Evaluation of two Sysmex XE-2100 analyzers in an HST-302 configuration

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Before introducing our new fully automated Sysmex HST-302 into routine use, we evaluated the performance of both XE-2100 hematology analyzers. New features of this analyzer are integrated nucleated red blood cell (NRBC) count, reticulocyte (RET) count, platelet (PLT) measurement by impedance and fluorescence, immature granulocyte (IG) count, and hematopoietic progenitor cell (HPC) count.

The Clinical Chemistry Laboratory of the University Hospital Rotterdam evaluated reproducibility, linearity, carryover, and lower detection limits using control material and patient blood. Correlation studies were performed against our routine analyzers Sysmex NE-8000 and R-3000. Reproducibility, linearity and carryover were excellent and within the manufacturer's specifications. Lower detection limits for WBC, RBC, PLT and HGB were very good. Good correlation between the NE-8000 and both XE-2100 analyzers was observed ($r > 0.9$). However, on both our instruments, readjustments in the calibration factors appeared necessary because after installation of the analyzers in our lab we found a lower RET count on the XE-2100 compared to the R-3000. Correlation between optical and impedance PLT counts and between closed sampler and open sampler modes were all good on the XE-2100. In conclusion, the XE-2100 shows excellent analytical performance characteristics.

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Key Words Automated Hematology Analyzer, XE-2100, HST-302, Evaluation, Comparison

INTRODUCTION

In clinical chemistry laboratories, a trend towards further automation can be observed, especially in large clinical centers. The increasing workload and the necessity to decrease the turn around time (TAT) imposes the need for automation. New in this field is the Sysmex HST-302. The HST-302 consists of the recently introduced automated hematology analyzer, the Sysmex XE-2100, and the SP-100, which automatically prepares and stains blood films. We recently installed a Sysmex HST-302 to replace our old Sysmex NE-8000 and R-3000 analyzers.

The XE-2100 is capable of measuring 32 parameters including white blood cell (WBC) 5-part differential, the immature granulocyte (IG) absolute and proportional counts (from the Diff channel), the hematopoietic progenitor cell (HPC) count from the Immature Information (IMI) channel, the reticulocyte (RET) count (including the different maturity fractions), the nucleated red blood cell (NRBC) count, and the "optical" fluorescence platelet (PLT-O) count. The WBC 5-part differential, NRBC, RET and PLT-O are measured using flow cytometry with a semi-conductor laser. Cells are differentiated on differences in side-scatter (granulation), forward scatter (volume) and fluorescence intensity after staining of nuclear RNA/DNA with specific dyes. RBC and PLT-I are measured using the sheath flow impedance (DC) method (hydrodynamic focusing method.) When interference with the DC method to measure PLT-I is suspected (low PLT count, abnormal platelet volume distribution), PLT-O measurement results from the fluorescence channel (RET channel) are provided to improve reliability. In the "research screen", the immature granulocyte (IG) count and hematopoietic progenitor cell (HPC) count are given. Hemoglobin (HGB) is measured using a cyanide-free method: binding of sodium lauryl sulfate (SLS) to hemoglobin results in the stable product SLS-methemoglobin, which is measured colorimetrically at 560 nm.

We thoroughly evaluated both XE-2100 analyzers by comparing them with our current analyzers (R-3000 and NE-8000) before we started using the HST-302.

MATERIALS AND METHODS

Between-day imprecision

For at least 20 consecutive days, 4 tubes of XE CHECK control (previous control material for XE-2100, used before August 2000.) ([C+D]-Low; [C+D]-Normal; [R]-1; [R]-2) were measured in duplicate (at 9.00 h. and 16.00 h.) on both analyzers according to the NCCLS EP5 protocol[1]. Samples were analyzed in the "closed auto mode" and the coefficient of variation (CV) for each parameter was calculated.

Within-day imprecision

Blood, drawn from a healthy volunteer, was analyzed 10 times on each analyzer in the "closed auto mode". The CV for each parameter was calculated. In a similar way, patient blood was used to verify imprecision for low WBC, PLT, and NEUT counts.

