

Validation of the Coulter LH 750 in a Hospital Reference Laboratory

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ABSTRACT

Validation of the Coulter LH 750 was carried out in our central hospital laboratory, which processes 1500 hemograms per day for patients admitted into the 4 hospitals of our hospital complex and their corresponding outpatient departments. It is the reference laboratory for the provincial health care area. The analytical quality and the practical use of the instrument were studied, and we obtained within-and between-run imprecision estimates with reference controls of between 0.24% and 2.56% and inaccuracies of between -1.32% and 3.07% for basic hemogram parameters. Similar results were obtained with specimens from patients, with imprecision estimates between 0.56% and 2.56%. Linearity estimates were between $0.3 \times 10^9/L$ and $380 \times 10^9/L$ for leukocytes and between $3 \times 10^9/L$ and $1900 \times 10^9/L$ for platelets. The instrument evaluation was completed with a study of interference by bilirubin, lipemia, hemolysis, platelet clumps, and heparin and an examination of other variables, including carryover, detection limits, and the correlation of results with those of the Coulter Gen•S. A special evaluation was made of the new erythroblast count feature; with reference controls, imprecision estimates for this count were 10% to 12%, and with patient specimens imprecision averaged 10.39% up to 4 erythroblasts per 100 leukocytes. We also studied the correlation of LH 750 results and interferences with those of the manual reference method. Finally, National Committee for Clinical Laboratory Standards protocols were used to test for suspect and confirmation

flags in leukocyte differential counts for 258 specimens representative of our routine. The practicability of the analyzer was studied in terms of technical difficulty, speed, and cost; also evaluated were new software elements for validation by source clinic and pathology and for reference values based on age. In conclusion, we analyzed the impact and improvements that may be expected in our laboratory (a user of Technicon and Coulter instruments) from introducing the new LH 750 analyzer into our routine. *Lab Hematol.* 2003;9:15-28.

KEY WORDS: Evaluation · Hematology automated analyzer · Clinical sensitivity · Specificity · Efficiency · VCS technology

INTRODUCTION

We evaluated the new Coulter LH 750 (Beckman Coulter, Fullerton, CA, USA) analyzer for 1 month in our central hospital laboratory, which uses Technicon (Advia 120; Bayer, Barcelona, Spain) and Coulter (Gen•S) equipment in its hematology area. The laboratory produces 1500 hemograms per day from samples submitted from 4 hospitals in our hospital complex (General, Traumatology, Maternity, and Children) and from the corresponding outpatient departments and the provincial health care area.

The protocols and methodologies used in this evaluation were those of the International Council for Standardization in Haematology (ICSH) and the National Committee for Clinical Laboratory Standards (NCCLS) for reference leukocyte differential counts and the evaluation of instrumental methods [1-3]. The analytical quality of the analyzer with respect to within-run and between-run precision and between-run accuracy was studied by using hematologic reference control

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samples with specific values and then making the same evaluations with hospital specimens obtained from patients with low, normal, and high values. We completed the evaluation with a study of other considerations, including interfering substances, linearity, carryover, correlation, and so on. The new erythroblast count feature, one of the technical innovations of this new analyzer, was the subject of a special evaluation.

A complete study was also carried out with suspect and confirmation flags by the processing of 258 specimens representative of our routine work load and having 2 qualified operators perform manual leukocyte differential counts on 200 cells. The practicability of the analyzer was studied in terms of technical difficulty, speed, and so forth; the new software possibilities of selective validation were explored on the basis of source clinic and pathology; and reference values were explored on the basis of age. Also carried out was an analysis of the expected impact of introducing the new analyzer into the hematologic routine of the laboratory.

MATERIALS AND METHODS

Specimens

The automatic module processed 380 patient specimens, 120 from patients admitted to the General Hospital, 30 from Maternity, 10 from Traumatology, and 190 from the outpatient departments. Additional specimens obtained from Pediatrics (30), hospitalized patients (15) and outpatients (15) were manually processed in the secondary module.

Specimens from adults were obtained with the Vacutainer system containing K₃EDTA (Becton Dickinson, Franklin Lakes, NJ, USA) and processed within 4 hours, as specified in the ICSH protocol [1]. The pediatric specimens were obtained by digital or venal puncture, depending on the patient's age, and collected in Becton Dickinson microtubes containing K₃EDTA anticoagulant.

Description of the Analyzer

The Beckman Coulter LH 750 analyzer is a totally automated system that provides a complete hematologic profile. The system can analyze up to 110 samples per hour in either the complete blood cell count (CBC) or the CBC + 6-part differential mode. The instrument possesses a number of new features that place it as the best model in the Beckman Coulter line; the principle new features include random access, an erythroblast count, and increased linearity ranges for counts of leukocytes (up to $400 \times 10^9/L$), erythrocytes (up to $8 \times 10^{12}/L$), and platelets (up to $3000 \times 10^9/L$) [4,5].

LH 750 CBC parameters are measured with Coulter AccuCount technology and an advanced analytical technique that combines the Coulter principle of impedance counting and sizing [6] with new, sophisticated mathematical algorithms for particle counting, wait-time counting, and estimating particle flight time. The new techniques improve the instrument's precision and expand the linear range for white

blood cell (WBC) and platelet counts. The WBC count is automatically corrected via a novel WBC interference algorithm for adjusting for interference by nucleated red blood cells, giant platelets, platelet clumps, unlysed red blood cells (RBCs), and RBC fragments. These unique features of the LH 750 analyzer are an improvement over the previous impedance technology of Gen•S analyzers. The nucleated red blood cell count is an integral part of the differential count and uses Beckman Coulter's VCS (volume, conductivity, scatter) technology, along with the advanced algorithm for correcting the WBC count.

The LH 750 uses 3 reagents for generating the hemogram and the erythroblast count. The latter requires no additional reagent or reagent consumption and is conducted as another parameter of the hemogram. The reticulocyte count requires a fourth reagent.

The capabilities of the LH 750 are further expanded with the addition of a SlideMaker and SlideStainer (Beckman Coulter) as a part of a trackless Coulter hematology work cell.

Sample Processing. Sample processing from the primary tube is entirely automatic and may be random if necessary. Agitation is by rocking (inversion), which is considered the ideal method for sample mixing while conserving sample integrity.

Maintenance and Special Procedures. Routine maintenance of the instrument is carried out from the analyzing unit with a simple, dedicated keyboard. These procedures are completely automated, require no operator time, and last approximately 6 minutes per day. In addition, the special correction functions are carried out automatically and efficiently from this keyboard and require no time-consuming and complicated manual procedures. The analyzer's new digital monitor has improved visual features for enhancing operator use and comfort and is protected from mobile phone interference.

Program. The analyzer offers user-adjustable flagging for reference ranges, action limits, critical limits, definitive flags, and the sensitivities of suspect flags. Based on its patient population and other requirements, a laboratory can input its own criteria or decide to use the built-in default criteria. Using customized decision rules, laboratories can easily program appropriate follow-up actions based on patient results. These advances allow the optimization of the postanalytic process.

Statistical Methods

Calculations of specificity, sensitivity, and efficiency of results were carried out with Passing-Bablok regression [7], and analysis of variance was performed with the aid of the SPSS (SPSS, Chicago, IL, USA) and MedCalc (MedCalc Software, Mariakerke, Belgium) computer programs.

