Laboratory Hematology 7:116–124

ISLH
Official Publication

Performance Evaluation of the Complete Blood Count and White Blood Cell Differential Parameters on the AcT 5diff Hematology Analyzer

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ABSTRACT

The Coulter AcT 5diff Hematology Analyzer from Beckman Coulter (Miami, FL) is a benchtop system designed for the low-volume laboratory. Its small sample size requirement, capability for complete blood count (CBC) with 5-part differential, and convenient reagents make it ideal for use in this environment. The system was evaluated to compare its CBC and 5-part white blood cell (WBC) differential performance with that of the midvolume Coulter HmX analyzer. The clinical utility and flagging performance of the automated analyzers were confirmed by 100-cell manual differential counts. The AcT 5diff analyzer was shown to have excellent precision and accuracy for the CBC parameters, with coefficients of correlation >0.95 for directly measured parameters. The WBC differential results from the AcT 5diff system compared well to other automated differential results, and flagged morphologically abnormal samples were confirmed as appropriate by the manual differential. An efficiency rate >80% means the laboratory will properly identify abnormal specimens for follow-up but will not be burdened with unnecessary manual differentials. *Lab. Hematol.* 2001;7:116-124.

KEY WORDS: AcT 5diff hematology analyzer · CBC parameters · WBC 5-part differential

Received June 4, 2001; accepted June 7, 2001

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INTRODUCTION

We conducted an evaluation to study the paired-sample precision of the complete blood count (CBC) parameters and the accuracy of the CBC and white blood cell (WBC) differential parameters on the Coulter AcT 5diff hematology analyzer (Beckman Coulter, Miami, FL). For accuracy, the CBC and WBC differential parameters obtained using the AcT 5diff analyzer were compared with those from the Coulter HmX hematology analyzer, and flagging of the WBC differential was confirmed by manual 100-cell differential count.

The study was designed to evaluate the performance of the AcT 5diff hematology analyzer over a clinical range that included both normal and morphologically abnormal samples and to evaluate the flagging rate and clinical utility of the system. A total of 111 samples were analyzed during the evaluation period.

MATERIALS AND METHODS

Instrumentation

The AcT 5diff hematology analyzer, shown in Figure 1, is a self-contained benchtop analyzer designed for use in the low-volume laboratory. It is capable of reporting a CBC with comprehensive red blood cell (RBC), WBC, and platelet (PLT) results, including a 5-part WBC differential. The AcT 5diff system is fully automated, including a cappierce feature on some models, and can analyze up to 60 samples per hour in either CBC or CBC/Diff mode. It can handle venous specimens collected into various types of tubes with K_3EDTA anticoagulant or capillary specimens collected into microcollection devices at volumes as low as 100 µL. The small sample volume, 30 µL in CBC mode and 53 µL in CBC/Diff mode, makes this system ideal for

FIGURE 1. AcT 5diff hematology analyzer.

pediatric and oncology patients from whom larger samples are difficult to obtain.

The AcT 5diff system uses a total of 5 conveniently packaged reagents:

AcT 5diff Diluent, to dilute the whole blood and stabilize cell membranes;

AcT 5diff Fix, to lyse RBCs, preserve leukocytes in their native state, and stain the granules of monocytes, neutrophils, and eosinophils with vital stain Chorazole Black E;

AcT 5diff WBC Lyse, to lyse RBCs for the leukocyte count and specifically differentiate basophils from other leukocytes;

AcT 5diff Hgb Lyse, to lyse blood cells and determine hemoglobin content; and

AcT 5diff Rinse, a rinsing agent.

These reagents are stable at room temperature and are preheated to 35°C before mixing with the sample in the reaction baths.

The unique reagent/sample delivery and mixing system on the AcT 5diff analyzer, called the sequential dilution system (SDS), is shown in Figure 2. This system accurately delivers specific volumes of samples and reagents to the baths in which the lytic or staining reactions occur. Of the 53 µL whole blood aspirated by the probe for the CBC/Diff mode, 10 µL is used for the WBC/basophil (WBC/BASO) dilution, 25 µL for the Diff dilution, and the remainder for hemoglobin (HGB) and RBC/PLT analyses. Sample and reagent are homogeneously mixed at specified times and delivered to the appropriate temperature-controlled baths where the reactions occur. From there, the reaction mixtures are transferred to the flow cells for analysis.

