Coulter Gen•S analyzer, the Abbott CellDyn 4000 analyzer, and the Bayer ADVIA 120 analyzer were used in the study. In this

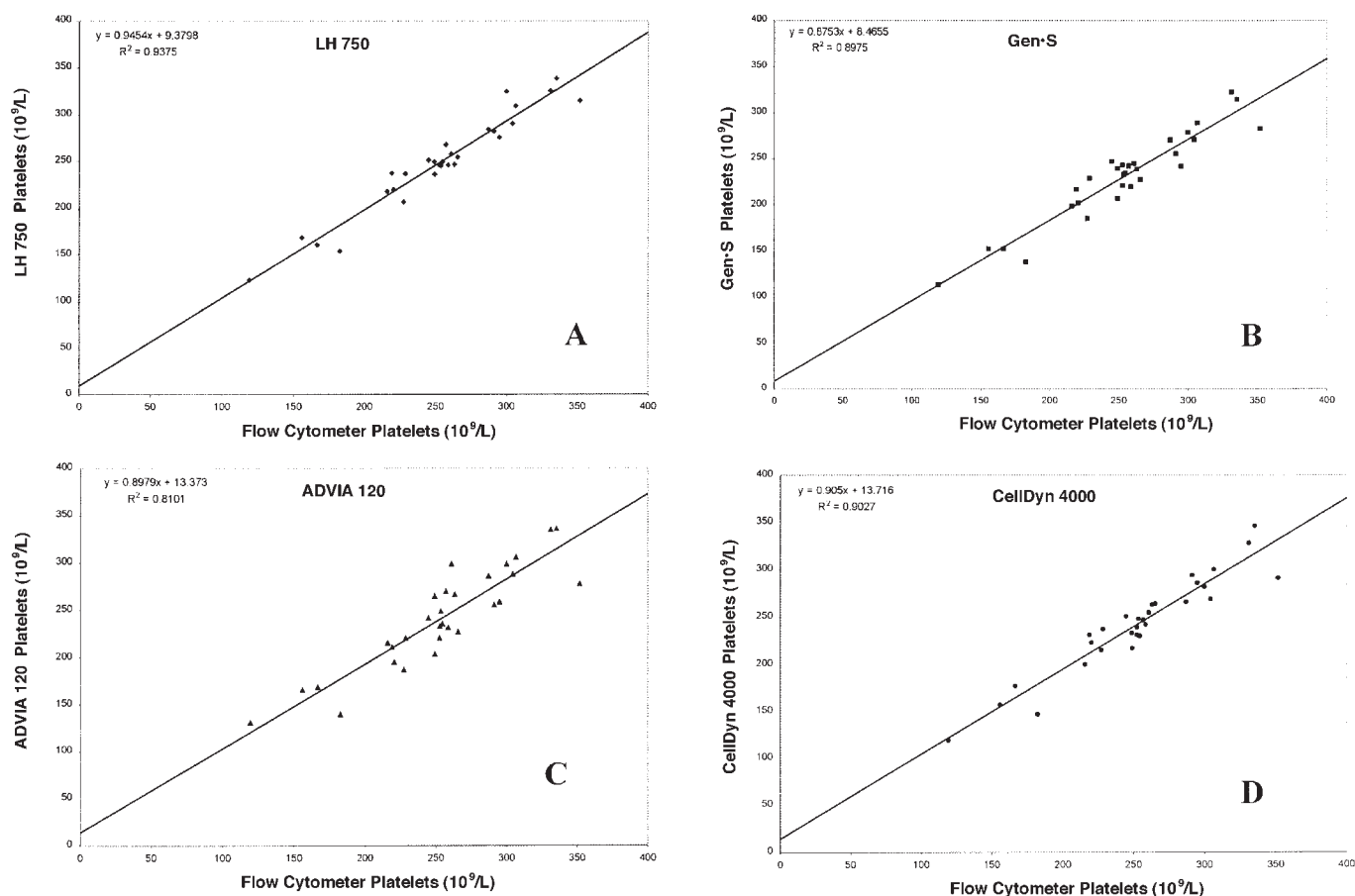


FIGURE 1. Comparison of control-group platelet count results from 4 hematology analyzers versus results from the flow cytometric method: A, LH 750; B, Gen•S; C, ADVIA 120; D, CellDyn 4000.