RESULTS

Analytical Quality

Within-Run Precision with Control Samples. The automatic sampler processed 3 known levels of hematologic and

TABLE 1. LH 750 Within-Run Precision with 3 Levels of Control Samples*

	Level 1		Level 2		Level 3		Mean CV
	Mean	CV	Mean	CV	Mean	CV	
WBC, $\times 10^9/L$	3.42	2.5	9.57	0.99	20.56	0.6	1.36
RBC, $\times 10^{12}/L$	1.86	0.56	5.37	0.37	4.25	0.53	0.49
Hgb, g/dL	5.35	2.29	16.21	0.74	12.96	0.63	1.22
MCV, fL	80.41	0.25	87.55	0.24	86.79	0.22	0.24
RDW, %	15.9	0.97	14.96	1.3	14.72	1.24	1.17
Plt, $\times 10^9/L$	74	1.4	223	1.5	445	1.75	1.55
MPV, fL	10.06	0.73	10.62	0.88	10.82	0.62	0.74
Neu, %	42.07	4.24	56.25	1.37	65.43	1.08	2.23
Lym, %	46.14	1.48	28.18	1.71	14.59	3.52	2.24
Mon, %	6.45	4.88	8.67	3.55	15.04	3.43	3.95
Eos, %	5.09	10.56	6.73	11.46	4.83	5.7	9.24
Bas, %	0.26	36.5	0.19	31.91	0.08	36.64	35.02
NRBC, /100 WBC	4.26	13.54	5.08	8.79	1.81	8.69	10.34
Ret, %	0.94	7.9	2.96	2.97	10.07	1.45	4.11
MRV, fL	108	1.94	119.3	1.27	121.1	0.68	1.30
IRF, ratio	0.36	9.29	0.71	4.01	0.79	2.01	5.10

*CV indicates coefficient of variation; WBC, white blood cells; RBC, red blood cells; Hgb, hemoglobin; MCV, mean corpuscular volume; RDW, red blood cell distribution width; Plt, platelets; MPV, mean platelet volume; Neu, neutrophils; Lym, lymphocytes; Mon, monocytes; Eos, eosinophils; Bas, basophils; NRBC, nucleated red blood cells; Ret, reticulocytes; MRV, mean reticulocyte volume; IRF, immature reticulocyte fraction.

reticulocyte control samples 20 times in batch mode on the same day. The results are presented in Table 1. A within-run precision study involving the processing of controls by the manual method produced similar results.

Within-Run Precision with Patient Samples. Three specimens containing 3 different levels of WBCs, hemoglobin, platelets, reticulocytes, and erythroblasts from patients

in the General Hospital area were processed 20 times in batch mode with the automatic sampler. The results of this test are presented in Table 2. A within-run precision study that processed the patient specimens by the manual method produced similar results.

Between-Run Precision with Control Samples. The automatic sampler processed 3 different levels of hematologic

TABLE 2. LH 750 Within-Run Precision with 3 Patient Specimens with Different Parameter Value Levels*

	Specimen 1		Specimen 2		Specimen 3		Mean CV
	Mean	CV	Mean	CV	Mean	CV	
WBC, $\times 10^9/L$	1.34	5.79	7.12	1.03	20.4	0.85	2.56
RBC, $\times 10^{12}/L$	2.43	0.64	4.4	0.56	4.22	0.49	0.56
Hgb, g/dL	8.15	1.49	13.3	0.46	12.8	0.91	0.95
MCV, fL	96.4	0.61	90.8	0.68	36.7	0.71	0.67
RDW, %	19	1.2	15.7	0.38	87	0.64	0.74
Plt, $\times 10^9/L$	50	2	228	1.04	14.8	1.28	1.44
MPV, fL	8.44	1.35	8.46	2.42	441	1.92	1.90
Neu, %	34.9	3.71	51.1	0.68	10.8	0.78	1.72
Lym, %	47.4	3.39	35.9	0.87	65.1	1.08	1.78
Mon, %	12.8	5.56	9.55	2.64	14.6	3.31	3.84
Eos, %	3.51	11.1	2.8	6.6	15.2	3.8	7.17
Bas, %	1.35	23.8	0.59	8.75	4.9	5.94	12.83
NRBC, /100 WBC	2.27	35.44	4.41	16.83	43.23	3.96	18.74
Ret, %	0.3	11.5	0.95	9.26	3.76	5.25	8.67
MRV, fL	98.6	1.24	111	3.42	137	1.62	2.09
IRF, ratio	0.26	19.3	0.29	9.36	0.6	3.08	10.58

*Abbreviations are expanded in the footnote to Table 1.

TABLE 3. LH 750 Between-Run Precision with 3 Levels of Control Samples*

	Level 1		Level 2		Level 3		Mean CV
	Mean	CV	Mean	CV	Mean	CV	
WBC, $\times 10^9/L$	3.5	3.58	9.5	1.03	20.2	0.85	1.82
RBC, $\times 10^{12}/L$	1.9	0.88	5.35	0.56	4.22	0.49	0.64
Hgb, g/dL	5.3	2.51	16	0.46	12.8	0.91	1.29
MCV, fL	81	0.36	88	0.38	87	0.64	0.46
RDW, %	15	0.99	15	1.04	14.8	1.28	1.10
Plt, $\times 10^9/L$	76	4.36	225	2.42	441	1.92	2.90
MPV, fL	10	1.41	10.6	1.10	10.8	0.78	1.10
Neu, %	43	4.27	57	0.88	65.1	1.08	2.08
Lym, %	46	1.49	28	2.64	14.6	3.31	2.48
Mon, %	6.5	5.49	8.6	6.60	15.2	3.80	5.30
Eos, %	4.5	7.05	6.7	8.75	4.9	5.94	7.25
Bas, %	0.26	36.00	0.19	34.85	0.1	26.20	32.35
NRBC, /100 WBC	4.5	12.05	5.2	10.00	1.19	9.20	10.42
Ret, %	1.1	11.90	3.1	3.81	10.6	1.63	5.78
MRV, fL	109	2.64	116	3.47	120	1.88	2.66
IRF, ratio	0.3	21.10	0.5	10.70	0.76	4.46	12.09

*Abbreviations are expanded in the footnote to Table 1.

control samples and 3 reticulocyte control samples over a period of 20 days. The results are presented in Table 3.

Between-Run Accuracy with Control Samples. Because the parameter values of control samples were known, the inaccuracies of averages obtained with respect to assigned values were calculated. These calculations yielded the percent bias of obtained values relative to known values. The results are shown in Table 4. A study of between-run accuracy with control samples processed by the manual method produced similar results.

Interference. We analyzed interference due to bilirubin, lipemia, hemolysis, heparin, hemoglobinopathies, cryoagglu-

tinins, and platelet clumps in the hemogram [8-11], especially with respect to the erythroblast count, this analyzer's main innovation.

(a) *Lipemia.* Given that lipemia constitutes a regular source of interference in our specimens, especially for those samples from the emergency department, a small study was made to define the level of lipemia interference and to determine what lipemia parameters affect this new model of analyzer.

We added increasing quantities of parenteral nutrition (Clinic Oleic 20%; Baxter International, Valencia, Spain) to a pool of routine specimens collected with K_3EDTA ,

TABLE 4. LH 750 Between-Run Accuracy with 3 Levels of Control Samples*

	Level 1		Level 2		Level 3		Mean Bias, %
	Mean	Bias, %	Mean	Bias, %	Mean	Bias, %	
WBC, $\times 10^9/L$	3.5	6.06	9.5	2.15	20.2	0.99	3.07
RBC, $\times 10^{12}/L$	1.9	1.09	5.4	0.75	4.2	1.69	1.18
Hgb, g/dL	5.3	0.00	16.0	-2.42	12.8	-1.54	-1.32
MCV, fL	81.0	1.00	88.0	1.50	87.0	1.75	1.42
RDW, %	15.0	5.96	15.0	3.45	14.8	3.50	4.30
Plt, $\times 10^9/L$	76.0	5.56	225.0	1.35	441.0	0.23	2.38
MPV, fL	10.0	1.00	10.6	0.95	10.8	1.89	1.28
Neu, %	43.0	-0.69	57.0	1.25	65.1	-0.15	0.13
Lym, %	46.0	-0.22	28.0	-2.11	14.6	0.69	-0.54
Mon, %	6.5	8.33	8.6	2.38	15.2	1.33	4.02
Eos, %	4.5	-2.17	6.7	-4.29	4.9	-5.77	-4.08
Bas, %	0.3	12.00	0.2	0.00	0.1	0.00	4.00
Ret, %	1.1	2.73	3.1	-4.06	10.6	0.48	-0.29

*Abbreviations are expanded in the footnote to Table 1.