Table 1 details the technologies used for CBC parameters on the AcT 5diff system and the flags that might be generated to alert technologists to an interference with the analysis. A series of interpretive messages provides information about the possibility of abnormal cell types or clinical conditions.

The AcT 5diff system uses the principles of absorbance cytochemistry and volume to provide a complete 5-part WBC differential. Lysis of the RBCs and cytochemical staining of the granular components of the monocytes, neutrophils, and eosinophils by the FIX reagent prepares the WBCs for differential analysis. Diluent is added to stabilize the reaction, and the sample is analyzed in the flow cell using dual focused flow (DFF) technology. This technology focuses cells in a stream of diluent and aligns them to pass through the flow cell singly. Cell volume, determined by the Coulter principle (impedance), and light absorbance are measured.

1) **Rinse Bath**
   a. 3 µL blood at beginning of cycle
   b. 7 µL blood in CBC mode at end of cycle
   c. 5 µL blood in CBC/DIFF mode at end of cycle
2) **First Dilution / Hgb Bath**
   a. 10 µL blood
   b. 1.7 mL Diluent
   c. Transfer 42.5 µL to Bath 4
   d. 0.4 mL Hgb Lyse
3) **Diff Bath**
   a. 25 µL blood
   b. 1 mL FIX
   c. Mix 12 sec
   d. 1 mL Diluent
4) **RBC Bath**
   a. 42.5 µL from Bath 2
   b. 2.5 mL Diluent
5) **WBC/BASO Bath**
   a. 10 µL blood
   b. 2 mL WBC Lyse

FIGURE 2. Sequential dilution system. Hgb indicates hemoglobin; DIFF, differential; RBC, red blood cell; WBC, white blood cell; BASO, basophil; CBC, complete blood count.

TABLE 1. Complete Blood Count Parameters*

Parameter	Technology	System Flag and Flag Description
WBC, n $\times 10^3$ cells/μL	Differential lysis; impedance	*WBC Interference from debris/particles between 0 and lower threshold V flag <3.0, 9%; >3.0, 7% +++ Over range (>100.0)
RBC, n $\times$ 10^6 cells/μL	Impedance	Micro/Macro Triggered when % of cells exceed set limits in microcytic (5%) or macrocytic (7.5%) regions of histogram V flag Voteout 8% ++++ Over range (>10.00)
HCT, %	Directly measured from RBC histogram	V flag Voteout 5% ++++ Over range (>80.0)
MCV, fL	Calculation (HCT/RBC $\times$ 10)	++++ Over range (>199)
RDW	Calculation (K $\times$ SD RBC/MCV) $\times$ 100	
HGB, g/dL	Cyanmethemoglobin absorbance at 550 nm	R flags >60 A/D difference between Blank Ref and Ref HGB Blank <2.5v; blank errors V flag >3% difference between 3 sample measurements …. No result +++ Over range (>30.0)
PLT, n $\times 10^6$ cells/μL	Impedance	R flag Interference at upper threshold MIC and SCH flags Microcytosis and schistocyte interference triggered by particles at the upper thresholds SCL Small cell interference triggered by particles at the lower threshold V flag Voteout <100, 20%; >100, 15% +++ Over range (>1500)
MPV, fL	Directly derived from PLT histogram	

*WBC indicates white blood cells; RBC, red blood cells; HCT, hematocrit; MCV, mean corpuscular volume; RDW, red cell distribution width; HGB, hemoglobin; PLT, platelet; MPV, mean platelet volume.

Lymphocyte populations are typically small and regular in shape, with a homogeneous gaussian volume distribution. Due to their lack of granules, they are lower in absorbance than the other cell types; therefore, they are positioned in the lower left portion of the differential plot (DiffPlot).