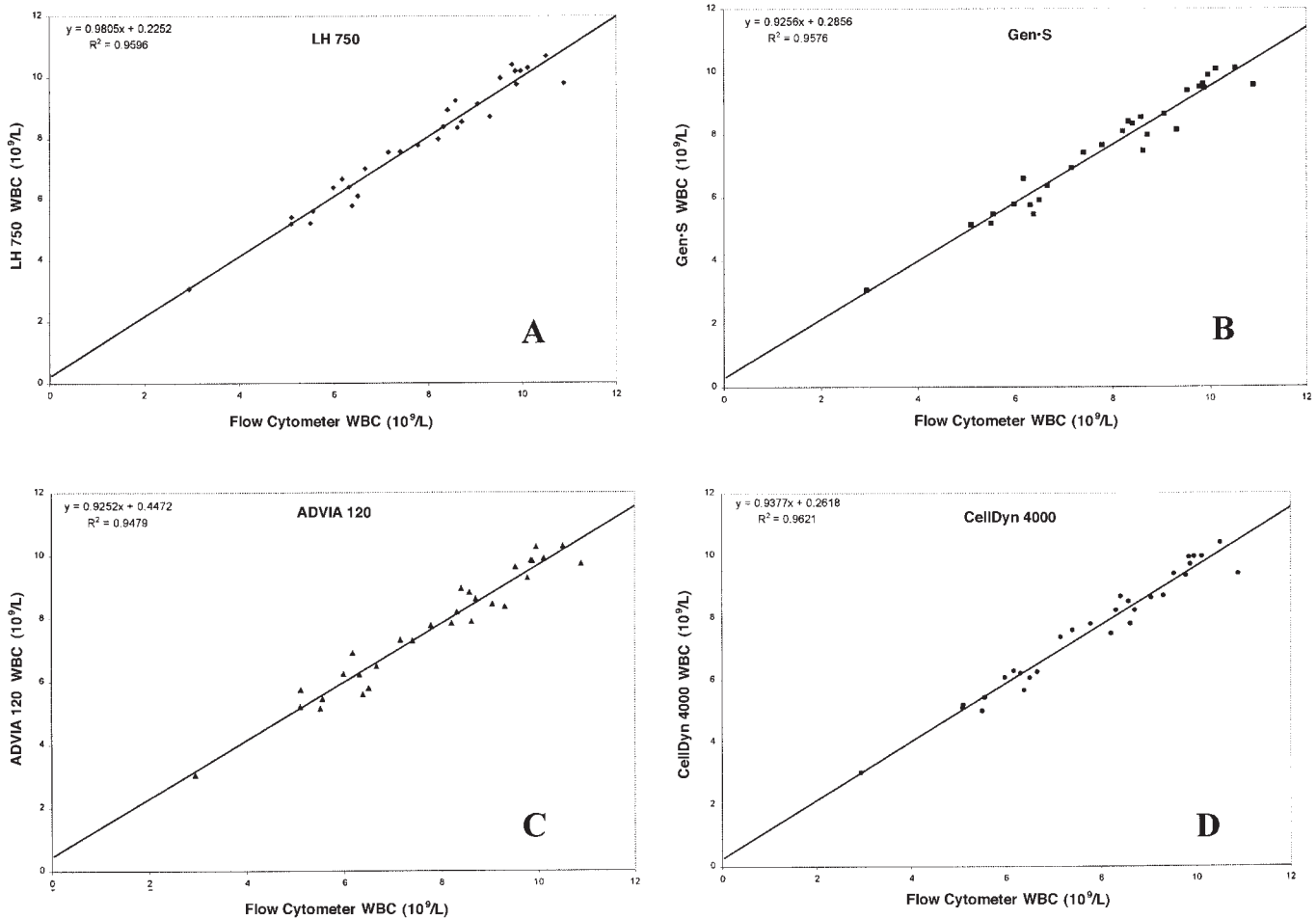


FIGURE 2. Comparison of control-group leukocyte count results from 4 hematology analyzers versus results from the flow cytometric method: A, LH 750; B, Gen•S; C, ADVIA 120; D, CellDyn 4000. WBC indicates white blood cell.

study, we used only the optical platelet count on the Cell-Dyn 4000, because we found low-level impedance platelet counts on this instrument to be unreliable. Also available to the investigators was an instrument in the final phase of development, the Coulter LH 750 analyzer. In this study, we compared the impedance platelet and WBC counts from the 2 Coulter analyzers and the optical platelet and WBC counts from the CellDyn 4000 and the ADVIA 120 analyzers with the reference methods for platelet and WBC counts. All instruments were operated with manufacturers' reagents and protocols for calibration and quality control.

Flow Cytometric Methods

The flow cytometric analyses were performed at the Beckman Coulter facility in Miami, Florida, using a Coulter EpicsXL. The flow cytometer operators were blinded to the results from the hematology analyzers. The platelet count was determined using a new reference method proposed by the International Society of Laboratory Hematology and the

International Council for Standardization in Haematology [12,13]. In this method, the platelets are labeled with 2 monoclonal antibodies, CD41-FITC and CD61-FITC (both antibodies from Immunotech, Marseilles, France), to assure identification of all platelets. RBCs were discriminated from platelets based on fluorescent and light-scatter signals. A ratio between RBCs and platelets was determined for each sample, and the final platelet count was determined by multiplying the platelet/RBC ratio by an RBC count obtained from a Beckman Coulter Gen•S.

A modification of the method of Schlenke et al [17] was used for the enumeration of leukocytes by flow cytometry. Leukocytes were identified by mixing an aliquot of whole blood with a combination of CD45-PC5 (leukocyte common antigen) and CD41-FITC (platelet surface antigen), both antibodies from Immunotech. Flow-count fluorospheres (Beckman Coulter) were added for quantitative leukocyte count. Leukocytes were counted after RBC lysis with ammonium chloride lysing solution.

TABLE 4A. Descriptive Statistics, Paired *t* test, and Regression Analysis of Low-Platelet Group*

Instrument	No. (Range)	Group Mean, Flow Cytometer†	Group Mean, Hematology Analyzer	P‡	Regression Analysis			S _{Y X} §
					Slope	Intercept	R ²	
Flow cytometer	31 (0.62-30.99)	14.97						
LH 750	19 (10.08-27.95)	17.92	21.04	<0.001	0.936	4.27	0.862	2.188
Gen•S	30 (2.20-25.50)	15.10	14.52	NS	0.863	1.49	0.842	3.109
ADVIA 120	25 (2.00-30.00)	14.38	17.40	<0.001	0.956	3.66	0.924	2.305
CellDyn 4000/optical	24 (3.05-30.90)	16.18	18.51	<0.001	1.012	2.14	0.878	2.596

*Results from flagged samples were deleted from analysis (group composition varies per analyzer). NS indicates not significant.