Linearity

The linearity for WBC, RBC, HGB, PLT, and RET was determined following the NCCLS EP6[2] protocol. Patient specimens at or above the upper limit of linearity specified for each parameter were diluted with CELLPACK. First, the sample was diluted to reach the upper linearity limit for each parameter (100% value). Then, from this 100% pool, 4:5 (80%), 3:5 (60%), 2:5 (40%), 1:5 (20%) serial dilutions were prepared and analyzed in triplicate on each analyzer in the "open manual mode". Also, a blank (CELLPACK only) was measured (0%). Linearity was evaluated using linear regression analysis.

Carryover

Carryover was determined for WBC, RBC, NEUT, HGB, and PLT using patient blood. On each analyzer, triplicate measurement of a high sample (H1, H2, H3) was followed by triplicate measurement of a low sample (L1, L2, L3). All samples were measured in the "open manual mode". Carryover percentage was calculated as followed, according to Broughton, et al.[3]

$$\text{Carryover (\%)} = \frac{L1 - L3}{H1 - H3} \times 100$$

Lower detection limit

Patient samples with low counts of WBC, RBC, HGB and PLT were serially diluted with CELLPACK. Samples were measured 6 times in the "open manual mode". The blank (CELLPACK) was measured 10 times and the mean and standard deviation were calculated. The lower detection limit was defined as that concentration of cells of which the mean value was just above the mean blank ± 3SD and with a coefficient of variation (CV) > 20%. Additionally, the % recovery for each dilution compared to the 100% pool was calculated. Recoveries of 100 ± 30% were considered acceptable in these low cellular concentration ranges. The detection limit was established using only one (XE-2100 (2)) analyzer.

Method comparison

Patient samples were analyzed in the "auto closed mode" on both XE-2100 analyzers and the NE-8000 or R-3000. According to NCCLS protocol EP9a[4], samples within a certain concentration range for each parameter were collected and measured. Also, agreement between the "manual open mode", "manual capillary mode" and the "auto closed mode" on each XE-2100 analyzer was determined. Agreement between analyzers and measuring modes was determined using Passing-Bablok regression analysis.

RESULTS

Between-day imprecision

The between-day imprecision (presented as %CV) for each parameter is presented in ***Tables 1a-b.*** For the basic parameters (WBC, RBC, HGB, PLT, MCV), imprecision was low (= 3%) and not different between both analyzers. Imprecision for RET varied between 3 and 6%.

Within-day imprecision

Results for within-day imprecision (presented as %CV) are given in ***Table 2.*** Within-day imprecision for the basic parameters (WBC, RBC, HGB, PLT, MCV) was = 2% on both analyzers. For all parameters, imprecision was lower than the manufacturer specifications. Also, for low counts of WBC, PLT, and NEUT, imprecision was low (4-8%).

Linearity

Linearity results for WBC, RBC, HGB, PLT, and RET are given in ***Fig. 1.*** For all parameters, linearity was very good to the upper limit specified by the manufacturer. Moreover, WBC count was even linear up to 625 $\times 10^9$/L (results not shown).

Carryover

Carryover was determined for WBC, RBC, NEUT, HGB, RET, and PLT using patient blood (***Tables 3a-b***). Carryover was negligible and smaller than the specifications of the manufacturer.

Lower detection limit

Lower detection limit data are presented in ***Table 4.*** Since the blank measured no cells, we considered 3x the mean SD of measured cell concentrations for each parameter as the lowest possible detection limit. The detection limit was excellent for WBC (0.04×10^9/L), RBC (0.04×10^{12}/L), PLT (2×10^9/L), and HGB (0.1 mmol/L).

Method comparison

The comparison between the XE-2100 and the NE-8000/R-3000 is given in ***Table 5a.*** The correlation between analyzers was good ($r^2 > 0.9$) except for RET (good correlation but lower values). RET on the XE-2100 was approximately 20% lower than on the R-3000. Correlation between both XE-2100 analyzers for RET was excellent. On each XE-2100, correlation between PLT-I (impedance) and PLT-O (optical) and between closed and open modes on the XE-2100 (***Table 5b***) were good.