TABLE 5. Lipemia Interference Study*

Parameter	Mean	Triglycerides, 736 mg/dL		Triglycerides, 1847 mg/dL		Triglycerides, 2030 mg/dL		Triglycerides, 2614 mg/dL	
		Mean	Bias, %	Mean	Bias, %	Mean	Bias, %	Mean	Bias, %
WBC, $\times 10^9/L$	4.1	4	0	4	-2.4	4.2	2.44	3.7	-9.75
RBC, $\times 10^{12}/L$	4.38	4.31	-1.6	4.32	-1.36	4.22	-3.65	4.14	-5.48
Hct, %	39.8	39	-2	37.9	-4.77	38.3	-3.77	37.6	-5.52
Hgb, g/dL	12.9	13.5	4.65	14.6	13.18	14.8	14.73	15.2	17.83
Plt, $\times 10^9/L$	366	364	-0.5	361	-1.36	379	3.55	362	-1.09
NRBC, /100 WBC	8	7	-12.5	8	0	9	12.5	10	25.00

*Hct indicates hematocrit; other abbreviations are expanded in the footnote to Table 1.

determined the hemogram, quantified the levels of cholesterol and triglycerides in the samples, and corrected for dilution, both before and after the study. Table 5 demonstrates that leukocyte counts, erythrocyte counts, hematocrits, platelet counts, and erythroblast counts are not affected up to the highest lipemia level studied (cholesterol levels of 550 mg/dL [14.24 mmol/L] and triglyceride levels of 2614 mg/dL [29.8 mmol/L]). We detected significant positive lipemia interference in the determination of hemoglobin, as has been previously described [8,9]. This interference in hemoglobin determination, which was quantified as percent increases, ranged from +4.65% for 736 mg/dL (8.39 mmol/L) triglycerides to +17.8% for 2614 mg/dL (29.8 mmol/L) triglycerides. In addition, leukocyte counts, erythrocyte counts, and hematocrit values were reduced, although not significantly, to levels of between -1.6% and -9.75%. Significant increases in erythroblast counts of approximately 25% at the maximum lipemia level were observed; however, additional studies of this parameter should be carried out, because the resulting pool presented only 8 erythroblasts/100 WBCs.

Finally, as a practical application of this feature, we encountered during routine processing a specimen from a lipemic patient with levels of 3342 g/dL (38.09 mmol/L) triglycerides and 552 mg/dL (14.29 mmol/L) cholesterol and who presented the following results in the RBC series: RBC, $4.33 \times 10^{12}/L$; hemoglobin, 17.6 g/dL; hematocrit, 39.8%; and mean corpuscular volume, 91.7 fL. On verifying that the rule of 3 was not fulfilled, we estimated a false increase in hemoglobin level of approximately 35% in this case, and this result was reported.

(b) *Hemolysis*. A study of the influence of hemolysis on hemogram results was carried out. The methodology [12] assessed was a controlled hemolysis of 10 samples with an ultrasound device (Sonicon 300; Hielscher Systems, Teltow, Germany). The samples were subjected to different frequencies and increasing periods of ultrasound scanning, which resulted in varying degrees of hemolysis. The degree of hemolysis was measured with a Hitachi 747 analyzer (Hitachi, Tokyo, Japan) to determine the hemoglobin concentration in the supernatant plasma after the sample had been centrifuged. Statistical analysis with the Student *t* test

for paired samples of parameter averages before and after hemolysis showed no significant differences ($P < .05$) for any parameter of the hemogram. The average degree of hemolysis obtained from the 10 samples was 175 mg/dL hemoglobin (hemolysis range, 100-800 mg/dL hemoglobin). We may deduce from these results that there is no interference in the hemogram parameters from hemolysis at these measured limits, except possibly for a slight reduction in erythrocyte counts and a small, insignificant increase in the red blood cell distribution width. Both of these phenomena were predictable, given the *in vitro* process of erythrocyte breaking that was carried out. Likewise, the noninterference of hemolysis in the erythroblast count was confirmed, even in the case of highest level of hemolysis (800 mg/dL hemoglobin), which may be considered an extremely hemolysed sample.

(c) *Heparin*. Heparin contamination is common in samples (catheters, hemodialysis, hemofilters, and so forth) destined for hemogram determination, so we thought it appropriate to study its potential for interfering in the determinations of hemograms and of the new erythroblast parameter in particular. Before the hemogram, increasing quantities of sodium heparin (1000 IU/mL; Rovi, Madrid, Spain) were added to 5 patient specimens (2 patient samples had erythrocyte levels of 4 and 9 per 100 WBCs) to final heparin concentrations of 1 to 5 IU/mL (therapeutic range, 0-1 IU/mL). If no interference is detected with these suprathreshold heparin concentrations obtained during extraction, we can infer the noninterference of heparin. Calculating the *t* test parameter values for paired samples, we detected no appreciable differences (for $P < .05$) in any of the hemogram parameter values before or after adding heparin, not even for platelets. Heparin interference was also not observed in erythroblast determinations in the 2 analyzed samples exhibiting 4 and 9 erythrocytes per 100 WBCs.

(d) *Bilirubin*. Interferences have been described for previous Coulter models in carrying out the formula, especially for samples from icteric patients [8,13-15]. In a sample of 16 formulas from our routine not carried out with the Coulter Gen•S analyzer, 12 were found to be icteric samples (75%) with an average of 11.88 mg/dL (20.19 mmol/L) total bilirubin (range, 1.64-33.06 mg/dL [0.94-56.2 mmol/L]).

The causes of the formula interferences in the remaining 4 samples (25%) were unknown.

We processed the same samples with the LH 750, and the instrument completed the formulas for 7 of the 12 icteric samples and for 3 of the 4 nonicteric samples. Thus, the LH 750 globally reduced the interference detected in the Coulter Gen•S analyzer by 62.5% and carried out the formula correctly in 10 of the 16 problematic patient samples. This result is a notable improvement over the previous Coulter Gen•S analyzer in resolving this source of interference.

During this study, we also noted that the simple action of diluting a sample, the formula of which the LH 750 had rejected, via the analyzer's Isoton function (dispenses 1 mL Isoton into the hemogram tube) and then reprocessing the hemogram resolved the interference. The analyzer correctly calculated the formula as a percentage, but the Isoton dilution effectively rendered the patient's tube useless. The dilution also required a manual correction of the formula's absolute value if the sample were to be processed online with a bar code.

(e) *Hemoglobinopathies*. Interference has been described with other analyzers in the hemolysis of samples from patients with hemoglobinopathies [8,9]. We processed samples from patients with various hemoglobinopathies (hemoglobins SS, SC, D, and E), and no interference was detected in the hemoglobin or RBC determinations.

(f) *Cryoagglutinins*. The same interference occurred in all analyzers for 4 patient specimens with cryoagglutinins. This source of interference was resolved by heating the sample to 37°C for a half hour [8], but in 1 case erythroblasts (6%) appeared in the formula after the sample had been heated. The casuistics of the cases studied therefore have to be increased to determine whether this event was a sporadic occurrence in a single sample or occurs more generally with the new erythroblast count technology.

(g) *Platelet Clumps*. For some samples with platelet clumps, large platelets, or EDTA dependencies, we found that this new technology produced false erythroblast counts, quantified as between 4% and 7% erythroblasts. The incidence in our routine was approximately 0.4% (2 specimens) in approximately 500 hemogram determinations. However, a decreased level of cell interferences has been recorded with the new LH 750 analyzer, compared with the previous Coulter Gen•S model.