†Per analyzer; nonflagged results only.

‡Paired *t* test; NS, *P* > 0.10.

§Standard error of estimate.

||Results requiring review were included.

TABLE 4B. Descriptive Statistics for All Samples in Low-Platelet Group, Unless Reanalysis Was Required by Analyzer*

Instrument	No. (Range)	Group Mean, Flow Cytometer	Group Mean, Hematology Analyzer	P†	Regression Analysis			S _{Y X} ‡
					Slope	Intercept	R ²	
Flow cytometer	31 (0.62-30.99)	14.97						
LH 750	30 (3.65-27.95)	14.43	17.07	<0.001	1.005	2.57	0.929	2.160
Gen•S§	30 (2.20-25.50)	15.10	14.52	NS	0.863	1.49	0.842	3.109
ADVIA 120	31 (2.00-31.00)	14.97	18.35	<0.001	0.978	3.72	0.926	2.262
CellDyn 4000/optical	29 (3.05-30.90)	14.86	17.30	<0.001	1.015	2.21	0.905	2.475

*Table includes results that were flagged for review (groups are more uniform). NS indicates not significant.

†Paired *t* test; NS, *P* > 0.10.

‡Standard error of estimate.

§Results requiring review were included.

Statistical Analysis

For statistical analysis, paired *t* tests, and correlation and regression analyses we used Microsoft Excel 98 (Redmond, WA).

RESULTS

Tables 2 and 3 show descriptive statistics of the control group for platelets and leukocytes as determined by flow cytometric analysis and by the 4 hematology analyzers. Also included in the tables are the regression analysis, correlation coefficients, and standard error of estimate of the various hematology analyzers versus the flow cytometer. Figures 1 and 2 show the results of the analyzers versus the flow cytometer. Table 4A and Figure 3 show similar analyses for the low-platelet group. Except for the Gen•S analyzer, patient data were deleted from analysis if they had been flagged indicating a result that was suspect or unacceptable. The Gen•S analyzer has an algorithm that essentially flags all specimens with platelet counts of $<20 \times 10^9/L$, and we

decided to include this data unless we deemed the results unacceptable according to the algorithm.

Analyzer flags can indicate that platelet count results may be abnormal or that results need to be reviewed before the low platelet count result can be released. For example, the review policy in our institution to verify an ADVIA 120 analyzer result with a "platelet noise" flag is to examine a smear confirming the platelet count before results are released. Similarly, in our institution a CellDyn 4000 analyzer result with an optical/impedance discordance flag requires a review process that includes a delta check of the previous sample. (As stated earlier, for the purpose of this study we used only the optical platelet results from the CellDyn 4000 analyzer.) Table 4B and Figure 4 contain the results from all analyzers that were flagged but were not deemed unacceptable by the analyzer. Typically, these samples may have required some form of review before the laboratory could release the test results. The flagged results showed no outliers when compared with the flow data, once they were included in the data (data not shown). Table 5 contains a tabulation of platelet flags by instrument.

