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Comparison of five automated hematology analyzers in a university hospital setting: Abbott Cell-Dyn Sapphire, Beckman Coulter DxH 800, Siemens Advia 2120i, Sysmex XE-5000, and Sysmex XN-2000

DOI 10.1515/cclm-2014-0945

Received September 24, 2014; accepted December 7, 2014

Abstract

Background: Various types of automated hematology analyzers are used in clinical laboratories. Here, we performed a side-by-side comparison of five current top of the range routine hematology analyzers in the setting of a university hospital central laboratory.

Methods: Complete blood counts (CBC), differentials, reticulocyte and nucleated red blood cell (NRBC) counts of 349 patient samples, randomly taken out of routine diagnostics, were analyzed with Cell-Dyn Sapphire (Abbott), DxH 800 (Beckman Coulter), Advia 2120i (Siemens), XE-5000 and XN-2000 (Sysmex). Inter-instrument comparison of CBCs including reticulocyte and NRBC counts and investigation of flagging quality in relation to microscopy were performed with the complete set of samples. Inter-instrument comparison of five-part differential was performed using samples without atypical cells in blood smear (n=292). Automated five-part differentials and NRBCs were additionally compared with microscopy.

Results: The five analyzers showed a good concordance for basic blood count parameters. Correlations between instruments were less well for reticulocyte counts, NRBCs, and differentials. The poorest concordance for NRBCs

with microscopy was observed for Advia 2120i (Kendall's $\tau_b=0.37$). The highest flagging sensitivity for blasts was observed for XN-2000 (97% compared to 65%–76% for other analyzers), whereas overall specificity was comparable between different instruments.

Conclusions: To the best of our knowledge, this is the most comprehensive side-by-side comparison of five current top of the range routine hematology analyzers. Variable analyzer quality and parameter specific limitations must be considered in defining laboratory algorithms in clinical practice.

Keywords: complete blood count; differential; flagging quality; hematology analyzer; inter-instrument comparison.

Introduction

Automated hematology analyzers are used for quantitative high-throughput analysis of complete blood counts (CBC), differentials, reticulocyte and nucleated red blood cell (NRBC) counts. They are also used for identification of pathological patient samples labeled by different flagging alerts for subsequent microscopic evaluation. A primary demand for hematology analyzers is to minimize the number of samples for which time- and personnel-intensive microscopic review is needed without increase of false-negative results. Therefore, all top level instruments combine different techniques, such as impedance and flow cytometry, some of them additionally use fluorescence techniques for optimized cell classification [1, 2]. Only few published studies have performed side-by-side testing of different hematology analyzers equipped with different techniques or different analytical algorithms and only few studies investigated a larger number of

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instruments in parallel [1–3]. Therefore, data about the diagnostic performance of different measurement technologies are scarce. Moreover, some of the previous evaluation studies were biased towards currently available instrumentation in that laboratory or towards selected patient groups, because testing was performed with flagged samples from routine analysis [2] or with samples from patients in a hematology setting [4].

The aim of our study was to perform an unbiased side-by-side comparison of five current top of the range routine hematology analyzers in the setting of a university hospital central laboratory, regarding inter-instrument comparison of CBC, differential, reticulocyte and NRBC counts and flagging quality.

Materials and methods

Evaluation protocol and specimens

Evaluation was performed during a two-month period, in which all five hematology analyzers were placed in the Institute of Laboratory Medicine, Hospital of Ludwig-Maximilians-University Munich. The XE-5000 (Sysmex, Kobe, Japan) was available in the laboratory as routine instrument, the Cell-Dyn Sapphire (Abbott, Santa Clara, USA), the DxH 800 (Beckman Coulter, Miami, USA), the Advia 2120i (Siemens Healthcare Diagnostics, Eschborn, Germany) and the XN-2000 (Sysmex, Kobe, Japan) were provided by the different companies. Inter-instrument comparisons, comparisons to manual microscopy and comparisons of flagging quality were designed following relevant aspects in International Council for Standardization in Haematology (ICSH) [5] or Clinical and Laboratory Standards Institute (CLSI) guidelines [6] or guidelines given by the International Consensus Group for Hematology Review [7]. Two technicians experienced in hematology were familiarized with each of the different analyzers by the companies during a 1-week period. Specimens were obtained from anonymized leftovers of K₂EDTA whole blood samples collected with the Sarstedt monovette system (Sarstedt, Nuembrecht, Germany) and submitted for routine blood count testing. Samples were stored at room temperature and all analyses in this study were completed within 6 h after sample entry into our laboratory. To permit unbiased testing of the five instruments, a total of 349 unselected study samples were taken randomly out of routine diagnostics without knowledge of clinical background or of results from potential prior blood count analysis. Up to 20 samples were randomly selected, processed each day and run on the instruments in auto-mode, samples without sufficient sample volume for analysis with the five analyzers were excluded. Blood smears were prepared from all samples with May-Gruenwald-Giemsa staining within 2 h of running on the XE-5000 as routine instrument. Specimens were analyzed on each instrument within a 4-h period, rotating the order of system application every day. Blood smear analysis was done by two experienced hematology technicians each performing a 200-cell manual differential as recommended by ICSH and CLSI guidelines [5, 6]. Potential observer dependent differences between the two technicians were evaluated with a set of 30 randomly collected patient samples, and no

significant observer bias was noted. Following the International Consensus Group for Haematology Review, blood smears were assessed to be positive for abnormal cells in case of presence of >0% blasts or presence of immature granulocytes (IGs) with $\geq 2\%$ metamyelocytes or $\geq 1\%$ myelocytes/promyelocytes [7]. Left shift was defined as a band count $\geq 8\%$ in blood smear. The manual NRBC count was expressed as number per 100 white blood cells (WBCs), samples were considered to be positive when optical microscopy showed ≥ 1 NRBC per 100 WBCs. A positive blood smear for variant lymphocytes was assessed in case of presence of $\geq 1\%$ plasma cells, $\geq 5\%$ atypical lymphocytes with suspected activation characteristics or $\geq 2\%$ abnormal lymphocytes with suspected malignant characteristics. To investigate a relationship between flagging sensitivity and concentration of abnormal cells, flagging sensitivity for presence of blasts and variant lymphocytes as clinically most relevant warning messages was further assessed using a cut-off of $\geq 5\%$ blasts or variant lymphocytes as criteria for positive blood smear.

In a sub-study, platelet (PLT) counts were additionally determined in 30 EDTA blood samples using an anti-CD61 based immunological method which is available in the Cell-Dyn Sapphire, in addition to conventional PLT testing.

Instrumentation, flagging parameters and instrument setting

The investigated hematology analyzers utilize different technologies for determination of CBC, differential, reticulocytes and NRBCs (Table 1) [4, 8–10]. Flagging messages for presence of immature cells, atypical lymphocytes and PLT clumps are similar between the five different instruments. As special features, DxH 800 further differentiates blasts with neutrophil, lymphocyte or monocyte character. Sysmex XE and XN instruments differentiate immature cells into blasts or blasts/abnormal lymphocytes and pathological lymphocytes into abnormal lymphocytes with suspected malignant characteristics or atypical lymphocytes with suspected activation characteristics. In the XN, a white progenitor cell (WPC) channel further allows discrimination between blast cells and abnormal lymphocytes. In the present study, differentiated blast flags in DxH 800 were not considered and the two lymphocyte flags in Sysmex instruments were assessed together as variant lymphocytes to ensure homogeneity with other instruments. Default settings for flagging notes and most favorable test algorithms were used for all three instruments. The fluorescence PLT (PLT-F) channel was used as principal PLT testing in XN-2000.