(h) *Old Samples*. Because the number of samples being sent from peripheral centers to the central laboratory is increasing, we carried out a simple study to evaluate the reliability of the LH 750 with specimens processed later than the day of extraction. We processed 10 specimens (3 of the samples with 203, 9, and 5 erythrocytes per 100 WBCs), kept them at 4°C, and processed them 24, 48, and 72 hours after collection. We tested the parameters and processing time with analysis of variance and the Fisher least significant difference test at a significance level of $P = .05$. Significant differences were found in a decrease in the formula's monocyte parameter (both percentages and absolute values) with initial mean levels

of 7.83% and final levels of 2.46%. A significant rise in mean platelet volume after 24 hours was also observed. Reticulocyte counts fell to significantly different levels at 24 hours but stabilized in subsequent determinations. With respect to erythroblasts, no significant reduction in the average count over time was observed despite the high variability in this parameter. In short, the LH 750 can process specimens sent to our laboratory up to 72 hours after extraction, if they are stored in the refrigerator and the demonstrated reductions in monocyte and reticulocyte levels are taken into account.

Carryover. The analyzer program is equipped with a function that allows analysis of this characteristic. The same sample containing high parameter values (H1, H2, H3) is processed 3 times and followed by 3 Isoton treatments (L1, L2, L3) or blanks. The analyzer then calculates the percent contamination in the third Isoton. Percent carryover is calculated according to the formula [5]: $H/L\% = [(L1 - L3)/H3] \times 100$.

Carryover was analyzed in duplicate by twice processing specimens from patients with high WBC counts ($350 \times 10^9/L$), and the WBC results indicated a carryover of 0.273%. Carryover results of tests conducted for other parameters were: hemoglobin (24 g/dL), 0.85% carryover; platelet count ($1959 \times 10^9/L$), 0.33% carryover; erythrocyte count ($7.90 \times 10^{12}/L$), 0.32% carryover; and, finally, erythroblast count (222/100 WBCs or $6.7 \times 10^9/L$), 0% carryover. All of these results were within the manufacturer's indicated specifications [4].

Detection Limit. Our hospital processes a significant number of specimens from patients in oncology and hematology departments and from patients who have received bone marrow transplants. These specimens are characterized by low leukocyte and platelet counts, and to assist in the clinical interpretation of these patients' hemogram results, we were especially interested in knowing the lower detection limits for these parameters in this analyzer. Another application of particular interest after the determination of each parameter's detection limit for such samples was establishing the reliability of the results of processing biological liquids (cerebrospinal, pleural, ascitic, arthritic, and other fluids), which present even lower values for leukocytes and erythrocytes. Given the elevated number of these cases in our hospital, we have been obliged to use automated methods to carry out their counting and differentiation.

Following the protocols established by the clinical chemistry committee for finding the detection limit of a parameter [16], we processed 10 times with Isoton. Then, samples diluted to varying degrees were processed 10 times until the lowest figure found for each parameter of the hemogram was significantly different ($P < .05$ by the t test) from the blank mean. The results show that the lower detection limits are as follows: leukocytes, $0.15 \times 10^9/L$; erythrocytes, $0.03 \times 10^{12}/L$; hemoglobin, 0.2 g/dL; platelets, $1.25 \times 10^9/L$; reticulocytes, 0.08% ($5000 \times 10^9/L$); and erythroblasts, 3.6/100 WBCs. Thus, values below the lower detection limits of the

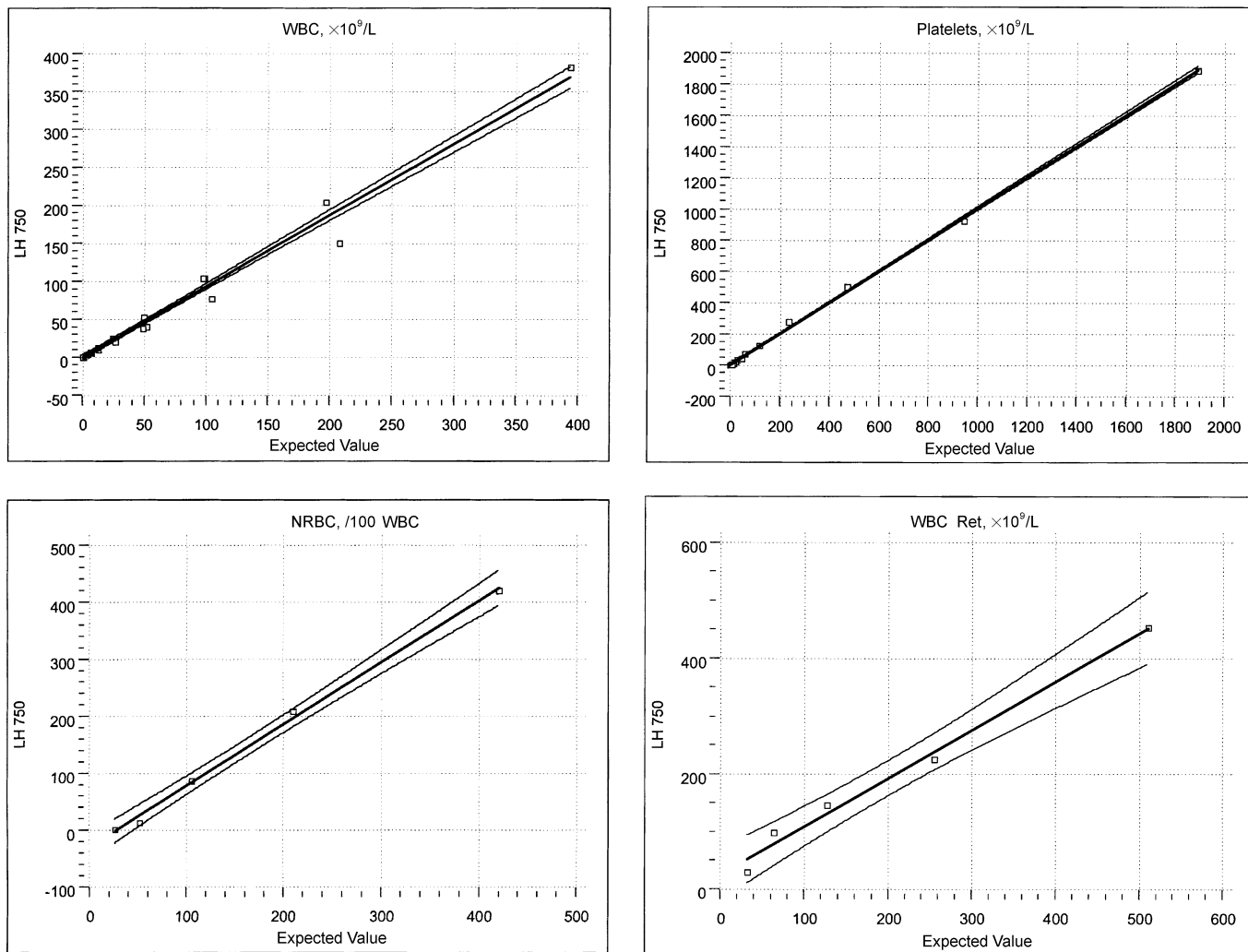


FIGURE 1. Linearity analysis of the Coulter LH 750 for leukocytes (WBC), platelets, nucleated red blood cells (NRBC), and reticulocytes (Ret).

instrument are not reliable, either for specimens of whole blood or for biological fluids. The linearity study in the following section of the evaluation establishes the analyzer's upper limit of reliability for each parameter.

Linearity. Instrument linearity was studied by carrying out serial dilutions with Isoton (whole blood with EDTA solution) of patient specimens from the routine that had the highest values for leukocytes, hemoglobin, platelets, reticulocytes, and erythroblasts. Figure 1 presents the most significant of these results. Our analysis of patient samples confirmed linearity from $0.3 \times 10^9/L$ to $380 \times 10^9/L$ for leukocytes, $3 \times 10^9/L$ to $1900 \times 10^9/L$ for platelets, 0.4 g/dL to 25 g/dL for hemoglobin, 0.5% to 72% for hematocrit, 8-604 $\times 10^9/L$ for reticulocytes, and 4/100 WBCs to 495/100 WBCs ($0.1-8 \times 10^9/L$) for erythroblasts.