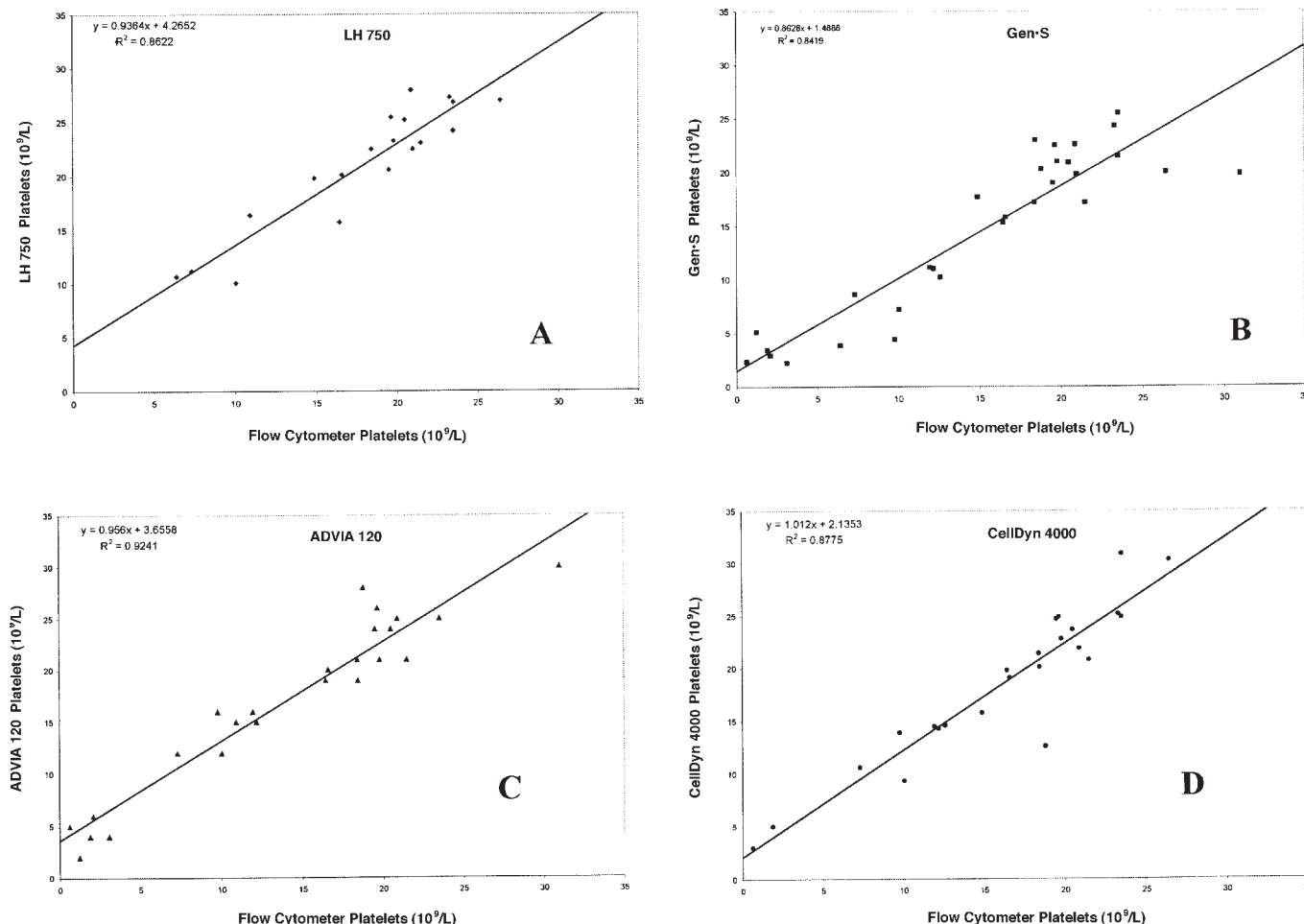


FIGURE 3. Comparison of low-platelet group results from 4 hematology analyzers versus results from the flow cytometric method: A, LH 750; B, Gen•S; C, ADVIA 120; D, CellDyn 4000. Flagged specimens, except for Gen•S, were not included.

Table 6 shows the effect of using reference-value cutoffs of $5, 10, 15,$ and 20×10^9 platelets/L as triggers for initiating platelet transfusions. For example, if a platelet count of 5×10^9 /L is the “reference” trigger, then the LH 750, Gen•S, ADVIA 120, and CellDyn 4000 analyzers would have identified correctly 4, 4, 3, and 3 patients of 5, respectively, in need of platelet transfusion. For this analysis, the flow cytometric data were assumed to be accurate and precise (see shaded area in Table 6).

Tables 7A and 7B represent a similar analysis. However, in these tables it is assumed that the real cutoff platelet count is either 5 or 10×10^9 /L, as determined by reference method, and this cutoff or trigger value is compared with analyzer data on the same samples. To investigate instances of inappropriately not administering transfusions to patients, we evaluated the hematology analyzers at the same or higher trigger values. At a reference trigger value for platelet transfusion of 5×10^9 /L and an analyzer trigger of $<10 \times 10^9$ /L, the analyzers correctly identified all patients who would require platelet transfusion

(Table 7A, shaded areas). One of the analyzers generated a flagged result in a single patient. In no case would an appropriate transfusion be withheld, but variable numbers of patients (1–4) would receive transfusions that were not indicated by the reference trigger. When the reference trigger value was set at 10×10^9 /L and the analyzer value at 15×10^9 /L, 3 analyzers correctly identified all patients in need of transfusion, with the same exception as mentioned earlier (Table 7B, shaded areas). One analyzer showed an unflagged value of 16×10^9 /L versus 9.8×10^9 /L by the reference method. The average number of patients who would have received transfusions inappropriately would have been 4 (range, 2–5). At these reference and analyzer trigger levels, the average number of patients correctly identified as not needing a platelet transfusion would be 19 (range, 17–21) of 29 to 31 total patients.

Table 8 and Figure 5 represent the results for the WBC interference group. Test results that were flagged by the various instruments as needing further evaluation were removed from analysis with 1 exception. ADVIA 120 analyzer results