Testing of required sample volume

For inter-instrument comparison of required sample volumes, different volumes of unselected K₂EDTA routine whole blood samples (500 μ L, 300 μ L, 200 μ L, 150 μ L) were transferred into four different tube types [2.7 mL and 1.2 mL K₂EDTA Sarstedt monovettes, 1.0 mL Siemens EZEE-nest tubes (Siemens, Munich, Germany), 1.5 mL Sarstedt micro tubes] and subsequently determined on different hematology analyzers. Any liquid (dissolved EDTA) contained in these tubes was discarded prior to adding the described volumes of anticoagulated blood. Blood count results of this experiment were otherwise not used in our study.

Table 1 Measurement technologies of different haematology analyzers.

Parameter	Instrument	DxH 800	Advia 2120i	XE-5000	XN-2000
	Cell-Dyn Sapphire				
Hemoglobin	Photometry	Photometry	Photometry	Photometry	Photometry
RBC count	Impedance/Light scatter	Impedance	Light scatter	Impedance	Impedance
PLT count	Impedance/Light scatter/Ab based	Impedance	Light scatter	Impedance/Light scatter/Fluorescence	Impedance/Light scatter/Fluorescence
WBC count	Light scatter/Fluorescence	Impedance	Light scatter/Peroxidase	Impedance/Light scatter/Fluorescence	Light scatter/Fluorescence
5-part differential	Light scatter/Fluorescence	Impedance/Conductivity/Light scatter	Light scatter/Peroxidase	Light scatter/Fluorescence	Light scatter/Fluorescence
Reticulocytes	Light scatter/Fluorescence	Impedance/Conductivity/Light scatter	Light scatter	Light scatter/Fluorescence	Light scatter/Fluorescence
NRBC count	Light scatter/Fluorescence	Impedance/Conductivity/Light scatter	Light scatter/Peroxidase	Light scatter/Fluorescence	Light scatter/Fluorescence

Ab, antibody; NRBC, nucleated red blood cell; PLT, platelet; RBC, red blood cell; WBC, white blood cell.

Data analysis and statistics

The complete set of randomly selected patient samples (n=349) was used for inter-instrument comparison of CBC parameters, NRBC and reticulocyte counts between different hematology analyzers. As blasts and variant lymphocytes are not included in the automated differential, inter-instrument comparison of five-part differential was performed using a subgroup of 292 samples without blasts or variant lymphocytes in blood smear. Automated five-part differentials of the sample subgroup and NRBC counts of the complete set of samples generated by the different analyzers were further compared with results of manual microscopy.

Analysis of correlation and regression analysis was done for measurements from each analyzer against the median of all five analyzers. For CBC parameters, differentials and reticulocyte counts method comparison was performed using Passing-Bablok regression [11]. Confidence intervals for slopes and intercepts were calculated to test the hypotheses slope=1 and intercept=0. The values 1 for slope and 0 for intercept not being enclosed in the respective interval indicated significant deviation. Regression equations could not be calculated for NRBCs, because for some instruments resulting slope was zero or infinity. As some investigated parameters showed significant outliers, correlations were calculated by means of Kendall's concordance coefficient τ_b [12], which is less sensitive to outliers than the commonly used correlation coefficient by Pearson. For comparison of automated NRBC counts with manual counts, automated NRBC values below 1% were set to zero. By slightly modifying the method of Bland and Altman, differences between single measurements and the median of all five analyzers were plotted against the median of all five analyzers [13]. Systematic deviation (bias) was estimated by the mean value of the differences. These differences not always corresponded to a Gaussian distribution. In addition to standard deviations, 95% limits of agreement were therefore calculated by the 2.5th and 97.5th percentile as a measure of scatter, which define the range containing 95% of the distribution. Thus, 95% limits of agreement are a measure of the degree to which an analyzer differs from the median of all instruments. We used absolute differences for all parameters except PLT counts, where a clear tendency of proportional bias could be observed and thus percentage differences were more appropriate. Furthermore, values for PLT counts were displayed on a logarithmic scale.

For inter-instrument comparison of flagging quality, all 349 samples were included, and results of microscopic evaluation were compared to flagging alerts of different analyzers. Flagging sensitivity and specificity were calculated by the number of true positives, true negatives, false positives and false negatives. Statistical analysis was performed using SAS (Version 9.3, SAS Institute Inc., Cary, NC, USA).

Results

Inter-instrument comparison of blood count parameters and reticulocyte counts (n=349)

Inter-instrument comparisons of directly analyzed CBC parameters showed good correlations with correlation coefficients $\tau_b \geq 0.93$ for all analyzers (Table 2,

Table 2 Inter-instrument comparison of blood counts, reticulocyte and NRBC counts (n=349) and comparison of automated NRBCs or PLTs to microscopy or CD61 (n=30).

System		τ_b	b	a	Mean	SD	95% limits of agreement
		Regression to median			Differences to median		
WBC, $10^3/\mu\text{L}$	Sapphire	0.98	1.00	0.00	-0.02	0.502	-0.70-0.60
	DxH 800	0.98	1.00	-0.00	0.03	0.309	-0.50-0.70
	Advia 2120i	0.97	1.05	-0.01	0.43	0.712	-0.17-1.70
	XE-5000	0.98	0.98	-0.02	-0.23	0.425	-1.00-0.08
	XN-2000	0.99	1.00	0.00	-0.05	0.187	-0.34-0.30
RBC, $10^6/\mu\text{L}$	Sapphire	0.98	1.00	0.00	0.01	0.037	-0.06-0.09
	DxH 800	0.97	0.97	-0.03	-0.13	0.049	-0.24 to -0.03
	Advia 2120i	0.96	1.00	0.06	0.06	0.062	-0.05-0.16
	XE-5000	0.97	1.00	0.00	0.01	0.087	-0.05-0.08
	XN-2000	0.96	1.03	-0.11	-0.02	0.078	-0.12-0.09
HGB, mmol/L	Sapphire	0.98	1.00	0.10	0.06	0.067	-0.06-0.19
	DxH 800	0.96	1.00	0.00	-0.03	0.113	-0.25-0.12
	Advia 2120i	0.98	1.00	0.00	0.01	0.070	-0.06-0.12
	XE-5000	0.96	1.00	-0.10	-0.07	0.249	-0.25-0.06
	XN-2000	0.98	1.00	0.00	-0.01	0.142	-0.12-0.12
HCT	Sapphire	0.96	1.00	0.00	-0.00	0.005	-0.01-0.01
	DxH 800	0.95	1.00	-0.01	-0.01	0.005	-0.02-0.00
	Advia 2120i	0.93	1.04	0.00	0.02	0.008	0.00-0.03
	XE-5000	0.96	1.00	-0.00	-0.00	0.008	-0.01-0.00
	XN-2000	0.96	1.02	-0.00	0.00	0.007	-0.01-0.01
MCV, fL	Sapphire	0.90	1.00	-1.20	-1.26	0.981	-3.30-0.00
	DxH 800	0.91	1.00	0.00	0.52	1.074	-1.50-3.00
	Advia 2120i	0.90	1.08	-4.54	2.35	1.145	0.20-5.00
	XE-5000	0.89	0.99	-0.74	-1.86	1.180	-4.50-0.00
	XN-2000	0.90	1.00	0.00	0.45	1.199	-2.40-3.10
MCH, pg	Sapphire	0.90	1.00	0.00	0.16	0.373	-0.40-1.10
	DxH 800	0.85	1.10	-1.93	0.95	0.588	0.00-2.10
	Advia 2120i	0.84	1.00	-0.50	-0.51	0.590	-1.40-0.40
	XE-5000	0.87	1.00	-0.40	-0.46	0.572	-1.50-0.20
	XN-2000	0.92	1.00	0.00	0.09	0.330	-0.50-0.80
PLT, $10^3/\mu\text{L}$	Sapphire	0.96	1.07	-2.96	3.44	7.840	-18.18-14.42
	DxH 800	0.96	0.94	-0.33	-5.05	8.219	-16.67-11.70
	Advia 2120i	0.95	1.10	-0.27	10.50	9.693	-0.78-29.63
	XE-5000	0.96	0.97	1.31	-1.32	9.834	-14.00-22.22
	XN-2000	0.97	1.00	0.00	-3.04	7.840	-25.00-7.89
RET, %	Sapphire	0.87	1.19	0.05	0.41	0.470	-0.10-1.20
	DxH 800	0.82	1.00	0.00	0.02	0.458	-0.60-1.30
	Advia 2120i	0.75	0.86	-0.14	-0.41	0.511	-1.80-0.30
	XE-5000	0.95	1.00	0.00	-0.04	0.152	-0.40-0.20
	XN-2000	0.91	1.00	0.00	0.07	0.196	-0.30-0.50
NRBC, %	Sapphire	0.57			-0.04	1.759	-0.80-1.00
	DxH 800	0.46			-0.09	1.752	-1.10-0.60
	Advia 2120i	0.47			0.27	5.636	-1.70-3.70
	XE-5000	0.85			0.24	1.768	0.00-1.40
	XN-2000	0.84			0.09	0.692	0.00-0.50
		Regression to microscopy			Differences to microscopy		
NRBC, %	Sapphire	0.54			-0.05	3.845	-2.00-1.10
	DxH 800	0.56			-0.23	3.481	-2.00-0.80
	Advia 2120i	0.37			0.26	6.062	-2.00-4.30
	XE-5000	0.63			0.20	3.297	-1.00-1.20
	XN-2000	0.66			0.03	2.701	-1.20-0.20
		Regression to CD61			Differences to CD61		
PLT, $10^3/\mu\text{L}$	Sapphire	0.92	1.04	0.21	7.72	22.95	-35.48-94.03
	DxH 800	0.91	0.91	3.05	23.84	47.24	-20.12-160.00