***Table 1a** Between-day imprecision presented as %CV*

Parameter	XE-2100 (1)		XE-2100 (2)	
	Normal ([C+D] - N)	Low ([C+D] - L)	Normal ([C+D] - N)	Low ([C+D] - L)
WBC	1.54	2.43	1.85	2.54
RBC	0.67	0.73	0.72	0.86
HGB	0.7	0.7	0.6	1.8
HCT	0.821	1.179	0.99	1.289
MCV	0.6	0.8	0.7	0.9
MCH	1	1	1	2
MCHC	0.9	1.3	1.0	1.8
PLT	2	3	2	3
PLT-O	2	3	3	4
RDW-SD	0.7	1.4	0.6	1.2
RDW-CV	1.2	1.4	1.2	1.2
MPV	0.8	1.5	0.9	1.4
P-LCR	3.5	9.3	3.8	8.5
PDW	1.5	3.5	1.6	3.2
PCT	2.50	8.00	2.50	8.00
NEUT#	2.57	3	2.22	3.06
LYMPH#	2.79	2.75	1.99	2.61
MONO#	9.31	26	7.86	15
EO#	5.48	6.25	5.48	6
BASO#	5.74	6.5	5.08	7
NEUT%	1.8	1.7	1.2	1.4
LYMPH%	2.3	1.4	0.7	0.9
MONO%	9.5	26.4	7.4	15
EO%	5.5	5.5	5.1	5.7
BASO%	5.3	6.1	4.7	6.4
IMI#	4	5	3	3
NRBC#	3.68	14.19	2.04	2.36
NRBC%	0	0	0	0

CV = coefficient of variation

***Table 1b** Between-day imprecision presented as %CV*

Parameter	XE-2100 (1)		XE-2100 (2)	
	Level 2 [R] - 2; normal	Level 1 [R] - 1; low	Level 2 [R] - 2; normal	Level 1 [R] - 1; low
RET%	3.85	6.74	3.28	6.08
RET#	3.98	6.84	3.46	6.08
RBC-O	4.51	1.10	1.26	1.50
IRF	7.6	10.9	8.8	14.6
LFR	2.6	2.7	2.2	2.6
MFR	7.1	11.2	8.5	15.0
HFR	19.1	33.0	19.6	43.7

CV = coefficient of variation

***Table 2** Within-day imprecision presented as %CV*

Parameter	(Mean ± SD)	XE-2100 (1)	XE-2100 (2)	Specification
WBC	(7.5 ± 0.1×10^9/L)	2.04	1.13	≤ 3
RBC	(5.60 ± 0.04×10^{12}/L)	0.47	0.68	≤ 1.5
HGB	(10.50 ± 0.09 mmol/L)	0.49	0.68	≤ 1.0
HCT	(0.49 ± 0.003 L/L)	0.44	0.68	≤ 1.5
MCV	(86.7 ± 0.18 fL)	0.08	0.20	≤ 1.0
MCHC	(21.6 ± 0.3 mmol/L)	0.51	1.10	≤ 1.5
PLT	(322 ± 11 ×10^9/L)	1.81	2.70	≤ 4
NEUT#	(5.90 ± 0.11×10^9/L)	2.23	1.41	≤ 8.0
LYMPH#	(1.16 ± 0.04×10^9/L)	3.60	3.87	≤ 8.0
MONO#	(0.36 ± 0.02×10^9/L)	3.79	6.29	≤ 20.0
EO#	(0.07 ± 0.01×10^9/L)	15.81	13.04	≤ 25.0
BASO#	(0.04 ± 0.007×10^9/L)	24.28	10.44	≤ 40.0
RET#	(0.043 ± 0.003×10^{12}/L)	7.82	8.17	≤ 15.0
RET%	(0.87 ± 0.07 %)	7.85	7.98	≤ 15.0
PLT	(10 ± 0.6×10^9/L)	5.77		
PLT	(14.17 ± 1.2×10^9/L)	8.42		
WBC	(0.91 ± 0.04×10^9/L)	4.77		
NEUT	(0.53 ± 0.04×10^9/L)	7.29		