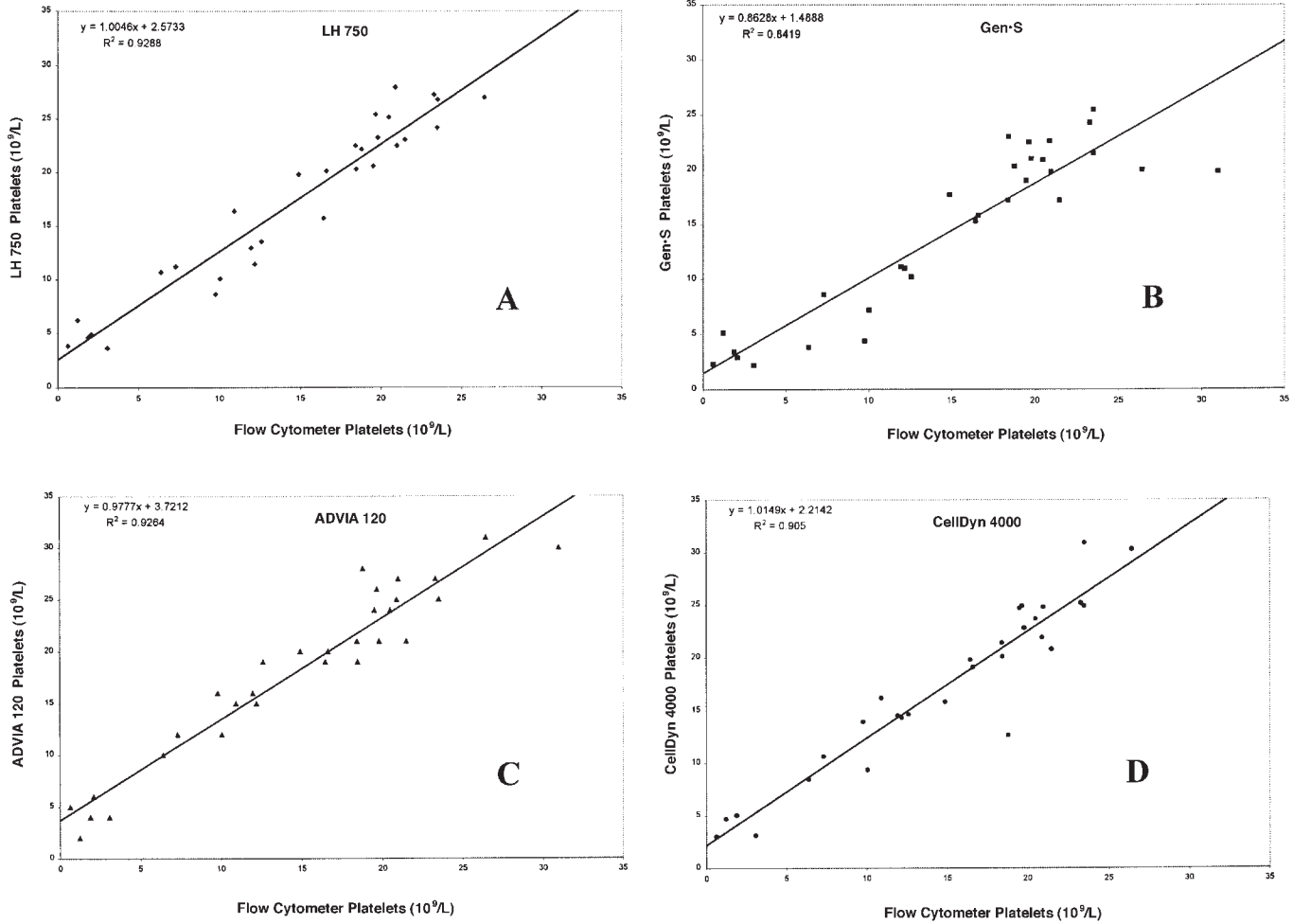


FIGURE 4. Comparison of low-platelet group results from 4 hematology analyzers versus results from the flow cytometric method: A, LH 750; B, Gen•S; C, ADVIA 120; D, CellDyn 4000. Specimens without flags that required reanalysis were included.

that were flagged for NRBCs were included because the manufacturer states that a manual differential should be done on these specimens, and, with the NRBC count thus obtained, a corrected WBC can be calculated. For 7 of the 10 samples identified on the ADVIA 120 analyzer, NRBC counts in blood smears reviewed by the clinical laboratory were used to determine a corrected WBC. In 2 of the remaining samples, the LH 750 and CellDyn 4000 analyzers did not identify nucleated erythrocytes, and a zero count for NRBCs was used in the correction of these ADVIA 120 analyzer results. One sample was excluded from statistical analysis because of other discrepancies in test results. Table 9 is a tabulation of the analyzer flags indicating that further intervention is required before a WBC count can be released.

DISCUSSION

It is important for clinicians to have hematology results that are accurate and without clinically significant bias. This is

especially true in this era of managed care, in which samples from one physician’s office may be sent to several different laboratories using different analyzers. Tables 2 and 3 show that, for the control group, there are statistically significant analyzer biases for both platelets and WBCs, which could be due to calibration. Care was taken that all analyzers were calibrated according to the manufacturers’ instructions, using the calibrator materials appropriate for each analyzer. In the normal to near-normal range, these differences do not appear to be of clinical importance. Furthermore, as Figures 1 and 2 show, there appear to be no anomalies or outliers in the data. Also included in Tables 2 and 3 is the standard error of the estimate ($S_{Y|X}$), which is a measure of the prediction of errors about the regression line, ie, 68% of the data points should fall within 1 $S_{Y|X}$ of the regression line. This statistic reflects the relative imprecision of the instruments studied [18]. There appears to be little difference between the instruments except that the ADVIA 120 analyzer shows relatively high $S_{Y|X}$ for platelets in the normal to near-normal range.

TABLE 5. Number of Results From the Low-Platelet Group Flagged by the Analyzers

	Analyzer			
	LH 750	Gen•S	ADVIA 120	CellDyn 4000/optical
Number of results with flags necessitating review and possible reanalysis	11	30	6	5
Number of results with flags necessitating reanalysis	1	1	0	2

Tables 4A and 4B show the descriptive statistics, paired *t* test, and regression and correlation analyses for the low-platelet group. With the exception of Gen•S analyzer results, all results in Table 4A are from specimens that were not flagged by the analyzers and as such could have been reported directly, without operator intervention. The flags were grouped according to the type of action required: (1) review and possible reanalysis and (2) obligatory reanalysis (Table 5). In Table 4B, the results include data from specimens that were flagged by the analyzers for review but not for reanalysis. It is interesting that for all 3 analyzers that had results excluded on the basis of flagging, the LH 750, ADVIA 120, and CellDyn 4000, the regression and correlation statistics compared to the reference method remain virtually the same (ADVIA 120 analyzer) or improve (LH 750 and CellDyn 4000 analyzers). Either way, with or without flagged specimens included, all analyzers showed excellent correlation with the reference method. Differences in performance between impedance (Gen•S and LH 750 analyzers) and optical platelet counts (ADVIA 120 and CellDyn 4000 analyzers) were shown to be minimal. It thus appears that analyzer flagging is set at quite conservative levels and that perhaps in the future a larger number of

low platelet counts from analyzers could get a “pass” rather than a review flag.