Monocytes are typically large cells with kidney-shaped nuclei and agranular cytoplasm that does not absorb large amounts of light. These cells are positioned high on the volume axis and low on the absorbance axis of the DiffPlot.

Neutrophils have cytoplasmic granules and segmented nuclei that cause them to absorb light more intensely than other cell types. Cells that are more complex, such as neutrophils, appear further to the right on the DiffPlot.

Eosinophils are stained more intensely than the other cells. Eosinophils show higher absorbance than the neutrophils although they are similar in volume.

Figure 3 shows the normal positions of the lymphocytes, monocytes, neutrophils, and eosinophils on the DiffPlot.

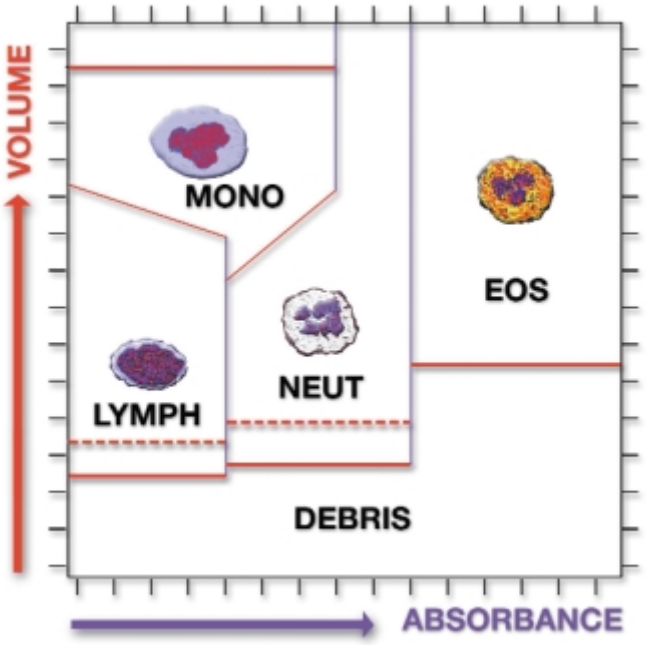

FIGURE 3. DiffPlot: normal cell positions. Mono indicates monocytes; AT, atypical lymphocytes; Lymph, lymphocytes; Neut, neutrophils; Eos, eosinophils.

Figure 3 also indicates where the debris might be positioned. A series of Diff Region flags indicate the possible presence of abnormal cell types or interference. These are shown in Figure 4.

Basophils are analyzed simultaneously with the WBC count in the WBC/BASO bath. Using specific cell lysis and impedance technology, in conjunction with histogram thresholds, the basophils are separated from the other WBCs and their percentage is determined.

Methods

A total of 111 specimens collected into K_3EDTA, were used in this study. The sample population was 50% normal and 50% abnormal. The normal specimens were obtained from a healthy blood-donor program and the abnormal specimens from a large tertiary-care hospital. All specimens were maintained at room temperature and analyzed on both the AcT 5diff and HmX systems within 1 hour of each other and within 8 hours of phlebotomy.

The Coulter HmX is a midvolume hematology analyzer. It was chosen as the reference (comparator) for the method comparison for both CBC and WBC differential parameters. In addition, to assess clinical utility and flagging performance of the WBC differential, a peripheral blood smear was prepared from each specimen using the wedge method. The blood smears were stained with Wright stain, and a 100-cell manual differential was performed under oil immersion. The performances of the comparator instrument and the manual WBC differential method are well established [1-3].

For the CBC parameters, paired-sample precision and accuracy, compared with the HmX analyzer, were evaluated over the clinical range of the system. WBC differential results from the AcT 5diff analyzer were also compared with results from the HmX analyzer. Clinical utility and flagging performance, compared with the manual differential, were used to determine the effectiveness of the flagging system.