CV = coefficient of variation
specification = %CV given by manufacturer

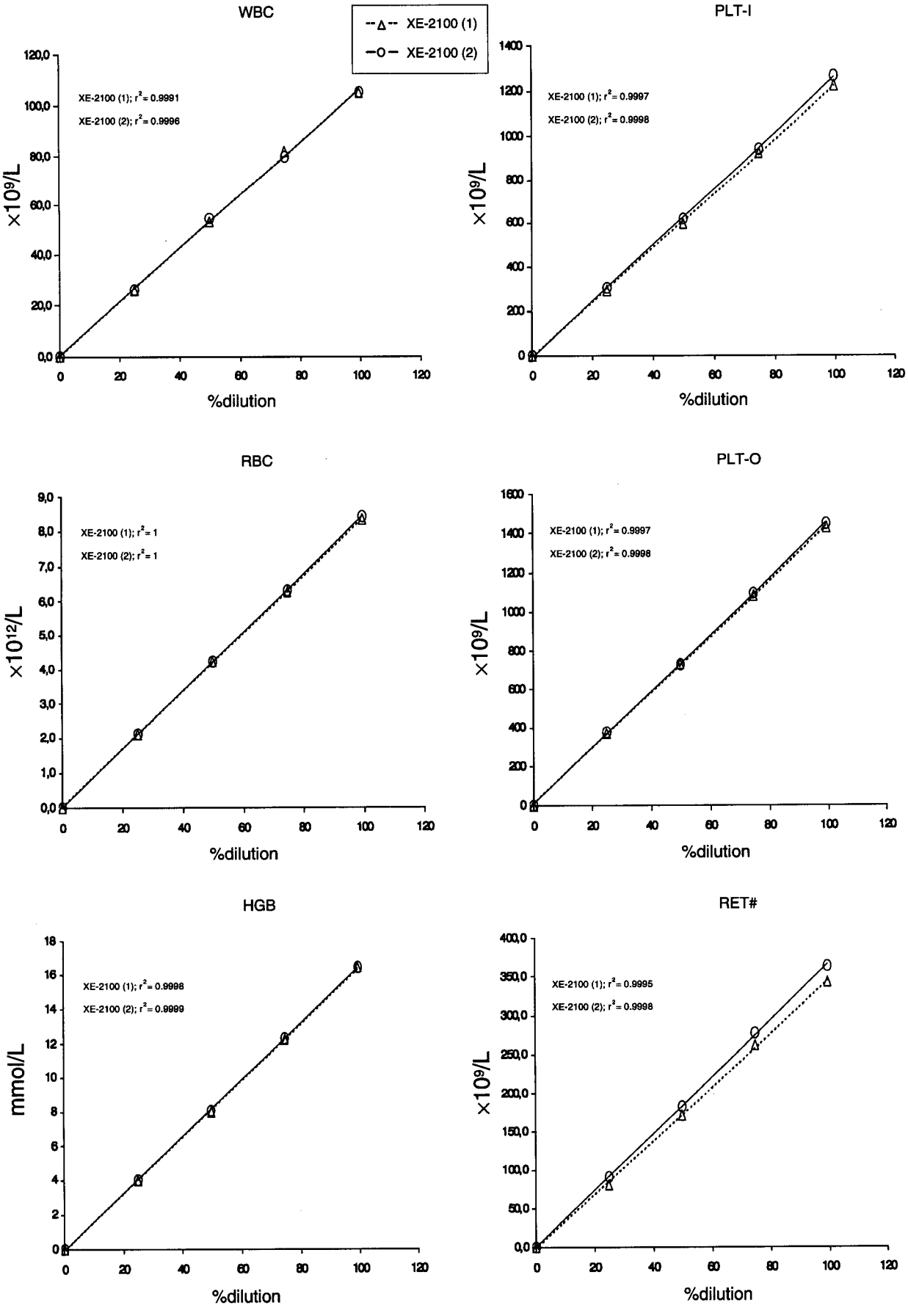

Fig. 1 Linearity studies XE-2100

Table 3a *Carryover (XE-2100 (1))*

Parameter	XE-2100 (1)			Specification (%)
	Mean (H1-H3)	Mean (L1-L3)	Carryover (%)	
WBC ($\times10^9$/L)	43.85	2.81	− 0.32	≤1.0
WBC ($\times10^9$/L)	181.59	0.39	0.06	≤1.0
RBC ($\times10^{12}$/L)	6.57	2.53	− 0.49	≤1.0
NEUT ($\times10^9$/L)	27.31	0.35	0.11	≤1.0
PLT-I ($\times10^9$/L)	714.67	81.33	0.47	≤1.0
PLT-O ($\times10^9$/L)	832.00	89.00	0.42	≤1.0
RET# ($\times10^9$/L)	348.50	115.77	− 1.20	n.g.
HGB (mmol/L)	14.07	4.70	0.00	≤1.0

H = high counts ; L = low counts ; specification = %CV given by manufacturer ; n.g. = not given.

Table 3b *Carryover (XE-2100 (2))*

Parameter	XE-2100 (2)			Specification (%)
	Mean (H1-H3)	Mean (L1-L3)	Carryover (%)	
WBC ($\times10^9$/L)	33.54	0.30	0.49	≤1.0
RBC ($\times10^{12}$/L)	6.29	2.20	0.00	≤1.0
NEUT ($\times10^9$/L)	29.78	0.21	0.65	≤1.0
PLT-I ($\times10^9$/L)	751.00	87.67	0.00	≤1.0
PLT-O ($\times10^9$/L)	844.00	91.33	0.95	≤1.0
RET# ($\times10^9$/L)	368.10	39.87	− 2.24	n.g.