Table 5 shows flagged platelet results per analyzer. These flags are divided into 2 categories. The first consists of results that may be valid but need to be reviewed, and, depending on the type of flag and the outcome of the review, the specimen may have to be reanalyzed, possibly by a different method. The second type indicates that the results are deemed invalid, the result may not even be displayed, and that reanalysis needs to be done. For example, a specimen from a patient with a low platelet count can be flagged because of curve-fitting issues or a high-mean platelet volume (first type of flag). If a delta check of the laboratory record indicates that the patient has had similarly low platelet counts in the past, some institutions may allow this type of result to be released. Flagged results of this nature can be reviewed by manual checking or by computer delta check and can be presented to the technologist or—if certain criteria are met—be released automatically. By automating such procedures, the laboratory can achieve 2 objectives: consistency of the review process and minimization of delays in reporting results. Obviously, if the flag indicates a result that must be rerun or reanalyzed, there will be a delay. Some analyzers, such as the Gen•S analyzer,

TABLE 6. Number of Subjects Identified or Misidentified by Hematology Analyzers at Various Trigger Criteria for Platelet Transfusions*

Trigger value for platelet transfusion ($\times 10^9/L$)	Number of Subjects Hematology Analyzer Correctly Identified as Requiring Transfusions				Number of Subjects Hematology Analyzer Incorrectly Identified as Not Requiring Transfusions				Number of Subjects Hematology Analyzer Incorrectly Identified as Requiring Transfusions				Number of Subjects Hematology Analyzer Correctly Identified as Not Requiring Transfusions			
	<5	<10	<15	<20	<5	<10	<15	<20	<5	<10	<15	<20	<5	<10	<15	<20
Platelet count on flow cytometer†	<5	<10	<15	<20	<5	<10	<15	<20	>5	>10	>15	>20	>5	>10	>15	>20
Platelet count on hematology analyzer	<5	<10	<15	<20	>5	>10	>15	>20	<5	<10	<15	<20	>5	>10	>15	>20
LH 750 (n = 30)	4	6	10	15	1	2	1	7	0	0	0	0	25	22	19	8
Gen•S (n = 30)	4	8	10	17	1	0	1	4	2	1	1	3	23	21	18	6
ADVIA 120 (n = 31)	3	5	8	15	2	3	3	7	1	1	0	0	25	22	20	9
CellDyn 4000/optical (n = 29)	3	5	9	16	1	2	1	5	0	1	1	0	25	21	18	8

*This table shows the effect of using reference-value cutoffs of 5, 10, 15, and 20×10^9 platelets/L as triggers for initiating platelet transfusions. For example (shaded area), if a platelet count of $5 \times 10^9/L$ is the reference trigger, then the LH 750, Gen•S, ADVIA 120, and the CellDyn 4000 analyzers would have identified correctly 4, 4, 3, and 3 patients of 5, respectively, in need of platelet transfusion.

†Flow cytometric data is assumed to be the correct platelet count.

TABLE 7A. Number of Subjects Identified or Misidentified by Hematology Analyzers at Various Trigger Criteria for Platelet Transfusions*

Trigger value for platelet transfusion ($\times 10^9/L$)	Number of Subjects Hematology Analyzer Correctly Identified as Requiring Transfusions					Number of Subjects Hematology Analyzer Incorrectly Identified as Not Requiring Transfusions					Number of Subjects Hematology Analyzer Incorrectly Identified as Requiring Transfusions					Number of Subjects Hematology Analyzer Correctly Identified as Not Requiring Transfusions				
	<5		<10			<5		<10			<5		<10			<5		<10		
	<5	<5	<5	<10	<10	<5	<5	<5	<10	<10	>5	>5	>5	>10	>10	>5	>5	>5	>10	>10
Platelet count on flow cytometer†	<5	<5	<5	<10	<10	<5	<5	<5	<10	<10	>5	>5	>5	>10	>10	>5	>5	>5	>10	>10
Platelet count on hematology analyzer	<10	<15	<20	<15	<20	>10	>15	>20	>15	>20	<10	<15	<20	<15	<20	>10	>15	>20	>15	>20
LH 750 (n = 30)	5	5	5	8	8	0	0	0	0	0	1	7	10	4	7	24	18	15	18	15
Gen•S (n = 30)	5	5	5	8	8	0	0	0	0	0	4	7	15	4	12	21	18	10	18	10
ADVIA 120 (n = 31)	5	5	5	7	8	0	0	0	1	0	1	4	10	2	7	25	22	16	21	16
CellDyn 4000/optical (n = 29)	4	4	4	7	7	0	0	0	0	0	2	8	12	5	9	23	17	13	17	13

*At a reference trigger value for platelet transfusion of $5 \times 10^9/L$ and an analyzer trigger value of $<10 \times 10^9/L$, the analyzers correctly identified all patients who would require platelet transfusion (shaded areas).
 †Flow cytometric data is assumed to be the correct platelet count.

TABLE 7B. Number of Subjects Identified or Misidentified by Hematology Analyzers at Various Trigger Criteria for Platelet Transfusions*