Erythroblasts. Because the determination of erythroblasts constitutes the analyzer's major innovation, the study of this application required special attention. Forty-six samples with

an erythroblast range of 1 to 110 erythroblasts per 100 WBCs were processed. The LH 750 performed erythroblast counts for 39 samples (85%) with a mean value of 13.69/100 WBCs (range, 0-94.6/100 WBCs; SD, 20.87/100 WBCs). Two members of our laboratory processed the same 39 samples by the reference manual method and obtained a mean erythroblast count of 19.35/100 WBCs (range, 1-110/100 WBCs; SD, 25.72/100 WBCs). We analyzed the manual and automated results with Passing-Bablok regression analysis (Figure 2). According to Bablock [7], results are exchangeable and present no significant differences if the 95% confidence interval (CI) of the slope encompasses the value 1 and the 95% CI of the ordinate intercept encompasses the value 0. In our analysis, the calculated slope was 0.7705 with a 95% CI between 0.6427 and 0.8759 (the CI does not encompass the value 1); the intersection with the ordinate was -1.5410 with a 95% CI between -3.6593 and -1.2693 (the CI does not encompass the value 0). These

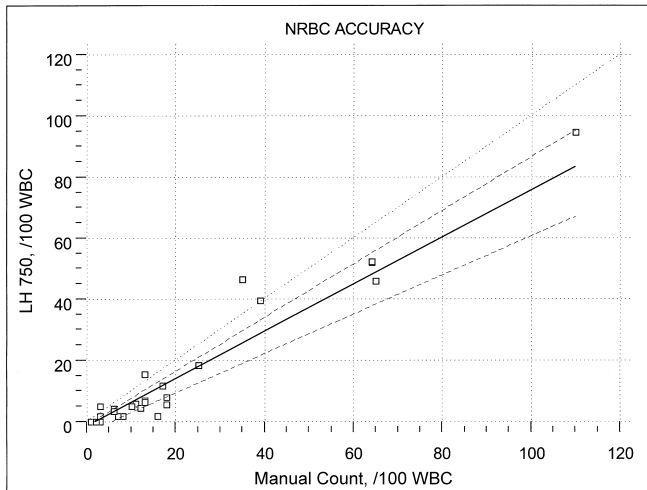


FIGURE 2. Accuracy of nucleated red blood cell (NRBC) counts determined with the Coulter LH 750. Indicated in the Passing-Bablok regression analysis plots are the calculated slope (solid line), the 95% confidence interval for the slope (dashed lines), and a slope of 1 (dotted line).

results demonstrate significant differences, as well as a lack of exchangeability, between the 2 methods. The LH 750 produces significantly lower erythroblast counts (mean, 13.69/100 WBCs) than the manual method (mean, 19.35/100 WBCs). However, although the LH 750 significantly underestimates the erythroblast count, the coefficient of variation of the automated LH 750 method is considerably lower (10%-12%) than that of the manual technique

which, as previously described, is approximately 20% to 25% [8]. The LH 750 also enables an automatic adjustment in the WBC count, which is currently carried out manually.

The erythroblast count, however, is linked to the formula, and some samples (15% in this study) do not allow an erythroblast count because they fail to appear in the formula. In addition, as was stated in the section on interferences, a small proportion of samples with platelet clumps gave false erythroblast counts with false erythroblast values (4%-7% erythroblasts). The fact that erythroblast determinations entail no costs, because the additional reagent required by other analyzers is not necessary in the LH 750, is a positive consideration.

Correlation. One hundred twelve patient specimens from outpatient and admissions departments were processed with both the Coulter LH 750 and the Gen•S analyzers and were calibrated with the same calibrator. Passing-Bablok regression was used to evaluate the 2 instruments' analyses of all parameters with respect to the exchangeability of results (Table 6). The results show significant differences between the 2 analyzers in WBC count, red blood cell distribution width, and mean reticulocyte volume, constant differences for both hemoglobin and percent reticulocytes, and proportional differences for platelet and monocyte percentages. The remaining hemogram parameters were perfectly exchangeable.

Leukocyte Differential Count. Following the corresponding NCCLS protocol [1-3] and the studies of other investigators [10,14,15], we processed 258 samples representative of our hematologic routine in the Coulter LH 750. Approximately 152 had normal formulas, and the other 106 exhibited pathologies according to the pathologic criteria established by the NCCLS; the results are reproduced in Table 7. Two labo-

TABLE 6. Method Comparison of LH 750 and Gen•S Analyzers*

Parameter	Gen•S	LH 750	Intercept, b	95% CI	Slope, p	95% CI
WBC, $\times 10^9/L$	7.74	7.73	0.19	0.119-0.268†	0.969	0.959-0.981†
RBC, $\times 10^{12}/L$	3.767	3.847	-0.0361	-0.08-0.01	1.006	0.995-1.02
Hgb, g/dL	11.665	11.98	0.2898	0.18-0.396†	1.003	0.99-1.013
Hct, %	35.03	34.62	-0.289	-0.719-0.23	0.997	0.98-1.01
MCV, fL	91.45	90.29	-0.664	-2.75-1.32	0.997	0.975-1.02
RDW, %	15.11	14.17	0.897	0.387-1.442†	0.956	0.918-0.992†
Plt, $\times 10^9/L$	227.9	223.13	2.31	-2.246-7.25	0.964	0.94-0.98†
MPV, fL	8.64	8.83	-0.054	-0.514-0.286	1.03	0.98-1.08
Neu, %	66.94	66.64	-0.837	-1.819-0.012	1.008	0.99-1.023
Lym, %	22.58	22.27	0.0024	-0.258-0.363	0.985	0.969-1.001
Mon, %	7.49	8.06	0.0128	-0.322-0.329	1.062	1.02-1.11†
Eos, %	2.55	2.56	0.0004	-0.042-0.062	1.004	0.97-1.04
Bas, %	0.43	0.46	-0.0675	-0.145-0.03	1.225	1.000-1.478
Ret, %	1.71	2.01	0.29	0.21-0.378†	1.002	0.94-1.06
MRV, fL	11.7	110.51	-14.81	-27.05-3.73†	1.12	1.027-1.235†
IRF, ratio	0.368	0.357	0.034	-0.005-0.069	0.898	0.80-1.00

*CI indicates confidence interval; Hct, hematocrit. Other abbreviations are expanded in the footnote to Table 1.

†Indicates significant difference ($P < .05$).

TABLE 7. NCCLS Criteria for the Clinical Sensitivity Study

	Proportional Count, %	Absolute Count, $\times 10^9/L$
Granulocytosis	>80	>9
Left shift (band form)	>6	>0.9
Granulocytopenia	<10	<1.5
Monocytosis	>10	>0.8
Eosinophilia	>7	>0.5
Lymphocytosis	>50	>3.5
Lymphopenia	<7	<1.0
Lymphocytes, atypical forms	>10	>0.7
Blasts	>2	>1.0
NRBC*	>2	>0.02

*NRBC indicates nucleated red blood cells.

ratory personnel (1 laboratory technician and 1 member of the medical staff) each performed manual formulas for 200 cells. In cases of discrepancies, a third equally qualified professional was called upon to evaluate the results.