System	τ_b	b	a	Mean	SD	95% limits of agreement
	Regression to CD61			Differences to CD61		
Advia 2120i	0.93	1.09	3.97	42.07	43.09	-1.15–173.33
XE-5000	0.90	1.01	1.98	19.75	33.60	-29.03–122.22
XN-2000	0.96	0.97	0.59	2.24	17.86	-25.93–56.67

τ_b , Kendall's τ_b ; b, slope (numbers in bold are significantly different from 1); a, intercept (numbers in bold are significantly different from 0); SD, standard deviation.

Supplemental Material, Figure 1, that accompanies the article <http://www.degruyter.com/view/j/cclm.2015.53.issue-7/cclm-2014-0945/cclm-2014-0945.xml?format=INT>). MCH and MCV revealed correlation coefficients $\tau_b \geq 0.84$. Systematic differences between the various analyzers were seen for WBC counts, which were slightly higher with Advia 2120i (slope 1.05), the latter also revealing the highest variability (95% limits of agreement -0.17–1.70) (Table 2, Figure 1A). Furthermore, as compared to other instruments, Advia 2120i revealed consistently higher MCV values (slope 1.08) (Table 2, Figure 1E). PLT measurements performed with Cell-Dyn Sapphire and Advia 2120i were significantly higher (slopes 1.07 and 1.10, respectively) and significantly lower for DxH 800 and XE-5000 (slopes 0.94 and 0.97, respectively) (Table 2, Figure 1G). Comparable results were found focussing on PLT counts $<50 \times 10^3/\mu\text{L}$ (data not shown). Comparison of anti-CD61 based PLT analysis with automated PLT analysis of different instruments was performed using 30 additional EDTA blood samples, of which 22 revealed CD61 based counts below $50 \times 10^3/\mu\text{L}$. Method comparison revealed τ_b values ≥ 0.90 , but also confirmed a maximum deviation to higher values using Advia 2120i (slope 1.09) (Table 2). The highest concordance to CD61 based PLT measurement could be shown for XN-2000 using PLF-F technology.

Reticulocyte counts determined with different instruments revealed correlation coefficients in the range between 0.75 and 0.95 (Table 2, Supplemental Figure 1H). Values were comparable between DxH 800, XE-5000 and XN-2000. The greatest systematic difference was found between Cell-Dyn Sapphire showing significantly higher values (slope 1.19) and Advia 2120i showing significantly lower values (slope 0.86) (Table 2, Figure 1H).

Inter-instrument comparison of NRBC counts and comparison to microscopy (n=349)

Inter-instrument comparison of NRBC counts resulted in a wide range of values for τ_b , with 0.57 for Cell-Dyn Sapphire,

0.46 for DxH 800, 0.47 for Advia 2120i, 0.85 for XE-5000 and 0.84 for XN-2000 (Table 2, Figure 2C). The highest variability in NRBC analysis (SD 5.64%) was found for Advia 2120i, with 95% limits of agreement between -1.70 and 3.70, (Table 2, Figure 2A). A rather low concordance was also observed between automated NRBC analysis performed with different analyzers and manual counts, with correlations of 0.54 for Cell-Dyn Sapphire, 0.56 for DxH 800, 0.37 for Advia 2120i, 0.63 for XE-5000 and 0.66 for XN-2000 (Table 2, Figure 2D). The greatest difference between automated and manual NRBC counts could be shown for Advia 2120i, with 95% limits of agreement between -2.0 and 4.30 (Table 2, Figure 2B). Sensitivities for the detection of NRBCs $\geq 1\%$ in manual count (n=30) were 43.3% for Cell-Dyn Sapphire, 46.7% for DxH 800, 40% for Advia 2120i, 60% for XE-5000, and 53.3% for XN-2000. Respective specificities ranged from 95% to 99%.

Inter-instrument comparison of WBC differential and comparison to microscopy (n=292)

As blasts and variant lymphocytes are not included in WBC differentials of hematology analyzers, comparisons were performed in a set of 292 out of 349 samples, after excluding samples containing blasts and variant lymphocytes in blood smears.

Neutrophil counts

Inter-instrument correlation of automated neutrophil counts revealed τ_b values ≥ 0.96 for all instruments (Table 3, Supplemental Figure 2A). The lowest variability in inter-instrument comparison (SD 0.15) with 95% limits of agreement between -0.30 and 0.20 was found for XN-2000 (Table 3, Figure 3A). Comparison of automated and manual neutrophil counts showed a somewhat lower correlation with τ_b values ≥ 0.93 (Table 3, Supplemental Figure 2B). For two samples, a difference