Trigger value for platelet transfusion ($\times 10^9/L$)	Number of Subjects Hematology Analyzer Correctly Identified as Requiring Transfusions					Number of Subjects Hematology Analyzer Incorrectly Identified as Not Requiring Transfusions					Number of Subjects Hematology Analyzer Incorrectly Identified as Requiring Transfusions					Number of Subjects Hematology Analyzer Correctly Identified as Not Requiring Transfusions				
	<5		<10			<5		<10			<5		<10			<5		<10		
	<5	<5	<5	<10	<10	<5	<5	<5	<10	<10	>5	>5	>5	>10	>10	>5	>5	>5	>10	>10
Platelet count on flow cytometer†	<5	<5	<5	<10	<10	<5	<5	<5	<10	<10	>5	>5	>5	>10	>10	>5	>5	>5	>10	>10
Platelet count on hematology analyzer	<10	<15	<20	<15	<20	>10	>15	>20	>15	>20	<10	<15	<20	<15	<20	>10	>15	>20	>15	>20
LH 750 (n = 30)	5	5	5	8	8	0	0	0	0	0	1	7	10	4	7	24	18	15	18	15
Gen•S (n = 30)	5	5	5	8	8	0	0	0	0	0	4	7	15	4	12	21	18	10	18	10
ADVIA 120 (n = 31)	5	5	5	7	8	0	0	0	1	0	1	4	10	2	7	25	22	16	21	16
CellDyn 4000/optical (n = 29)	4	4	4	7	7	0	0	0	0	0	2	8	12	5	9	23	17	13	17	13

*At a reference trigger value of $10 \times 10^9/L$ and an analyzer trigger value of $15 \times 10^9/L$, 3 analyzers correctly identified all patients in need of transfusion (shaded areas).
 †Flow cytometric data is assumed to be the correct platelet count.

have very high flagging rates for low platelet counts, requiring review and sometimes a microscope review of blood smears, with associated costs and reporting delays. However, as the regression and correlation statistics show, inclusion of such results in the comparison with the reference method does not adversely affect results.

As expected from the results in the control group, the majority of the results from hematology analyzers also have a positive bias in the low-platelet group compared with results from flow cytometric analysis. One effect of a positive bias is that the use of analyzer trigger values of 5 or $10 \times 10^9/L$ platelets might lead to patients inappropriately not receiving platelets. It is, of course, expected that some mis-

classification will occur, because values near the trigger values will be on one side or the other of the cutoff because of Poisson error. However, inspection of the data shows, as anticipated, that the 3 analyzers with a positive bias to the reference method would have more instances of providing results that would lead to incorrectly withholding transfusions than incorrectly giving transfusions (Table 6). In a different analysis, using the flow cytometric cutoff of either 5 or $10 \times 10^9/L$ platelets (assuming the flow cytometric data to be accurate and precise), we set analyzer triggers at different levels and correlated those with flow cytometric results. Tables 7A and 7B show how patients would be classified as to whether or not they required transfusions when platelet

TABLE 8. Descriptive Statistics, Paired *t* Test, and Regression Analysis of the White Blood Cell–Interference Group*

Instrument	No. (Range)	Group Mean, Flow Cytometer†	Group Mean, Hematology Analyzer	<i>P</i> ‡	Regression Analysis			
					Slope	Intercept	<i>R</i> ²	<i>S</i> _{Y X} §
Flow cytometer	30 (1.81-26.04)	10.54						
LH 750	30 (1.88-25.81)	10.54	10.41	NS	1.005	-0.182	0.984	0.679
Gen•S	23 (1.81-18.24)	9.58	9.20	.002	0.927	0.311	0.985	0.467
ADVIA 120‡	29 (1.69-26.98)	10.53	10.54	NS	0.994	0.065	0.986	0.635
CellDyn 4000	27 (1.83-27.30)	10.80	10.77	NS	0.999	-0.025	0.985	0.681

*NS indicates not significant.

†Paired *t* test; NS, *P* > .10.

‡Samples with nucleated red blood cells are corrected for and included.

counts of 10, 15, or 20 × 10⁹/L are used as the triggers for each of the 4 analyzers. At a reference trigger of 5 × 10⁹/L, all patients with platelet counts conforming to this criterion will be identified if the analyzer threshold is set at <10 ×

10⁹/L or higher, whereas only a small number of patients would receive platelet transfusions not indicated by their platelet counts (Table 7A). When the reference trigger is set at <10 × 10⁹/L, all patients with platelet counts conforming