First, we studied the correlation of the automated formulas to the means of results obtained by laboratory personnel and calculated the Passing-Bablok regression line [7]. These regression lines are shown in Figure 3, and the Passing-Bablok parameter values are presented in Table 8. To make this comparison possible, we added the immature granulocyte value (myelocytes, metamyelocytes, bands) to the segmented granulocyte value in the manual neutrophils section and compared the result with the neutrophil mean of the automated formula. Analysis of these regression data indicates that the LH 750 neutrophil results correlate perfectly

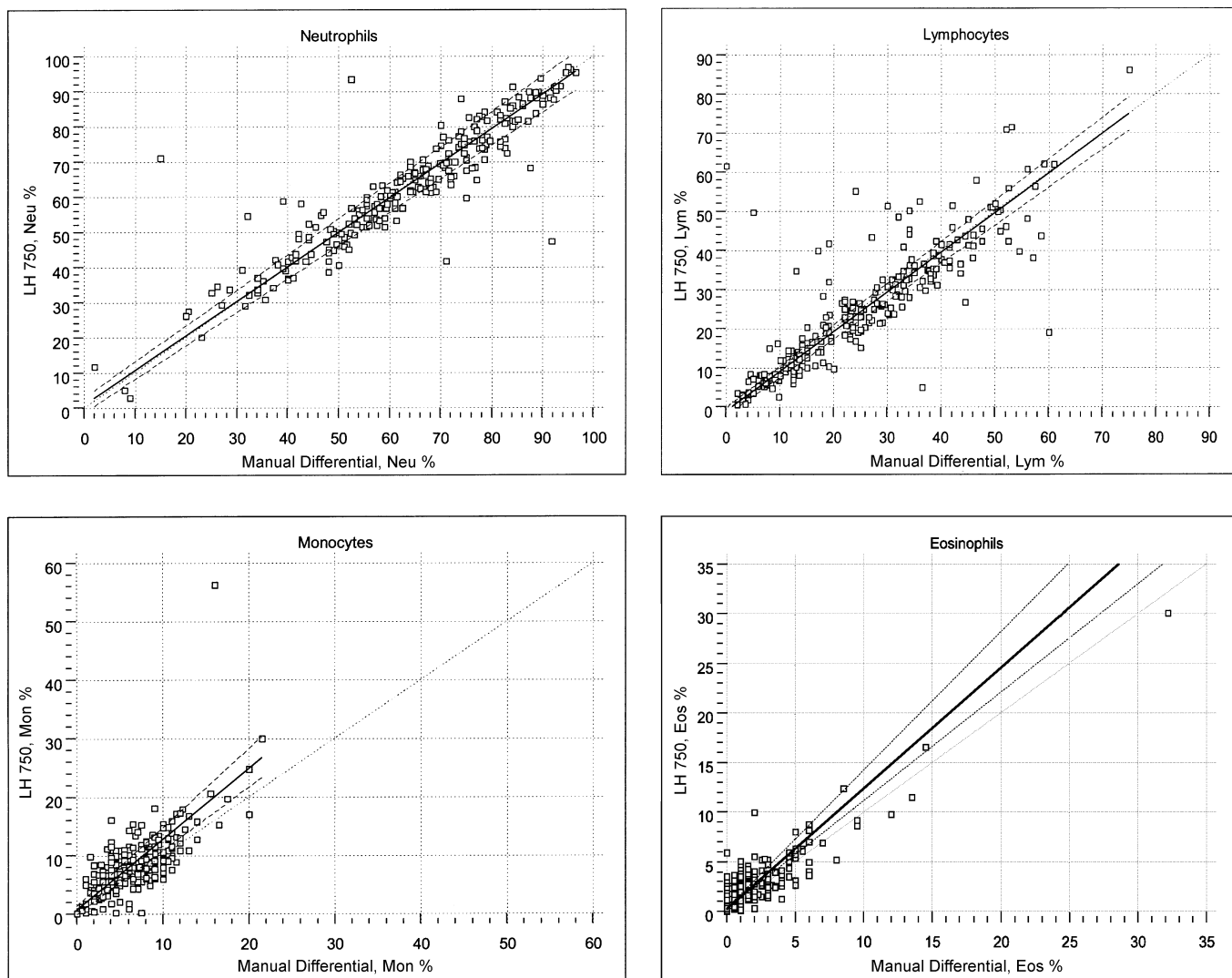


FIGURE 3. Leukocyte differential counts determined manually and with the Coulter LH 750. Indicated in the Passing-Bablok regression analysis plots are the calculated slope (solid line), the 95% confidence interval for the slope (dashed lines), and a slope of 1 (dotted line).

TABLE 8. Comparison of Manual versus LH 750 Determinations of Leukocyte Differential*

Parameter	Manual	LH 750	Intercept, b	95% CI	Slope, p	95% CI
Bas, %	0.27	0.4	0.21	0-0.2	—	—
Eos, %	1.84	1.93	0.2623	0.2-6.32†	1.216	1.09-1.39†
Neu, %	62.84	62.63	0.732	-1.43-2.78	0.982	0.95-1.02
Lym, %	25.76	25.67	-1.24	-2.24-0.28†	1.01	0.97-1.06
Mon, %	6.51	8.61	0.685	-0.07-1.4	1.21	1.08-1.34†

*CI indicates confidence interval; other abbreviations are expanded in the footnote to Table 1.

†Indicates significant difference ($P < .05$).

with the mean neutrophil results of the manual formula; no significant differences were found between the 2 results. With respect to the lymphocyte determination, the confidence interval of the ordinate intercept does not encompass the value 0, a result that according to Bablock indicates a significant difference ($P < .05$) of a constant nature between the 2 methods. Slightly lower values were obtained with the LH 750 instrument than when the formula was determined manually. This result is a statistically significant value, but in accordance with our criteria, it is of little clinical importance. With respect to monocytes, the slope CI does not encompass the value 1, a result that according to Bablock indicates proportional differences between the 2 methods, with the LH 750 overestimating the percentage of monocytes compared with the manual technique. This finding coincides with the observations of other investigators [13-15] in evaluations of previous Coulter Gen•S models. The degree of overestimation, however, has been considerably reduced in the new LH 750 model. With respect to eosinophils, neither the intercept CI nor the slope CI encompasses the value 0 or 1, respectively. This result indicates according to Bablock that significant differences exist between the 2 methods, with the LH 750 presenting higher values, principally at high

eosinophil values. It would therefore be interesting to widen this study with the analysis of samples containing high levels of eosinophils to establish some precision on this point. Finally, with respect to the results obtained in the basophil count, the manual method yielded a mean of 0.27%, and the automated method gave a mean of 0.40%. As some published evaluations have indicated [8,9], the application of this type of regression analysis to such low percentages is not particularly valid, and the results have no statistical validity.

In a second phase of this part of the evaluation, we studied the reliability of the definitive morphologic flags (granulocytosis, eosinophilia, monocytosis, and so forth) programmed by the users on the basis of their patient populations, specimen sources, pathologies, and so on [17,18]. For the purposes of the evaluation, we initially set the flags at 80% for neutrophils, 50% for lymphocytes, 10% for monocytes, and 7% for eosinophils. We determined the values for sensitivity, specificity, positive predictive value (PPV), negative predictive value, and efficiency presented by the analyzer for these preestablished values and used the manual formula and the criteria of Table 7 as a reference. The results obtained with our patient population are shown in Table 9 and demonstrate that the granulocytosis flag presents an efficiency of 88.66%.

TABLE 9. Sensitivity and Specificity of Definitive Flag Alarms*

	Alarm Criteria	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Efficiency, %
Granulocytosis (n = 30)	Neu >80%	86.67	88.94	52	97.97	88.66
	Plus Neu $>9 \times 10^9/L$	86.67	97.24	81.25	98.14	96.02
Granulocytopenia (n = 4)	Neu <10%	66.67	100	100	99.59	99.6
	Plus Neu $<1.5 \times 10^9/L$	66.67	100	100	99.59	99.6
Monocytosis (n = 13)	Mon >10%	76.92	73.5	13.89	98.29	73.68
	Plus Mon $>0.8 \times 10^9/L$	76.92	90.6	31.25	98.6	89.88
Eosinophilia (n = 6)	Eos >7%	85.5	97.49	53.85	99.57	97.17
	Plus Eos $>0.5 \times 10^9/L$	75	98.33	60	99.16	97.57
Lymphocytosis (n = 4)	Lym >50%	33.33	97.96	80.95	84	96
	Plus Lym $>3.5 \times 10^9/L$	27.45	100	100	84.12	85.02
Lymphopenia (n = 14)	Lym <7%	90	93.25	36	99.55	93.12
	Plus Lym $<1.0 \times 10^9/L$	90	94.09	39.13	99.55	93.93

*PPV indicates positive predictive value; NPV, negative predictive value. Other abbreviations are expanded in the footnote to Table 1.