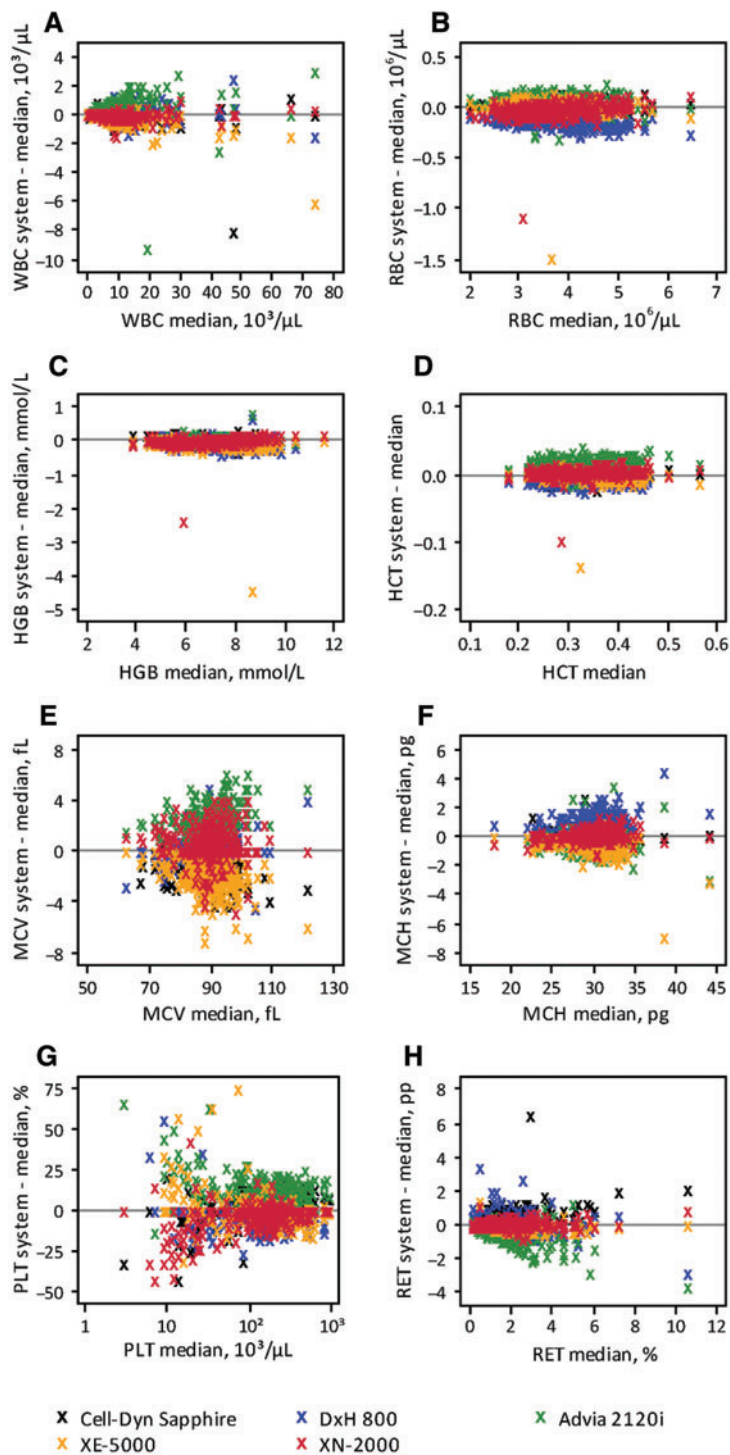


Figure 1 Difference plots for blood count parameters and reticulocyte counts.

Inter-instrument comparisons of blood and reticulocyte counts were determined in 349 routine samples. Differences between single measurements and the median of all five analyzers were plotted against the median of all analyzers. (A) WBC count, (B) RBC count, (C) hemoglobin concentration, (D) hematocrit, (E) MCV, (F) MCH, (G) PLT, (H) reticulocyte count.

of more than $5 \times 10^3/\mu\text{L}$ between automated and microscopic neutrophil counts was observed for all instruments, due to misclassification of smudge cells or IGs to neutrophils.

Lymphocyte counts

Inter-instrument correlation of automated lymphocyte counts showed τ_b values ≥ 0.94 (Table 3, Supplemental

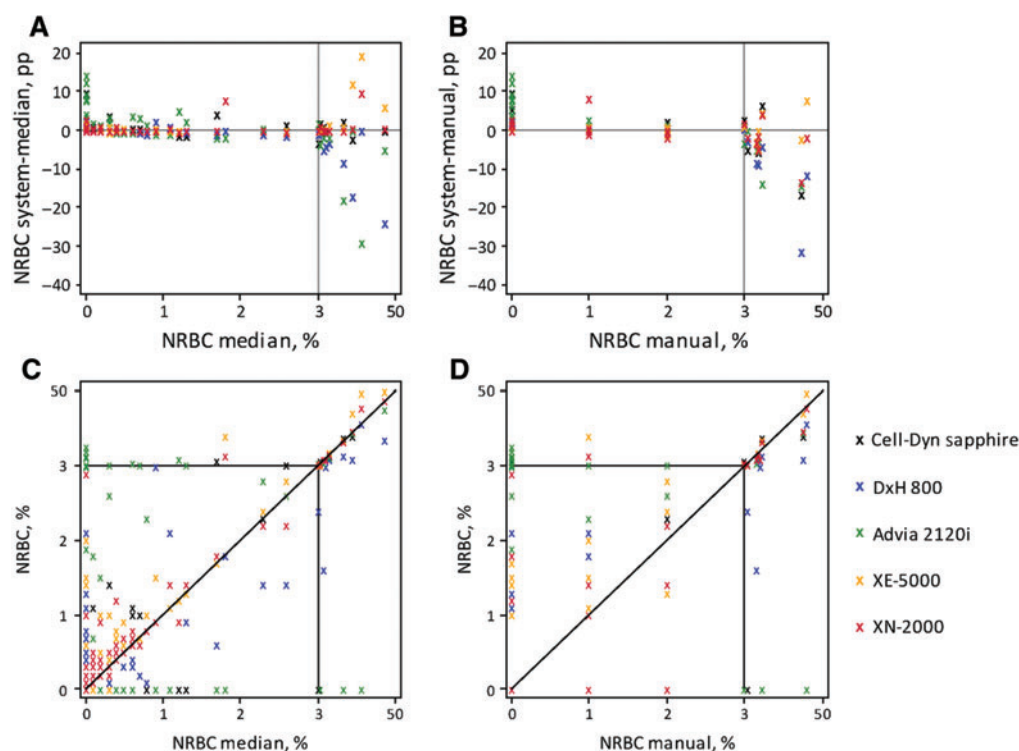


Figure 2 Inter-instrument comparison of NRBC counts and comparison to microscopy.

Inter-instrument comparison and comparison between automated and manual NRBC counts were performed in 349 routine samples. (A) Differences between single measurements of each analyzer and the median of all five analyzers plotted against the median of all analyzers. (B) Differences between automated and manual NRBC counts plotted against manual NRBC counts. (C) Single NRBC measurements of each analyzer compared to median of all five analyzers. (D) Single NRBC measurements of each analyzer compared to single manual counts. The line in the scatter plot represents identity. Axes were scaled to include all data except one data point of 94% NRBCs measured by Advia 2120i and results of one sample with manual NRBC count of 54% and automated counts between 81% and 100%.

Figure 2C), comparison with microscopy revealed τ_b values ≥ 0.80 (Table 3, Supplemental Figure 2D).

Monocyte counts

Inter-instrument correlation of automated monocyte counts revealed τ_b values between 0.85 (Advia 2120i) and 0.95 (DxH 800 and XN-2000), with Advia 2120i tending to lower values (slope 0.80) (Table 3, Figure 3E, Supplemental Figure 2E). Comparing automated and manual monocyte counts, correlation coefficients in the range of 0.66 (Advia 2120i)–0.72 (XN-2000) were found, with Advia 2120i revealing the lowest mean of differences to manual values (mean 0.06) (Table 3, Figure 3F, Supplemental Figure 2F).

Eosinophil counts

Inter-instrument correlation of automated eosinophil counts showed τ_b values between 0.90 (Cell-Dyn

Sapphire) and 0.97 (XE-5000, XN-2000) (Table 3, Supplemental Figure 2G), comparison with microscopy revealed τ_b values in the range of 0.76–0.79 (Table 3, Supplemental Figure 2H).

Basophil counts

A poor inter-instrument correlation for automated basophil counts with τ_b values ranging from 0.37 to 0.82, and between automated basophil counts and microscopic examination with τ_b values ranging from 0.17 to 0.35 was observed (Table 3).

Inter-instrument comparison of flagging quality in unselected samples (n=349)

For inter-instrument comparison of flagging quality, results of microscopic evaluation were compared to flagging alerts of different analyzers. Defining a >0% cut-off

Table 3 Inter-instrument comparison of differential blood count and comparison to manual count (n=292 out of 349 samples, excluding samples with blasts and atypical lymphocytes).