Paired-Sample Precision

Paired-sample precision was measured on both the test and comparator instruments. This method provides information about total system precision including random sample and instrument error. Patient specimens were processed in duplicate, and the differences between the 2 analyses were calculated for each system. Samples with system flags were eliminated from the data analysis. (System flags include any of the following: AcT 5diff analyzer: Hgb R flags, +++ over range, *WBC, WBC, RBC, and/or Plt V, and . . . . . incomplete; HmX analyzer: WBC *R, RDW R, Plt and MPV R flags, WBC, RBC, Plt *V and V flags, and ….. incomplete or ++++ over range.) The mean and SD of the differences were calculated.

Method Comparison: CBC Parameters

CBC results from the test method (y), the AcT 5diff analyzer, were compared with those from the reference method

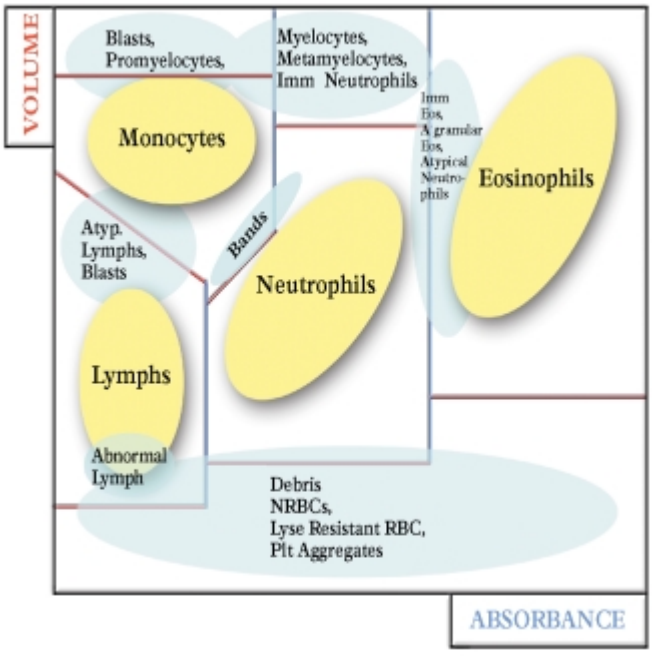

FIGURE 4. DiffPlot cell populations. Imm indicates immature; Eos, eosinophils; Atyp, atypical; Lymphs, lymphocytes; NRBCs, nucleated red blood cells; RBC, red blood cells; Plt, platelet.

TABLE 2. Paired-Sample Precision*

Parameter	Number of Samples	Mean	Mean of Differences		SD of Differences	
			HmX	AcT 5diff	HmX	AcT 5diff
WBC, $\times 10^3$ cells/μL	90	10.06	–0.08	0.01	0.53	0.38
RBC, $\times 10^6$ cells/μL	109	4.108	–0.009	0.001	0.06	0.04
HGB, g/dL	110	12.68	0.00	0.00	0.10	0.10
MCV, fL	110	87.6	0.00	–0.10	0.90	0.65
HCT, %	109	35.92	–0.01	0.02	0.67	0.46
RDW	107	16.5	0.01	–0.01	0.23	0.48
PLT, $\times 10^6$ cells/μL	87	223.3	0.2	1.4	10.78	15.54
MPV	87	8.63	0.00	0.00	0.20	0.19

*WBC indicates white blood cells; RBC, red blood cells; HGB, hemoglobin; MCV, mean corpuscular volume; HCT, hematocrit; RDW, red cell distribution width; PLT, platelet; MPV, mean platelet volume.

(*x*), the HmX analyzer. The comparison included CBC parameter results for WBC, RBC, HGB, mean corpuscular volume (MCV), hematocrit (HCT), red cell distribution width (RDW), PLT, and mean platelet volume (MPV).

Patient specimens were analyzed on both the AcT 5diff and HmX systems within 1 hour of each other. The data set for each parameter included only unflagged results; sample sets were eliminated from the calculations if system flags were present on results from either analyzer. The CBC results were compared by regression analysis, and the coefficients of correlation, slope and intercept of the regression line, and the mean difference between methods were reported.