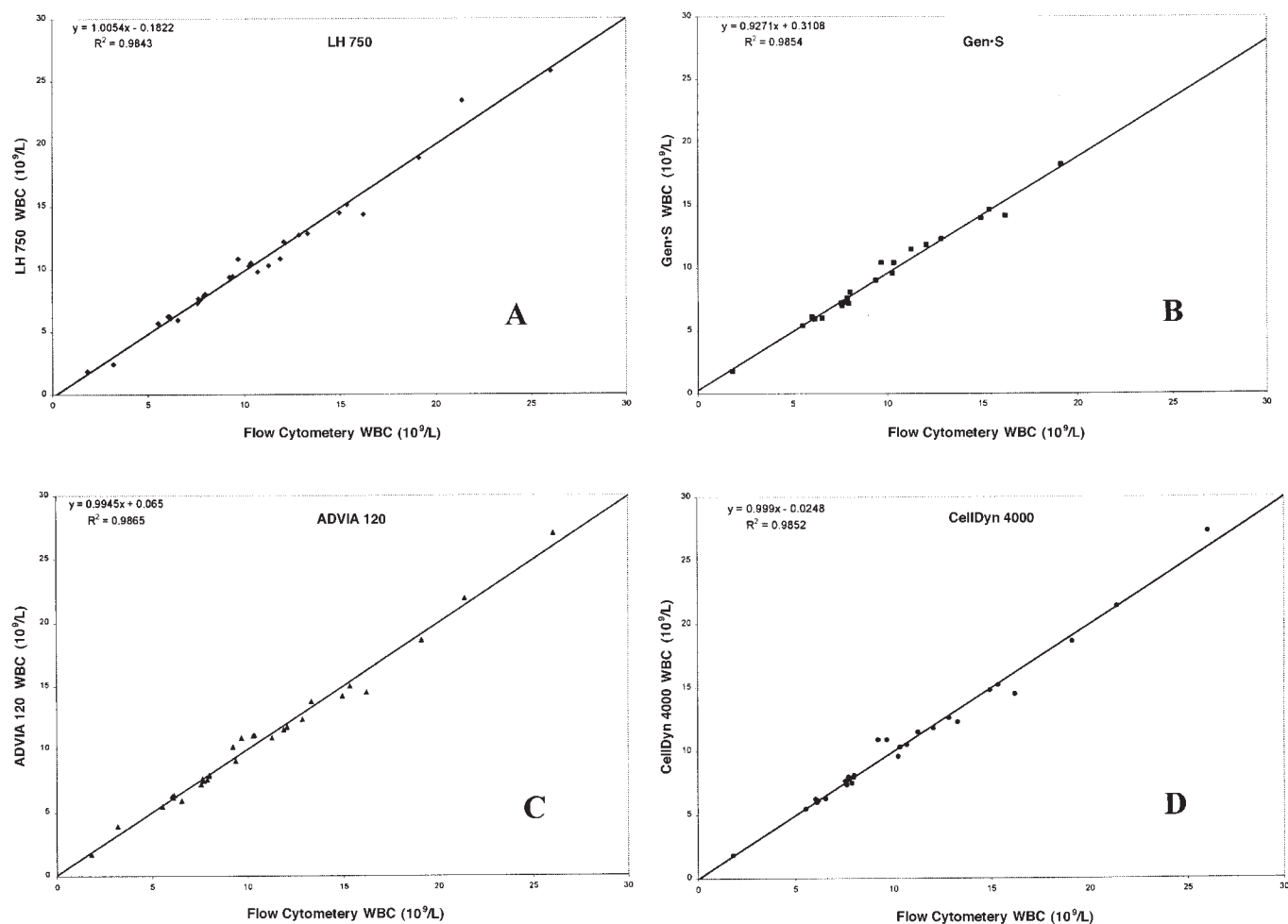


FIGURE 5. Comparison of white blood cell (WBC)-interference group leukocyte count results from 4 hematology analyzers versus results from the flow cytometric method: A, LH 750; B, Gen•S; C, ADVIA 120; D, CellDyn 4000.

TABLE 9. Tabulation of Analyzer White Blood Cell Flags*

	Number	Hematology Analyzer					
		LH 750	Gen•S	ADVIA 120		CellDyn 4000	
		Incomplete RBC Lysis	Incomplete RBC Lysis	Nucleated RBCs	Incomplete RBC Lysis	Incomplete RBC Lysis	Other
Sickle disease	10	0	6	5	1	1	1
Thalassemia	10	0	0	3	0	0	0
Liver/renal	10	0	1	2	0	1	0
Total	30	0	7	10	1	2	1

*RBC indicates red blood cell.

to this level are identified by 3 of the 4 instruments if analyzer thresholds of $<15 \times 10^9/L$ or higher are used (Table 7B). Our data thus indicate that there would be patients incorrectly subjected to transfusion, but that the clinically potentially catastrophic circumstance of not administering required transfusions is unlikely to occur. At an analyzer cutoff of $<15 \times 10^9/L$, only a single analyzer result would lead to not providing a necessary transfusion, yet many fewer patients would receive transfusions than when a $<20 \times 10^9/L$ analyzer cutoff was used. Using an analyzer cutoff of $<15 \times 10^9/L$ compared to $<20 \times 10^9/L$ would result in a reduction of unnecessary transfusions from an average of 9 to 4 transfusions, or a reduction of 55% (range, 43%-71% for the different analyzers) (Table 7B). It is, of course, recognized that such extrapolations are of limited value, because of the sample size and design of our study. However, in our opinion these data support the undertaking of a prospective large-scale study exploring the clinical effects of lowering the platelet-count threshold for platelet transfusions in nonbleeding patients with thrombocytopenia.

The descriptive statistics, paired *t* test, and regression and correlation analyses for the group with anticipated WBC count interference are given in Table 8. The higher $S_{Y|X}$ of the WBC interference set compared to that of the control group is consistent with its higher mean. Although the regression and correlation statistics were very similar among the 4 analyzers, they showed quite different patterns in flagging test results (Table 9). Some analyzers showed problems with samples from sickle cell patients and others with samples from thalassemia patients or patients with renal and/or liver disease. The ADVIA 120 and Gen•S analyzers showed the highest number of specimens flagged, whereas the LH 750 analyzer did not flag a single specimen. As can be seen from the regression and correlation analysis, which was based on unflagged specimens only, the lack of flagged specimens did not influence the LH 750 WBC count. For unflagged specimens, all analyzers showed excellent correlation with the flow cytometric reference WBC method (Figure 5). The main difference found was in the flagging rates, which obviously would affect any resulting review processes and possible reanalysis accordingly.

CONCLUSION

The counting performance of 4 hematology analyzers was tested for platelet counts at normal levels and various decision levels such as those commonly used in oncology patients. All analyzers except 1 showed positive bias against reference counts but provided clinically useful results, mostly without excessive flagging. These findings indicate that analyzer results can be used for medical decision making without long delays for repeat and confirmatory testing.

Interference of WBC counting showed an incidence ranging from 0% to 37% for different analyzers. By clinical condition, the incidence ranged from 0% to 60% for the samples from sickle cell patients, from 0% to 20% for samples from thalassemia patients, and from 0% to 10% for the samples from liver/renal patients.

Overall, this study shows that counting performance by hematology analyzers for the platelet and WBC parameters is robust and clinically useful.

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