System		τ_b	b	a	Mean	SD	95% limits of agreement
		Regression to median			Differences to median		
NEU, 10 ³ /μL	Sapphire	0.98	1.00	0.00	−0.04	0.158	−0.40–0.20
	DxH 800	0.98	1.00	0.00	0.06	0.205	−0.20–0.60
	Advia 2120i	0.96	1.08	0.02	0.45	0.400	−0.10–1.40
	XE-5000	0.98	0.98	−0.04	−0.17	0.191	−0.60–0.10
	XN-2000	0.99	1.00	0.00	−0.03	0.147	−0.30–0.20
LYM, 10 ³ /μL	Sapphire	0.96	1.00	0.00	0.05	0.123	−0.10–0.30
	DxH 800	0.97	1.00	0.00	−0.04	0.085	−0.20–0.10
	Advia 2120i	0.94	1.00	0.10	0.14	0.154	0.00–0.40
	XE-5000	0.97	1.00	0.00	−0.01	0.081	−0.20–0.20
	XN-2000	0.97	1.00	0.00	−0.05	0.077	−0.20–0.00
MON, 10 ³ /μL	Sapphire	0.94	1.00	0.00	−0.01	0.078	−0.20–0.10
	DxH 800	0.95	1.00	0.00	0.00	0.064	−0.10–0.10
	Advia 2120i	0.85	0.80	0.02	−0.12	0.157	−0.50–0.10
	XE-5000	0.94	1.00	0.00	0.01	0.077	−0.10–0.20
	XN-2000	0.95	1.00	0.00	0.03	0.061	−0.10–0.20
EOS, 10 ³ /μL	Sapphire	0.90	1.00	0.00	0.02	0.060	−0.10–0.10
	DxH 800	0.96	1.00	0.00	−0.01	0.029	−0.10–0.00
	Advia 2120i	0.93	1.00	0.00	0.02	0.042	−0.00–0.10
	XE-5000	0.97	1.00	0.00	0.00	0.031	−0.10–0.00
	XN-2000	0.97	1.00	0.00	−0.01	0.028	−0.10–0.00
BAS, 10 ³ /μL	Sapphire	0.37	1.00	0.00	0.00	0.057	−0.10–0.10
	DxH 800	0.62	1.00	0.00	0.01	0.048	−0.10–0.10
	Advia 2120i	0.82	1.00	0.00	0.01	0.033	0.00–0.10
	XE-5000	0.67	1.00	0.00	−0.01	0.036	−0.10–0.00
	XN-2000	0.80	1.00	0.00	0.01	0.038	0.00–0.10
		Regression to microscopy			Differences to microscopy		
NEU, 10 ³ /μL	Sapphire	0.94	1.00	−0.05	−0.03	0.606	−0.90–0.90
	DxH 800	0.94	1.00	0.00	0.07	0.714	−0.90–1.30
	Advia 2120i	0.93	1.05	0.04	0.46	0.760	−0.30–2.10
	XE-5000	0.94	0.98	−0.05	−0.18	0.506	−1.00–0.60
	XN-2000	0.93	1.00	−0.10	−0.02	0.633	−0.90–1.00
LYM, 10 ³ /μL	Sapphire	0.82	1.00	0.20	0.19	0.301	−0.30–0.90
	DxH 800	0.84	1.00	0.10	0.09	0.275	−0.40–0.70
	Advia 2120i	0.80	1.05	0.19	0.28	0.327	−0.30–1.10
	XE-5000	0.83	1.00	0.10	0.13	0.281	−0.40–0.80
	XN-2000	0.83	1.00	0.10	0.09	0.285	−0.40–0.80
MON, 10 ³ /μL	Sapphire	0.70	1.14	0.09	0.17	0.215	−0.20–0.60
	DxH 800	0.70	1.14	0.10	0.18	0.237	−0.20–0.70
	Advia 2120i	0.66	1.00	0.10	0.06	0.205	−0.40–0.50
	XE-5000	0.69	1.17	0.08	0.19	0.235	−0.10–0.70
	XN-2000	0.72	1.14	0.13	0.21	0.238	−0.10–0.70
EOS, 10 ³ /μL	Sapphire	0.76	1.00	0.00	0.04	0.109	−0.10–0.20
	DxH 800	0.78	1.00	0.00	0.02	0.096	−0.20–0.20
	Advia 2120i	0.76	1.00	0.00	0.04	0.099	−0.10–0.20
	XE-5000	0.78	1.00	0.00	0.02	0.094	−0.20–0.20
	XN-2000	0.79	1.00	0.00	0.02	0.094	−0.20–0.20
BAS, 10 ³ /μL	Sapphire	0.17	1.00	0.00	0.01	0.065	−0.10–0.10
	DxH 800	0.26	1.00	0.00	0.01	0.065	−0.10–0.10
	Advia 2120i	0.34	1.00	0.00	0.01	0.062	−0.10–0.10
	XE-5000	0.35	1.00	0.00	−0.01	0.049	−0.10–0.10
	XN-2000	0.30	1.00	0.00	0.01	0.066	−0.10–0.10

τ_b , Kendall's τ_b ; b, slope (numbers in bold are significantly different from 1); a, intercept (numbers in bold are significantly different from 0); SD, standard deviation.

for presence of blasts in blood smear, cut-offs $\geq 1\%$ plasma cells, $\geq 5\%$ atypical lymphocytes or $\geq 2\%$ abnormal lymphocytes for presence of variant lymphocytes, cut-offs $\geq 2\%$ metamyelocytes or $\geq 1\%$ myelocytes/promyelocytes for presence of IG and $\geq 8\%$ bands for presence of left shift, microscopy identified 34 samples with blasts, 25 samples with variant lymphocytes, 90 samples with IGs and 76 samples with left shift out of 349 unselected samples. Seven samples revealed PLT clumps in microscopy. A complete overview of samples with pathological findings in microscopy, the number of true-positive and false-positive flagging alerts in automated analysis with different instruments and respective sensitivities and specificities are shown in Table 4.

Blast flagging

All except one out of 34 blast positive samples could be identified with a blast flag by XN-2000, whereas 8–12 blast positive samples could not be correctly identified by other instruments (Table 4). The one not identified sample by XN-2000 and 4, 7, 3 and 6 not identified samples by Cell-Dyn Sapphire, DxH 800, Advia 2120i and XE-5000, respectively, revealed WBC counts $< 2 \times 10^3/\mu\text{L}$. Two out of eight samples showing blasts in microscopy but without blast flagging in Cell-Dyn Sapphire revealed a variant lymphocyte flag, four an IG flag. Five out of nine samples without correct blast flagging in DxH 800 revealed a variant lymphocyte flag, two an IG flag. Six out of 12 blast containing samples were flagged to be positive for variant lymphocytes in Advia 2120i, five to be positive for IGs. Two out of 12 samples showing blasts in microscopy but without blast flagging in XE-5000 revealed a variant lymphocyte flag, five an IG flag. Two samples with blasts in microscopy were not even flagged by Cell-Dyn Sapphire and DxH 800, respectively, one sample by Advia 2120i and XN-2000, respectively, and four samples by XE-5000.

A blast flagging without verification of blasts in microscopy was found in 21 samples for Cell-Dyn Sapphire, in 15 samples for DxH 800, in 12 samples for Advia 2120i, in six samples for XE-5000 and in 14 samples for XN-2000. Flagging sensitivity was highest for XN-2000 (97%) and markedly lower for all other instruments (Cell-Dyn Sapphire, 76%; DxH 800, 74%; Advia 2120i and XE-5000, 65%). Setting the cut-off from > 0 to $\geq 5\%$ presence of blasts in blood smear, slightly better sensitivities could be shown for different instruments (Cell-Dyn Sapphire, 90%; DxH 800, 84%; Advia 2120i, 74%; XE-5000, 79%; XN-2000, 100%). Flagging specificity was comparable between instruments (93% to 98%).