Method Comparison: WBC Differential Parameters

The WBC differential results from the test method (*y*), AcT 5diff analyzer, were compared with results from the reference method (*x*), the HmX analyzer. This comparison included the WBC differential results, neutrophils, lymphocytes, monocytes, and eosinophils. The data set for each parameter included only results from samples that were unflagged. In this laboratory, flagged samples are followed up with a manual differential. The WBC differential results were compared by regression analysis, and the coefficients of correlation, slope and intercept of the regression line, and the mean difference between methods were reported.

Clinical Utility and Flagging Performance

The clinical utility of the AcT 5diff analyzer was determined by comparing the instrument positive results (those with WBC differential flags) from the AcT 5diff analyzer with the results from the 100-cell manual differential count. A differential was considered positive whenever 1 or more of the following interpretive messages or conditions was present:

- AcT 5diff anaylzer: DiffPlot flags (DB, SL, SL1, NL, MN, UM, LN, UN, NE, ATL, IMM), NRBC flag, and WBC parameter interference flags.

TABLE 3. Method Comparison: Complete Blood Count Parameters*

Parameter	Number of Samples	Population Range	Mean		Mean of Difference (*y* – *x*)	Slope	Intercept	Coefficient of Correlation
			x	*y*				
WBC, $\times 10^3$ cells/μL	96	0.5-37.1	10.0	10.0	0.0	1.028	–0.257	0.999
RBC, $\times 10^6$ cells/μL	109	2.57-6.11	4.31	4.11	–0.20	0.967	–0.063	0.995
HGB, g/dL	111	7.7-18.3	13.1	12.7	–0.4	1.018	–0.630	0.998
MCV, fL	111	75.3-106.2	89.7	87.5	–2.20	0.975	0.034	0.960
HCT, %	109	23.0-54.7	38.6	35.9	–2.7	0.981	–1.919	0.991
RDW†	110	11.2-22.4	13.5	12.8	–0.7	0.499	6.065	0.878
PLT, $\times 10^6$ cells/μL	89	88-654	255	225	–30	0.894	–3.076	0.986
MPV, fL	89	6.3-10.4	7.8	8.7	0.9	0.824	2.245	0.932

*WBC indicates white blood cells; RBC, red blood cells; HGB, hemoglobin; MCV, mean corpuscular volume; HCT, hematocrit; RDW, red cell distribution width; PLT, platelet; MPV, mean platelet volume.

†Technology difference.