TABLE 10. Sensitivity and Specificity of Suspect Flag Alarms*

WBC Manual Differential	n	LH 750 Alarm†	Alarm Level	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Efficiency, %
Imm gra 1%-3%	37	Imm Ne 1	L3	14.29	35.14	55.56	6.74	17.41
Imm gra 3%-6%	21	Imm Ne 1	L3	61.90	81.86	24.07	95.85	80.16
Imm gra >6%	16	Imm Ne 1	L3	68.75	81.39	20.37	97.41	80.57
Imm gra 1%-3%	37	Imm Ne 2	L3	2.38	64.86	27.78	10.48	11.74
Imm gra 3%-6%	21	Imm Ne 2	L2	4.76	93.81	6.67	91.38	86.23
		Imm Ne 2	L3	14.29	93.36	16.67	92.14	86.64
Imm gra >6%	16	Imm Ne 2	L1	50.00	98.27	66.67	96.60	95.14
			L2	56.25	97.40	60.00	96.98	94.74
			L3	62.50	96.54	55.56	97.38	94.33
Blasts >2%	4	Neu Blas	L1	0.00	99.59	0.00	98.37	97.98
			L2	75.00	99.59	75.00	99.59	99.19
			L3	100.00	99.59	80.00	100.00	99.60
		Lym Blas	L1	0.00	99.60	0.00	98.44	98.05
			L2	25.00	99.60	50.00	98.82	98.44
		Mon Blas	L1	0.00	99.18	0.00	98.37	97.57
Atyp lym 1%-5%	19	Var Lym	L2	5.26	98.25	20.00	92.56	91.09
			L3	10.53	93.86	12.50	92.64	87.45
Atyp lym 5%-10%	8	Var Lym	L3	12.45	97.91	20.00	96.69	94.74
Atyp lym >10%	5	Var Lym	L3	20.00	98.35	20.00	98.35	96.76
Reac lym 1%-5%	17	Var Lym	L3	5.88	93.48	6.25	93.07	87.45
Reac lym 5%-10%	8	Var Lym	L3	12.50	98.33	20.00	97.11	95.55
Reac lym >10%	4	Var Lym	L2	50.00	98.77	40.00	99.17	97.98
			L3	75.00	94.65	18.75	99.57	94.33

*PPV indicates positive predictive value; NPV, negative predictive value; Imm gra, immature granulocytes; Atyp lym, atypical lymphocytes; Reac lym, reactive lymphocytes.

†LH 750 alarm codes are: Imm Ne 1 and Imm Ne 2, immature neutrophils; Neu Blas, neutrophil blasts; Lym Blas, lymphocyte blasts; Mon Blas, monocyte blasts; Var Lym, variant lymphocytes.

If we also add the absolute value as a criterion, as indicated by the NCCLS, we obtain an efficiency of 96.02%. Thus, given the demonstrated improvements brought about by joining the 2 criteria, we set the criteria for granulocyte flag generation in our patient population for samples that simultaneously present a neutrophil percentage >80% and a neutrophil count $>9.0 \times 10^9/L$.

Despite the low incidence of granulopenia in our study (4 cases), we set the granulopenia flag for <10% neutrophils and obtained an efficiency of 99.6%. When we added the NCCLS absolute value criterion (neutrophil count $<1.5 \times 10^9/L$), the same 99.6% level of diagnostic efficiency was obtained. We therefore think it would be interesting to widen the casuistics of these cases.

When the monocytosis flag is programmed to be generated when the sample exceeds 10% monocytes, the instrument presents an efficiency of 73.68%. If we add the NCCLS absolute value criterion (monocyte count $>0.8 \times 10^9/L$), the efficiency rises to 89.88%, although with low PPV values of approximately 31.25%.

When the flag for eosinophilia in a specimen is set at >7% eosinophils, the efficiency is 97.17%. If we add the NCCLS absolute value criterion (eosinophil count $>0.5 \times 10^9/L$), efficiency is slightly improved to 97.5% with a PPV of 60%.

Setting the lymphocytosis flag to >50% lymphocytes results in a diagnostic efficiency of 96%, and if we add the NCCLS absolute value criterion (lymphocyte count $>3.5 \times 10^9/L$), the efficiency falls to 85% but results in a better PPV (100%). When the lymphopenia flag is set at <7% lymphocytes, an efficiency of 93.12% is obtained, and if the NCCLS absolute value criterion is added (lymphocyte count $<1.0 \times 10^9/L$), the efficiency remains the same but results in a slightly higher PPV (39.13%).

Finally, the reliability of the morphologic suspect flags (Imm Ne 1, Imm Ne 2, Var Lym, blasts) provided by the analyzer was studied.

To evaluate the diagnostic efficiency of the Imm Ne 1 and Imm Ne 2 flags, we divided the manual formulas into 3 levels for the purposes of this study with a bias to the left according to their level of pathogeny: (1) immature granulocytes, 1% to 3%; (2) immature granulocytes, 3% to 6%; and (3) immature granulocytes, >6%. The preliminary analysis of these results (Table 10) reveals that the Imm Ne 1 flag has little diagnostic efficiency (17%) in detecting specimens with immature granulocytes between 1% and 3% but is highly sensitive in detecting immature granulocytes between 3% and 6% (80.16% efficiency). The Imm Ne 1 flag is equally sensitive in detecting immature granulocytes >6% (81% efficiency). Furthermore, it

was confirmed that no significant differences exist in the 3 levels of flags that the analyzer allows to be programmed (L1, L2, L3).

The Imm Ne 2 flag is not very sensitive (2.38%; efficiency, 11.7%) for specimens with immature granulocytes between 1% and 3%. Sensitivity rises to 14.29% (86% efficiency) for specimens with immature granulocytes between 3% and 6%, and this flag reaches maximum sensitivity in detecting specimens with levels of immature granulocytes >6%. Sensitivity is 62%, and diagnostic efficiency is 94%, especially at level L3, although there are no large differences among the 3 levels (Table 10) that the analyzer allows to be programmed. In summary, with respect to the diagnostic efficiency of flags for left bias, the Imm Ne 1 flag is highly sensitive for detecting specimens with immature granulocytes levels between 3% and 6%, and the Imm Ne 2 flag is more sensitive in detecting the presence of >6% immature granulocytes. Values of >6% immature granulocytes generate both flags.

With respect to the blasts alarms (neutrophil, lymphocyte, and monocyte blasts), Table 10 shows that the neutrophil blasts flag at level L3 has a maximum sensitivity of 100%, with a 99% efficiency in detecting specimens with more than 2% blasts. The lymphocyte and monocyte blast flags demonstrated very low sensitivity, however, given the small number of cases ($n = 4$), the casuistic will have to be increased to verify this point.

Finally, with respect to the lymphocyte variants alarms, the NCCLS has pointed out the morphologic difficulties presented by lymphocyte classification because of the huge variety of lymphocytes in existence (activated, granular, cleaved, chronic lymphocytic leukemia-type, and reactive lymphocytes, prolymphocytes, and so forth). It is thus unlikely that analyzers will be capable of discriminating lymphocytes, and our study has confirmed this view. The LH 750 showed little sensitivity in detecting varieties of lymphocytes, including granular lymphocytes, prolymphocytes, cleaved lymphocytes, and chronic lymphocytic leukemia-type lymphocytes. Sensitivities varied between 5% and 12%, with manual lymphocyte varieties in a range of 1% to 10%, although when formulas presented values for these lymphocyte varieties of greater than 10%, the sensitivity increased to 20% (96.76% efficiency). In addition, a higher sensitivity was obtained in the detection of reactive or activated lymphocytes, which are typical of infectious mononucleosis and, to a lesser degree, of viral infections. Sensitivities ranged from 6% to 75% for specimens with values above 10% (94% efficiency). The Lym Blas flag proved to be equally sensitive when more than 10% reactive lymphocytes are present.