Variant lymphocyte flagging

Samples described as positive for variant lymphocytes in microscopy ($n=25$) clearly revealed activation or atypical characteristics. Fourteen, 16 or 18 variant lymphocyte positive samples were correctly identified by Cell-Dyn Sapphire, DxH 800 or Advia 2120i. XE-5000 and XN-2000 each correctly identified 20 positive samples (Table 4). Two out of 11 samples showing variant lymphocytes in microscopy but without variant lymphocyte flagging in Cell-Dyn Sapphire revealed a blast flag, two an IG flag. Two out of nine samples that were not correctly flagged by DxH 800 for variant lymphocytes revealed a blast flag. One out of seven samples without correct variant lymphocyte flagging was classified as IG positive by Advia 2120i. One or four out of five samples showing variant lymphocytes in blood smear but without variant lymphocyte flagging in XE-5000 or XN-2000 were flagged to be positive for blasts, three samples were flagged to be positive for IGs by XE-5000. Seven or six samples with variant lymphocytes in microscopy were not even flagged by Cell-Dyn Sapphire, DxH 800 or Advia 2120i, and one sample by XE-5000 and XN-2000, respectively.

A variant lymphocyte flag without verification in microscopy was found in 18 samples for Cell-Dyn Sapphire and DxH 800, in 40 samples for Advia 2120i, in 17 samples for XE-5000 and in 14 samples for XN-2000. Flagging sensitivity was highest for XE-5000 and XN-2000 (80%) and markedly lower for other instruments (Cell-Dyn Sapphire, 56%; DxH 800, 64%; Advia 2120i, 72%). Setting the cut-off to $\geq 5\%$ presence of variant lymphocytes in blood smear resulted in clearly better sensitivities (Cell Dyn Sapphire, DxH 800, XN-2000, 78%, respectively; Advia 2120i, 89%; XE-5000, 100%). Except Advia 2120i (88%), flagging specificity was comparable between instruments (94% to 95%).

Combining the clinically most relevant flags for blasts and variant lymphocytes resulted in sensitivities from 74% (Cell-Dyn Sapphire) to 96% (XN-2000), and in specificities from 94% to 96% for different instruments (Table 4).

IG and left shift flagging

Out of 90 samples revealing IGs (promyelocytes, myelocytes or metamyelocytes) in microscopy, 49 samples were correctly flagged by Cell-Dyn Sapphire, 60 samples by DxH 800, 35 samples by Advia 2120i, 72 samples by XE-5000 and 82 samples by XN-2000 (Table 4). Numbers of false-positive IG flags were 24 for Cell-Dyn Sapphire, 16 for DxH 800, 11 for Advia 2120i, 21 for XE-5000 and

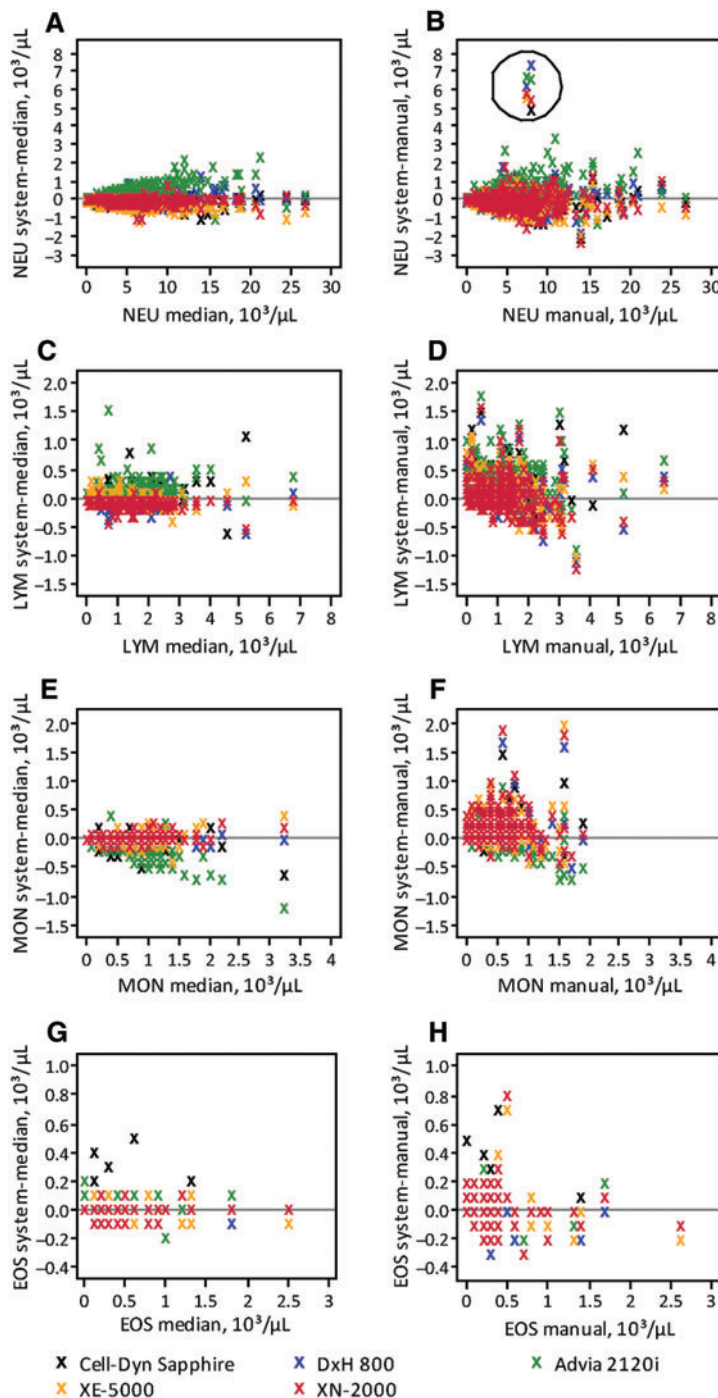


Figure 3 Difference plots for differential blood counts.

Automated and manual differential blood counts were compared in samples without blasts or variant lymphocytes in blood smear ($n=292$). A, C, E, and G show differences between automated counts and the median of all five analyzers plotted against the median. B, D, F, and H show differences between automated and manual counts plotted against manual count. (A, B) neutrophil count, (C, D) lymphocyte count, (E, F) monocyte count, (G, H) eosinophil count. Samples with a difference of more than $5 \times 10^3/\mu\text{L}$ between automated and microscopic neutrophil counts are circled.

35 for XN-2000. Out of 76 samples revealing left shift in microscopy, 39 were correctly flagged by Cell-Dyn Sapphire, 64 by DxH 800, 39 by Advia 2120i, 38 by XE-5000

and 36 by XN-2000. As compared to microscopy, a false-positive left shift flag occurred in 13 samples for Cell-Dyn Sapphire, in 27 samples for DxH 800, in 14 samples for

Table 4 Inter-instrument comparison of pathological flaggings in 349 samples taken randomly out of routine analysis.