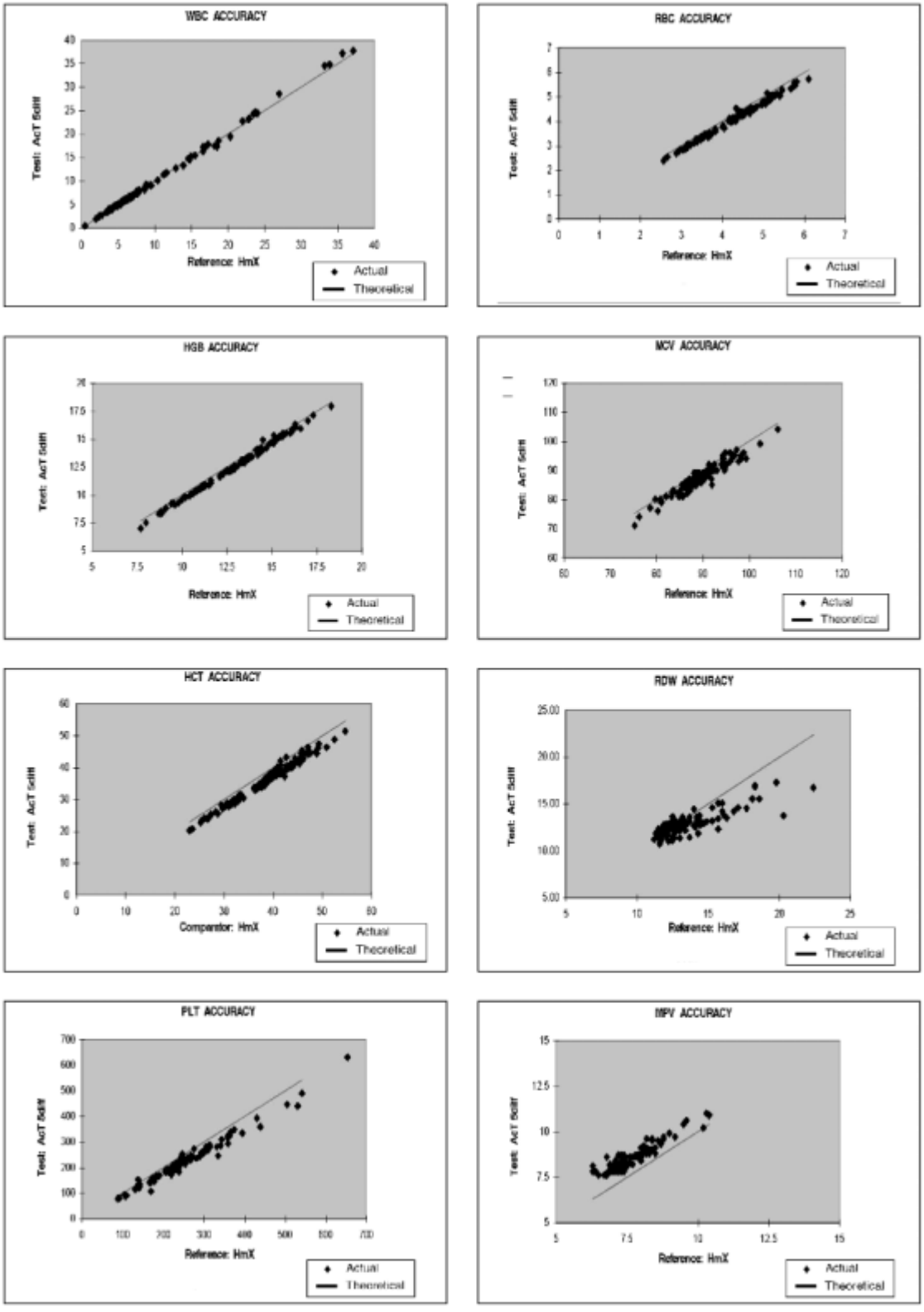

FIGURE 5. Complete blood count (CBC) parameters: *x*, *y* regression plots. WBC indicates white blood cells; RBC, red blood cells; HGB, hemoglobin; MCV, mean corpuscular volume; HCT, hematocrit; RDW, red cell distribution width; PLT, platelets; MPV, mean platelet volume.