HGB (mmol/L)	10.40	4.00	0.00	≤1.0

H = high counts ; L = low counts ; specification = %CV given by manufacturer ; n.g. = not given.

Table 4 *Lower detection limit of XE-2100 (CELLPACK diluted)*

% cells (dilution)	WBC ($\times10^9$/L)			*RBC ($\times10^{12}$/L)			PLT ($\times10^9$/L)			HGB (mmol/L)		
	Mean ± SD	CV%	%Rec.	Mean ± SD	CV%	%Rec.	Mean ± SD	CV%	%Rec.	Mean ± SD	CV%	%Rec.
Blank (n=10)	0 ± 0			0 ± 0			0 ± 0			0 ± 0		
100% (n=6)	0.42 ± 0.02	4.7	−	0.10 ± 0.00	0	−	12.2 ± 1.0	8.1	−	5.5 ± 0.0	0	−
80% (n=6)	0.33 ± 0.03	7.6	97	0.08 ± 0.00	0	100	8.3 ± 1.2	14.5	85	4.4 ± 0.0	0.9	100
60% (n=6)	0.25 ± 0.02	8.6	100	0.06 ± 0.00	0	100	6.2 ± 0.5	7.2	85	3.3 ± 0.0	0	100
40% (n=6)	0.16 ± 0.01	8.4	94	0.04 ± 0.00	0	100	4.3 ± 0.8	18.8	88	2.1 ± 0.0	0	95
20% (n=6)	0.11 ± 0.01	8.0	138	0.01 ± 0.00	0	50	2.0 ± 0.0	0.0	83	1.0 ± 0.0	4.0	91
10% (n=6)	0.04 ± 0.01	35.4	100				1.0 ± 0.0	0.0	83	0.5 ± 0.0	0	83
5% (n=6)	0.02 ± 0.00	0	100							0.3 ± 0.0	15.8	100
2.5% (n=6)	0.02 ± 0.00	22.3	200							0.1 ± 0.0	0	100

Blank = CELLPACK ; %Rec. = % recovery ; CV = coefficient of variation ; * = diluted in AB plasma.