Instrument flagging	Pathological samples in microscopy, n	Instrument	True positives, n	Sensitivity 95% CI, %	False positives, n	Specificity 95% CI, %
Blasts	34	Sapphire	26	76 (59–89)	21	93 (90–96)
		DxH 800	25	74 (56–87)	15	95 (92–97)
		Advia 2120i	22	65 (46–80)	12	97 (94–98)
		XE-5000	22	65 (46–80)	6	98 (96–99)
		XN-2000	33	97 (85–100)	14	96 (93–98)
Variant lymphocytes	25	Sapphire	14	56 (35–76)	18	94 (91–97)
		DxH 800	16	64 (43–82)	18	94 (91–97)
		Advia 2120i	18	72 (51–88)	40	88 (84–91)
		XE-5000	20	80 (59–93)	17	95 (92–97)
		XN-2000	20	80 (59–93)	14	95 (93–98)
Immature granulocytes	90	Sapphire	49	54 (44–64)	24	91 (87–94)
		DxH 800	60	67 (56–76)	16	94 (90–96)
		Advia 2120i	35	39 (29–50)	11	96 (93–98)
		XE-5000	72	80 (70–88)	21	92 (88–95)
		XN-2000	82	91 (83–96)	35	86 (82–90)
Left shift	76	Sapphire	39	51 (40–63)	13	95 (92–97)
		DxH 800	64	84 (74–92)	27	90 (86–93)
		Advia 2120i	39	51 (40–63)	14	95 (92–97)
		XE-5000	38	50 (38–62)	1	99 (98–100)
		XN-2000	36	47 (36–59)	7	97 (95–99)
Platelet clumps	7	Sapphire	4	57 (18–90)	8	98 (96–99)
		DxH 800	6	86 (42–100)	7	98 (96–99)
		Advia 2120i	4	57 (18–90)	6	98 (96–99)
		XE-5000	4	57 (18–90)	8	98 (96–99)
		XN-2000	4	57 (18–90)	4	99 (97–100)
Blasts and/or variant lymphocytes	57	Sapphire	42	74 (60–84)	16	95 (91–97)
		DxH 800	46	81 (68–90)	15	95 (92–97)
		Advia 2120i	44	77 (64–87)	18	94 (90–96)
		XE-5000	43	75 (62–86)	11	96 (93–98)
		XN-2000	55	96 (88–100)	18	94 (90–96)
Blasts and/or variant lymphocytes and/or immature granulocytes	103	Sapphire	70	68 (58–77)	29	88 (84–92)
		DxH 800	80	78 (68–85)	29	88 (84–92)
		Advia 2120i	66	64 (54–73)	26	89 (85–93)
		XE-5000	88	85 (77–92)	30	88 (83–92)
		XN-2000	101	98 (93–100)	54	78 (72–83)

CI, confidence interval; n, number.

Advia 2120i, in one sample for XE-5000 and in seven samples for XN-2000. The highest flagging sensitivity for the presence of IGs could be shown for XN-2000 (91%), Advia 2120i revealed the lowest (39%). Flagging sensitivity and specificity for left shift were comparable among different instruments (47%–51%; 95%–99%), except DxH 800 revealing a sensitivity of 84% and a specificity of 90% (Table 4).

PLT clump flagging

PLT clumping was identified by microscopy in seven samples. Out of these, six were correctly flagged by DxH

800, and four samples were correctly flagged by the other instruments (Table 4). Four out of seven samples with PLT clumps in microscopy revealed automated PLT counts in the range of $30\text{--}100 \times 10^3/\mu\text{L}$ and manually corrected PLT counts considerably above $100 \times 10^3/\mu\text{L}$, and were therefore of potential clinical relevance. Each of these four samples was correctly flagged by DxH 800, three were correctly identified by Cell-Dyn Sapphire, and only two by Advia 2120i, Sysmex XE-5000 and XN-2000, respectively. A PLT clump flag without evidence for PLT clumping in microscopy occurred in eight samples for Cell-Dyn Sapphire, in seven samples for DxH 800, in six samples for Advia 2120i, in eight samples for XE-5000 and in four samples for XN-2000.

Inter-instrument comparison of false-positive flags in samples without pathological criteria in microscopy (n=198)

In 198 out of 349 randomly selected samples, microscopy did not reveal pathological criteria. In this selection of samples, numbers of false-positive flags for blasts and variant lymphocytes did not differ significantly between the different instruments, but were significantly higher for IGs with the XN-2000 (n=18) compared to all other instruments (χ^2 -test, Table 5).

Inter-instrument comparison of required sample volume

Minimal volumes required for CBC analysis including WBC differential by different instruments using different tubes are presented in Supplemental Table 1. Using 2.7 mL Sarstedt tubes, Cell-Dyn Sapphire required the smallest volume (300 μ L), while the other analyzers required a minimal volume of 500 μ L. Using 1.2 mL Sarstedt tubes, XN-2000 required the smallest volume (200 μ L), while Cell-Dyn Sapphire and DxH 800 required minimal volumes of 300 μ L, Advia 2120i and XE-5000 of 500 μ L. Using 1.0 mL Siemens EZEE-nest tubes or 1.5 mL Sarstedt micro tubes, Cell-Dyn Sapphire, Advia 2120i and XN-2000 required a minimal volume of 150 μ L, respectively. DxH 800 required minimal volumes of 200 μ L using both tubes, XE-5000 required a minimal volume of 150 μ L or 300 μ L, respectively.

Discussion

To the best of our knowledge, this is the most comprehensive side-by-side comparison, investigating the diagnostic performances of five current top of the range routine

hematology analyzers in the setting of a large university hospital central laboratory. Samples were collected randomly from routine diagnostics (n=349), permitting unbiased testing of the five instruments. A good correlation for CBC parameters was found in inter-instrument comparison, however, slight systemic differences of instruments were observed (Table 2, Figure 1, Supplemental Figure 1). A consistently poorer inter-instrument concordance could be shown for reticulocyte counts, with Cell-Dyn Sapphire showing significantly higher and Advia 2120i showing significantly lower values (Table 2, Figure 1H, Supplemental Figure 1H). A rather low concordance for NRBC counts was observed between different instruments, the poorest concordance for automated NRBC counts with microscopy was found for Advia 2120i (Table 2, Figure 2). Without the use of a true reference method for most of the investigated parameters, interpretation of results of different analyzers is difficult. For inter-instrument comparison we therefore used the median of all five analyzers as a reference. Correlations of differential blood counts between instruments and with microscopy were less well, however, clear differences between the various instruments were not noted.

A major expectation of the clinical laboratory from any automated hematology analyzer is to decrease review-rates, requiring the highest possible flagging specificity and sensitivity. In our analysis of 349 randomly selected routine samples, XN-2000 outperformed the other instruments in blast flagging, while specificity was comparable between instruments (Tables 4 and 5).

It is difficult to compare our findings with previous published work, because different analyzers were used in each paper. The closest publication might be by Hotton et al. [3], where three of the instruments, the Cell-Dyn Sapphire, DxH 800 and XN-2000, also tested in our study, were used. In agreement with our work, these authors did not find relevant differences for blood count between the three analyzers, except slightly higher PLT counts in Cell-Dyn Sapphire. It must be noted that the Advia 2120i,

Table 5 Inter-instrument comparison of false-positive flags in 198 samples without pathological criteria in microscopy.

Instrument	Instrument flagging (n)					
	Blasts	Variant lymphocytes	Immature granulocytes	Left shift	PLT clumps	Other
Sapphire	1	0	6	6	2	3 ^a
DxH 800	1	0	7	12	3	
Advia 2120i	0	2	5	11	3	
XE-5000	0	0	5	0	0	
XN-2000	1	1	18	4	2	2 ^b

n, number; ^aunidentified fluorescence cells; ^bWBC abnormal distribution.

which showed a slightly lower concordance of WBCs, MCV and PLTs compared to other analyzers in our work, was not tested in that study. Unfortunately, there are no current side-by-side comparisons of Advia 2120i, except for NRBC analysis [14, 15] and flagging performance [16, 17], which are however, not informative with regard to CBC parameters. In our study, we were able to show a slightly lower concordance of PLTs for Advia 2120i systematically tending to higher counts compared to DxH 800, XE-5000 and XN-2000 and to a lesser extent compared to Cell-Dyn Sapphire (Table 2). Previous studies also observed higher PLT counts with Cell-Dyn Sapphire and Advia 120, the predecessor model of Advia 2120i, when compared with DxH 800, XE-2100 the predecessor model of XE-5000, and XN-2000 [1–3]. Considerably higher PLT counts were also confirmed in a recent publication comparing Advia 2120i with CD61 based PLT counts and an immunological reference method [18].