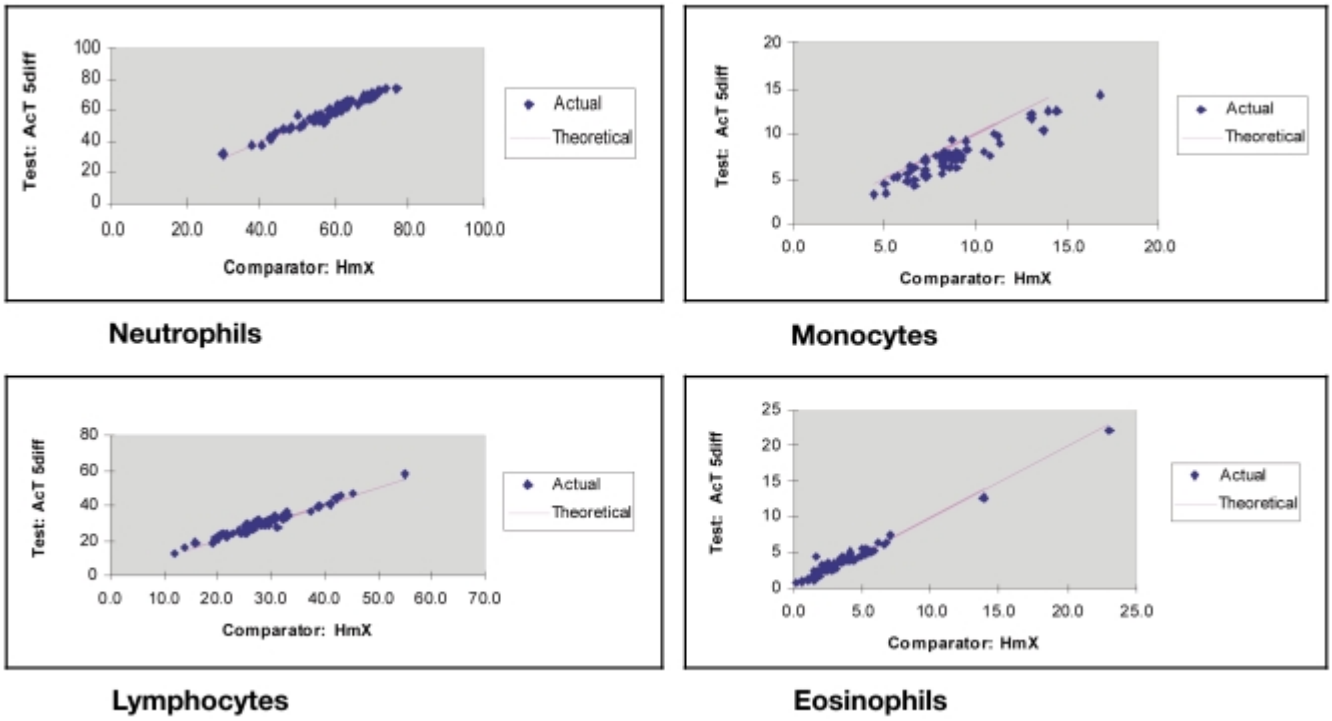

FIGURE 6. White blood cell (WBC) differential parameters: *x*, *y* regression plots.

- Manual WBC differential: bands >10.0%, metamyelocytes >1.0%, myelocytes >1.0%, promyelocytes >1.0%, blasts >1.0%, and NRBCs >1.0%.
- Instrument-generated system flags for RBCs and PLTs, excluding NRBCs, were not considered.

Flagging performance was assessed using the WBC Suspect flags compared with the routine laboratory protocol. For this evaluation, the laboratory protocol required a scan of the stained blood smear for all samples without flagged results. If abnormal cells were observed on the scan, a complete WBC manual differential was performed. A 100-cell manual differential, with morphology, was performed on all samples with flagged results.

RESULTS

Paired-Sample Precision

Results from the CBC paired-sample precision study for the AcT 5diff and HmX analyzers are shown in Table 2. The mean and SD of the differences for all parameters were well within the precision specifications for each system.

Method Comparison: CBC Parameters

The CBC parameters were compared by regression analysis, and the results are shown in Table 3. The *x*, *y* regression plots are shown in Figure 5. The HmX results are on the *x*-axis (reference), and the AcT 5diff results are on the *y*-axis (test).

WBC, RBC, HGB, and HCT had coefficients of correlation of >0.99, MCV and PLT of >0.96, and MPV of >0.93. Only RDW had a low coefficient of correlation of 0.88, likely because of technology differences between the 2 systems in the determination of this parameter.

Method Comparison: WBC Differential Parameters

The WBC differential parameters, neutrophils, lymphocytes, monocytes, and eosinophils, were compared by regres-

TABLE 4. Method Comparison: White Blood Cell Differential Parameters

Parameter	Number of Samples	Population Range	Mean		Mean of Difference ($y - x$)	Slope	Intercept	Coefficient of Correlation
			x	*y*				
Neutrophils	60	29.8-76.5	58.4	58.1	–0.3	0.97	1.55	0.984
Lymphocytes	60	12.1-55.3	28.7	30.0	1.3	1.00	1.35	0.983
Monocytes	60	4.4-16.8	8.5	7.2	–1.3	0.86	–0.15	0.942
Eosinophils	60	0.2-23.0	3.6	3.9	0.3	0.91	0.62	0.986