Practicability

This practicability of using the LH 750 constitutes an important part of this evaluation, and several points regarding the practical use of the analyzer [8,9] are considered.

Technical Difficulty. Start-up and operation are simple, especially for users of previous Coulter models, and opera-

tor difficulty is at the level of a middle-grade laboratory technician.

Speed. Sample processing speed is approximately 90/hour without reticulocyte determinations and 56/hour with reticulocyte determinations.

Cost. The cost is 0.90 euros for a complete hemogram with reticulocyte and erythroblast determinations.

Contamination. The use of cyanmethemoglobin reagent for hemoglobin determinations requires selective waste disposal in accordance with the regulations of the country or autonomous community where the instrument is located, because this reagent is considered potentially hazardous.

Information Technology System, Software/Computer. Computer support incorporates Windows NT technology in all 2001 laboratory analyzers of the latest generation, and this feature facilitates learning the instrument's operation and the handling of samples. The instrument program is easy to learn and use and features icons and programs similar to those of the previous Gen•S model. The operation of and familiarization with the computer system is easy for users of the Coulter Gen•S, and we confirmed this observation with the laboratory technician who carried out the evaluation. The incorporation of an intermediate station of Remysol system validation (not evaluated) helps in the result-processing and validation routine. The analyzer also allows a bidirectional online connection. The incorporation of a digital monitor improves the visual features and operator use of the new analyzer. With respect to validation, we confirmed that the incorporation of specific validation ranges for pathologies and source areas and the introduction of reference values for age constitute important advances in the quality of medical validation.

Reagents. The reagents are not specific for the Coulter LH 750 and may be used with the Coulter Gen•S, but Gen•S reagents cannot be used in the LH 750. The new reagents require no special preservation, are equally stable, and have incorporated different-colored indicators in their composition to avoid confusion. Unlike other analyzers, the erythroblast determination requires no additional reagent.

Specimen Volume. The dead volume is 1 mL for the automatic system. This is the minimum specimen volume required for the determination of 300 μ L of sample; the manual method requires 225 μ L. The processing of pediatric specimens is not more difficult than the processing of any manual sample by the secondary mode.

Technical Service. No technical problem occurred during the course of the evaluation.

CONCLUSIONS

The central laboratory of our hospital complex processes 1500 hemograms a day (1000 routine, 500 emergency) and is the provincial reference laboratory. The evaluation of analyzers constitutes an important part of its function as a refer-

ence laboratory and ensures the proper functioning of its automated area in the hematology department. The evaluation of the Coulter LH 750 analyzer was carried out over a 1-month period, and the results in the "Analytical Quality" section showed coefficients of variation with control samples that ranged from 1.36% for leukocytes, 1.22% for hemoglobin, 0.49% for erythrocytes, 1.55% for platelets, and 4.1% for reticulocytes to 10.34% for the new erythroblast parameter. Similar results were obtained with specimens from patients. The different sources of interference that this technology may present were analyzed. We quantified positive lipemia interference in hemoglobin determinations and determined that erythroblast counts in specimens presenting platelet clumps can be underestimated by approximately 4% to 6%. We determined detection limits for the various hemogram parameters and established the following sensitivities: leukocytes, $0.15 \times 10^9/L$; erythrocytes, $0.03 \times 10^{12}/L$; and platelets, $1.25 \times 10^9/L$. Given the reliability of the established lower detection limits, especially for leukocytes and erythrocytes, these sensitivities even enable the processing of biological fluids. Also noted was an improvement in linearity of up to $380 \times 10^9/L$ for leukocytes and up to $1900 \times 10^9/L$ for platelets. The correlation study of results obtained with the LH 750 and Gen•S instruments revealed significant differences in the counting of leukocytes, which may reflect an improvement in the LH 750 in the counting of difficult specimens, as was established in the parallel processing of such samples with the Advia 120. The coefficient of variation (10%-12%) seemed acceptable for the counting of erythroblasts, although a comparison of this result with the manual reference method indicates that the LH 750 underestimates this count, but not significantly. However, performing the leukocyte correction automatically, avoiding troublesome and error-prone calculations (especially with specimens containing high erythroblast levels as can occur with premature babies), and the zero cost of the erythroblast determination (because, unlike other analyzers, no additional reagents are required) also constitute positive points when considering the cost of a complete hemogram. Significant improvements in the performance of formulas for samples from icteric and lipemic patients were also observed.

Evaluation of the reliability of the new flags merited special attention, as did their incidence in the work flow in the area of hematologic automation. Comparison of LH 750 leukocyte differential counts with manual counts showed good correlations in the neutrophil and lymphocyte counts and a lower correlation in the monocyte counts. The LH 750 overestimated the eosinophil counts, a finding that would be interesting to follow up in a complementary study. We demonstrated that the definitive flags (for granulocytosis, monocytosis, and so on) acquire maximum efficiency when the analyzer is set to combine the percent elevation criterion with the absolute value criterion. On the basis of these

results and for the sake of our patient population, we set these flags at their maximum efficiency. With respect to the suspect flags (Imm Ne 1, Imm Ne 2, blasts, and Var Lym), our exhaustive study demonstrates that the Imm Ne 1 flag reaches maximum detection efficiency with specimen values of greater than 3% immature granulocytes. The maximum detection efficiency for the Imm Ne 2 flag occurs with specimen values in excess of 6% immature granulocytes. Both of these flags are activated for specimen values greater than 6% immature granulocytes. Equally efficient was the neutrophil blast flag at level L3 for detecting specimen values in excess of 2% blasts (100% sensitivity).

Finally, the analyzer showed little detection sensitivity (5%-10%) for lymphocyte variants (granular, cleaved, chronic lymphocytic leukemia-type, and so on). Sensitivity increased with the reactive lymphocytes typical of mononucleosis and approached 75% when lymphocyte levels exceed 10%.

With respect to evaluating the practical use of the LH 750, several aspects stand out. As users of the previous model, the Coulter Gen•S, we found that adapting to the new features of the LH 750 was fast. The new instrument incorporates the same methodology and computer system and has been updated. It uses the same computer icons, which facilitate its operation by users of the previous model. Thus, the incorporation of the LH 750 into the laboratory routine presented no problems and required only a little complementary training on the differences of the new model.

Operation of the analyzer is simple, and the instrument presented no technical problems during the month of evaluation in terms of the automatic and manual sampler, the formulas, instrument calibration, quality control, processing speed, and the computer system.

The zero cost of the new erythroblast determination is a positive factor in comparisons with other analyzers, which require additional reagents and costs to perform this test. Incorporating the possibility of technical and medical validation with alarm or panic values, which vary depending on the age of the patient (premature babies, infants), the sample source (intensive care unit, hematology, bone marrow transplantation units, oncology), and pathology (sepsis, burns, nephrology), constitutes one of the most interesting novelties of the analyzer. This feature may significantly improve the quality of validations and reduce the work flow time in the automated area. Processing pediatric specimens involved no additional processing difficulties. The reduced volume of these specimens is the limiting factor, because the new analyzer requires 225 μL for a complete hemogram.

In conclusion, the principal advantages of the new Coulter LH 750 are its sturdiness and efficiency in processing significant numbers of specimens, both as a matter of routine and in emergencies. This instrument has a notably improved linearity, avoids manual dilutions, and significantly reduces the icteric and lipemic interferences registered

in the previous Coulter Gen•S instrument. The incorporation of erythroblast counting with no additional hemogram costs constitutes one of the principal attractions of the new model. These features, together with the advances in the area of validation, position the LH 750 as one of the best analyzers in hematologic automation.

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