The application of PLT-F channel as first-line PLT testing in XN-2000 in our study may not reflect routine algorithms, because the impedance method is normally used as first-line diagnostics followed by fluorescence based testing in case of abnormal scattergram or low PLT count. However, based on the fact that impedance and optical PLT counts are measured with the same technology in Sysmex XE-5000 and XN-2000, we decided to define the PLT-F channel as first-line testing in XN-2000 in order to investigate a potential benefit of PLT-F technology in the present study. We could not find clear differences of PLT counts generated by PLT-F based technology as compared to XE-5000, but we were able to confirm recent studies showing a slightly better concordance of PLT-F based analysis as compared to CD61 based analysis (Table 2) [19].

Regarding differential blood count, we found an overall high inter-instrument correlation of neutrophil counts (Supplemental Figure 2A), lymphocyte counts (Supplemental Figure 2C) and eosinophil counts (Supplemental Figure 2G) and with microscopy (Supplemental Figure 2B, D, H), also observed in previous studies [1, 2]. In our study monocyte counts between different instruments revealed a considerably lower agreement with Advia 2120i showing the maximum deviation to consistently lower values (Table 3, Figure 3E,). Except Advia 2120i, automated differentials revealed higher percentages of monocytes in comparison with microscopy (Table 3, Figure 3F). Lower percentages of monocytes in automated analysis have already been described for Advia 120 and were suspected to be due to underestimation by the peroxidase staining [2, 20]. As in previous validation studies, we were able to show low correlations of basophil counts between automated and manual counts (Table 3) [1, 2]. However, largely

due to statistical uncertainty because of missing samples with higher basophil counts, the informative value of this analysis is quite limited.

The presence of NRBCs in peripheral blood is an indicator for pathologic conditions and the number of NRBCs was shown to be associated with the prognosis in neonates and in critically ill patients [21, 22]. Furthermore, accurate identification and quantification of NRBCs is a prerequisite for adequate correction of WBC counts. Here, we found a very limited concordance of NRBC counts in inter-instrument comparison and in comparison with microscopy (Table 2, Figure 2). Using linear regression analysis, a recent publication showed a good correlation of NRBC analysis between Cell-Dyn Sapphire, DxH 800 and microscopy [1, 23], however, overall concordance was also limited. Unlike Tan et al., we calculated Kendall's τ_b , which in our view better reflects the distribution of NRBC values with the majority in a very low range. In comparison to Pearson's correlation, τ_b resulted in lower correlation coefficients. Other studies showed a clear inferiority of Advia 2120i compared to DxH 800 [15] and XE-5000 [14] in NRBC analysis. Taking all instruments evaluated in our study into consideration, the worst relationship to microscopy as reference method was also found for Advia 2120i, other instruments revealed comparable quality in NRBC analyses.

An essential role of modern routine hematology analyzers in clinical practice is to screen for samples containing pathological cell types and therefore requiring microscopic evaluation. For blast flagging as one of the clinically most relevant warning messages, a significant improvement of sensitivity was found for XN-2000 in comparison to other instruments (Table 4). These data confirm a recent study showing sensitivities to detect blasts of 89%, 72% and 100% for Cell-Dyn Sapphire, DxH 800 and XN-2000 in a limited population of 18 blast positive samples [3]. The higher sensitivity of blast flagging in XN instruments might be due to the newly introduced white cell differential (WDF) and white cell nucleated red blood (WNR) channel allowing optimized identification of blast cells [4]. Our data revealing limited sensitivity of XE-5000 in blast flagging is in line with Eilertsen et al. showing 11 false negative out of 37 blast positive samples [24]. In addition, these authors showed a high variability of blast flagging in different XE-5000 instruments. The limited sensitivity of blast flagging in different instruments other than the XN-2000 was not restricted to samples with leucopenia. Despite an association of the three flags for blasts, variant lymphocytes or IGs for detection of blasts, different instruments still missed blast positive samples. It must also be noted, that in contrast to the study by Hotton

et al. [3], the XN-2000 still overlooked blasts in one out of 34 samples, thereby not providing 100% certainty for detecting these cells.

The highest sensitivity for flagging of variant lymphocytes was found for XE-5000 and XN-2000, continuously decreasing sensitivities were found for Advia 2120i, DxH 800 and Cell-Dyn Sapphire (Table 4). In their inter-instrument comparison between Cell-Dyn Sapphire, DxH 800 and XN-2000, in accordance with our data, Hotton et al. showed the highest lymphocyte flagging sensitivity for the XN-2000, however, remarkably lower sensitivities were shown for other instruments [3].

IGs are increased in various clinical conditions [25]. As microscopic evaluation of patient samples for presence of IGs is time consuming, identification of the respective samples in automated blood count analysis gains increasing importance in clinical practice. Comparing IG flagging of different instruments with presence of IGs in blood smear analysis, we were able to show a superior sensitivity for XN-2000 (91%) compared to other instruments with sensitivities ranging from 80% for XE-5000 to 39% for Advia 2120i (Table 4). Our findings are consistent with Hotton et al. showing considerably higher IG flagging sensitivities for XN-2000 compared with Cell-Dyn Sapphire and DxH 800 and with Meintker et al. showing lowest sensitivity for Advia 120 as compared to Cell-Dyn Sapphire and DxH 800 [2, 3].

There is only limited information available about the quality of PLT clump flagging of different hematology analyzers, however, a high flagging quality would be of great interest as agglutination of PLTs is associated with false low PLT counts in automated analysis. With the exception of DxH 800, all of the analyzers had limitations in the discrimination of PLT clumps (Table 4). Our data are in accordance with Sandhaus et al. describing only very limited sensitivities for PLT clump flagging in LH 750, XE-2100 and Advia 120, as predecessor models of DxH 800, XE-5000 and Advia 2120i [26].

Beside high flagging sensitivity, low numbers of false-positive flagging alerts are a prerequisite to reduce unnecessary manual smear reviews. As already described in recent publications, flagging parameters most contributing to the number of false-positive samples were IGs followed by left shift and PLT clumping [1–3, 17]. Combining flagging alerts for presence of blasts, variant lymphocytes and IGs, XN-2000 revealed the lowest specificity (78%) among all investigated instruments primarily due to a relatively high number of false-positive IG warnings. In accordance with existing publications comparing Cell-Dyn Sapphire versus DxH 800 [1] and DxH 800 versus Advia 2120i [17], we were able to show similar specificities

ranging from 94% to 96% for the two most clinically relevant warning alerts (presence of blasts and variant lymphocytes) in inter-instrument comparison (Table 4).

The required sample volume of different analyzers for CBC analysis and differential is of great interest especially for pediatric samples. Inter-instrument comparison revealed that the minimal volume required by different instruments depended on usage of different tubes (Supplemental Table 1).

In conclusion, side-by-side testing of five current top of the range hematology analyzers, revealed a good concordance for blood count parameters, correlations between instruments were less well for reticulocyte counts, NRBCs, and differentials. With respect to automated NRBC analysis Advia 2120i revealed main limitations compared to microscopic analysis. Regarding flagging quality between different instruments, the highest overall sensitivity for presence of blasts, variant lymphocytes and IGs was found for XN-2000. Variable analyzer quality has to be taken into consideration in defining laboratory algorithms in clinical practice.

Acknowledgments: We thank Abbott, Beckman Coulter, Siemens and Sysmex for their support of the study by providing their instruments for evaluation free of charge.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Financial support: None declared.

Employment or leadership: None declared.

Honorarium: None declared.

Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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Supplemental Material: The online version of this article (DOI: 10.1515/cclm-2014-0945) offers supplementary material, available to authorized users.