TABLE 5. Clinical Utility

	Calculation	Result
True Negative (TN)	n	60
True Positive (TP)	n	29
False Negative (FN)	n	3
False Positive (FP)	n	19
Total	n	111
Reportable, %	[(TN + FN)/Total] × 100	56.8
Review, %	[(TP + FP)/Total] × 100	43.2
Sensitivity	[TP/(TP + FN)] × 100	90.63
Specificity	[TN/(TN + FP)] × 100	75.95
Efficiency	[(TP + TN)/Total] × 100	80.18
Predictive value of positive	[TP/(TP + FP)] × 100	60.42
Predictive value of negative	[TN/(TN + FN)] × 100	95.24
False positive, %	(FP/Total) × 100	17.12
False negative, %	(FN/Total) × 100	2.70
Flagging rate HmX	[(FN + TP)/Total] × 100	28.83
Flagging rate AcT 5diff	[(FP + TP)/Total] × 100	43.24

sion analysis, and the results are shown in Table 4. The *x*, *y* regression plots are shown in Figure 6. The HmX analyzer results are on the *x*-axis (reference), and the AcT 5diff results are on the *y*-axis (test).

Coefficients of correlation were >0.98 for neutrophils, lymphocytes, and eosinophils and >0.94 for monocytes. Similar results comparing the AcT 5diff analyzer to the 100-cell manual differential have been reported by another investigator [4].

Clinical Utility and Flagging Performance

Results of the clinical utility analysis are summarized in Table 5. In this study, the AcT 5diff system demonstrated a false-negative rate of 2.7%. Three false-negative samples were noted in the comparison. The false-positive rate of 17.1% included 19 false-positive samples. Details of the false-positive and false-negative samples for the AcT 5diff system are described in Table 6. Sensitivity was calculated at 90.6%, specificity at 76.0%, and efficiency at 80.2%.

Using the laboratory's protocol for handling WBC differential results, a total of 48 samples (43.2%) would have required follow-up with a manual WBC differential, and 63 samples (56.8%) would have required only a scan of the stained blood smear. This is consistent with the sample population mix of 50% abnormal and 50% normal used in the study.

TABLE 6. False-Negative and False-Positive Specimens

False Negatives, n	False Positives, n	Abnormal Cells	Flag
1		Bands (14%)	None
1		Bands (14%), metamyelocytes (1%)	None
1		Bands (17%), metamyelocytes (1%)	None
	12	None	IMM*
	2	None	Atyp Lym*
	5	None	SL, SL1, Cold Agg

*Triggered based on user-definable flagging levels; false-positive ratio may be reduced by adjusting those levels.

DISCUSSION

The Beckman Coulter AcT 5diff hematology analyzer provides accurate and precise results through high-quality technology at an affordable price. The results of this evaluation demonstrate the AcT 5diff analyzer's precision and accuracy of performance compared with a more complex midvolume hematology system for the directly measured CBC parameters. The AcT 5diff system also provides the low-volume laboratory with an automated 5-part WBC differential that is both accurate and able to properly identify patient specimens requiring follow-up.

Paired-sample precision measured on the AcT 5diff and HmX analyzers was considered similar when comparing the mean difference and SD. Only the platelet parameter showed a slightly higher mean difference and SD on the AcT 5diff analyzer; this result was within the precision specifications and was not considered to be clinically significant.

Instrument-to-instrument agreement of the CBC parameters demonstrates good correlation for the WBC, RBC, HGB, HCT, and PLT parameters. Correlation coefficients for these parameters were >0.99, except for PLT, which had a correlation coefficient of >0.96. Correlation coefficient for MCV was very good at >0.96. However, the mean differences for both MCV and HCT were somewhat high, indicating a bias (–2.2 and –2.65, respectively). This result can be attributed to differences in technology and calibration between the 2 instrument systems for these parameters: on the AcT 5diff, the HCT is directly measured and the MCV calculated; on the HmX, the MCV is directly measured and the HCT calculated.

The AcT 5diff hematology analyzer competently performs the WBC differential and screens appropriately for morphologically abnormal samples. An efficiency rate of >80% means the laboratory will properly identify abnormal specimens for follow-up but will not be burdened with excessive numbers of unnecessary manual differentials.

The AcT 5diff hematology analyzer is reliable and gives results that are precise and accurate. It provides the low-volume laboratory with high-quality results comparable to those of higher-volume, more complex systems.

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