Table 5a *Correlation and regression (y = ax + b; Passing-Bablok) between analyzers (XE-2100 vs. NE-8000/R-3000)*

Parameter	Comparison	r	a	b	N
WBC	XE-2100 (1) *vs.* NE-8000	0.999	0.986	−0.039	106
(×10⁹/L)	XE-2100 (2) *vs.* NE-8000	0.999	0.960	−0.006	105
	XE-2100 (1) *vs.* XE-2100 (2)	0.999	0.970	0.019	105
RBC	XE-2100 (1) *vs.* NE-8000	0.999	1.000	0.120	101
(×10¹²/L)	XE-2100 (2) *vs.* NE-8000	0.999	1.027	0.014	101
	XE-2100 (1) *vs.* XE-2100 (2)	0.999	1.030	−0.115	101
PLT-I	XE-2100 (1) *vs.* NE-8000	0.998	0.974	−1.1	106
(×10⁹/L)	XE-2100 (2) *vs.* NE-8000	0.998	0.975	−0.4	105
	XE-2100 (1) *vs.* XE-2100 (2)	0.999	1.002	0.9	105
HGB	XE-2100 (1) *vs.* NE-8000	0.999	1.000	0.20	100
(mmol/L)	XE-2100 (2) *vs.* NE-8000	0.999	1.023	−0.09	100
	XE-2100 (1) *vs.* XE-2100 (2)	0.999	1.000	−0.10	100
RET# *	XE-2100 (1) *vs.* R-3000	0.951	0.802	0.65	68
(×10¹²/L)	XE-2100 (2) *vs.* R-3000	0.958	0.827	0.57	68
	XE-2100 (1) *vs.* XE-2100 (2)	0.996	1.062	0.00	68
RET *	XE-2100 (1) *vs.* R-3000	0.975	0.794	0.000	68
(%)	XE-2100 (2) *vs.* R-3000	0.977	0.825	0.005	68
	XE-2100 (1) *vs.* XE-2100 (2)	0.997	1.053	0.003	68
HFR	XE-2100 (1) *vs.* R-3000	0.572	1.174	0.41	68
(%)	XE-2100 (2) *vs.* R-3000	0.647	0.818	0.00	68
	XE-2100 (1) *vs.* XE-2100 (2)	0.806	0.783	−0.07	68
MFR	XE-2100 (1) *vs.* R-3000	0.730	0.934	1.25	68
(%)	XE-2100 (2) *vs.* R-3000	0.760	0.893	−0.41	68
	XE-2100 (1) *vs.* XE-2100 (2)	0.885	0.949	−1.75	68
LFR	XE-2100 (1) *vs.* R-3000	0.714	0.958	2.51	68
(%)	XE-2100 (2) *vs.* R-3000	0.762	0.905	9.78	68
	XE-2100 (1) *vs.* XE-2100 (2)	0.896	0.927	9.10	68
IRF	XE-2100 (1) *vs.* R-3000	0.714	0.958	1.65	68
(%)	XE-2100 (2) *vs.* R-3000	0.762	0.905	−0.32	68
	XE-2100 (1) *vs.* XE-2100 (2)	0.896	0.927	−1.83	68
NEUT#	XE-2100 (1) *vs.* NE-8000	0.993	1.000	−0.050	61
(×10⁹/L)	XE-2100 (2) *vs.* NE-8000	0.994	0.987	−0.074	58
	XE-2100 (1) *vs.* XE-2100 (2)	1.000	0.984	0.000	57
PLT	I *vs* O: XE-2100 (1)	0.994	0.977	−0.5	48
(×10⁹/L)	I *vs.* O: XE-2100 (2)	0.986	1.037	−2.8	48
	O *vs.* O: XE-2100 (1) *vs* (2)	0.997	1.042	1.6	48
NEUT%	XE-2100 (1) *vs.* NE-8000	0.973	1.063	−6.65	100
	XE-2100 (2) *vs.* NE-8000	0.975	1.091	−8.64	100
	XE-2100 (1) *vs.* XE-2100 (2)	0.994	1.017	−1.15	100
LYMPH%	XE-2100 (1) *vs.* NE-8000	0.991	1.003	−0.03	100
	XE-2100 (2) *vs.* NE-8000	0.992	1.063	−0.63	100
	XE-2100 (1) *vs.* XE-2100 (2)	0.993	1.040	−0.64	100
MONO%	XE-2100 (1) *vs.* NE-8000	0.689	1.657	−2.07	100
	XE-2100 (2) *vs.* NE-8000	0.680	1.800	−3.03	100
	XE-2100 (1) *vs.* XE-2100 (2)	0.955	1.000	0.00	100
EO%	XE-2100 (1) *vs.* NE-8000	0.987	1.000	0.00	100
	XE-2100 (2) *vs.* NE-8000	0.983	1.000	0.00	100
	XE-2100 (1) *vs.* XE-2100 (2)	0.984	1.000	0.00	100
BASO%	XE-2100 (1) *vs.* NE-8000	0.434	0.500	0.20	100
	XE-2100 (2) *vs.* NE-8000	0.472	0.600	0.18	100
	XE-2100 (1) *vs.* XE-2100 (2)	0.885	1.000	0.00	100

1) *: Before readjustment

2) Data of correlation between XE-2100 and R-3000 will be changed after readjustment as follows;

XE-2100 (1) vs. R-3000: y = 0.994x−0.89

XE-2100 (2) vs. R-3000: y = 0.961x−1.82

Table 5b *Correlation and regression (y = ax + b; Passing-Bablok) between measuring modes on the XE-2100*

Parameter	Comparison	r	a	b	N
WBC	Manual open *vs.* Closed XE-2100 (1)	0.995	0.965	0.129	20
(×10⁹/L)	Capillary open *vs.* Closed XE-2100 (1)	0.988	0.914	0.384	20
	Manual open *vs.* Closed XE-2100 (2)	0.998	0.993	0.075	20
	Capillary open *vs.* Closed XE-2100 (2)	0.996	1.051	−0.032	20
RBC	Manual open *vs.* Closed XE-2100 (1)	0.999	1.052	−0.205	20
(×10¹²/L)	Capillary open *vs.* Closed XE-2100 (1)	0.999	1.068	−0.278	20
	Manual open *vs.* Closed XE-2100 (2)	0.999	1.038	−0.186	20
	Capillary open *vs.* Closed XE-2100 (2)	0.997	1.020	−0.099	20
MCV	Manual open *vs.* Closed XE-2100 (1)	0.999	1.000	0.55	20
(fL)	Capillary open *vs.* Closed XE-2100 (1)	0.996	1.000	−0.10	20
	Manual open *vs.* Closed XE-2100 (2)	0.998	1.000	−0.30	20
	Capillary open *vs.* Closed XE-2100 (2)	0.997	0.999	−0.18	20
HGB	Manual open *vs.* Closed XE-2100 (1)	0.999	1.000	0.10	20
(mmol/L)	Capillary open *vs.* Closed XE-2100 (1)	0.994	1.037	−0.14	20
	Manual open *vs.* Closed XE-2100 (2)	0.998	1.027	−0.20	20
	Capillary open *vs.* Closed XE-2100 (2)	0.997	1.000	0.00	20
PLT-I	Manual open *vs.* Closed XE-2100 (1)	0.988	1.047	−4.0	20
(×10⁹/L)	Capillary open *vs.* Closed XE-2100 (1)	0.981	1.000	−0.5	20
	Manual open *vs.* Closed XE-2100 (2)	0.992	1.031	−3.3	20
	Capillary open *vs.* Closed XE-2100 (2)	0.991	0.951	−4.2	20

DISCUSSION

Both within-run and between-day analytical precision of the XE-2100 are excellent and confirm the manufacturer specifications. Results for within-day precision are comparable to recent results obtained by Tsuruda, et al.[5] and better than those reported by Gould, et al.[6] Also, linearity is excellent to the upper limit specified by the manufacturer. The WBC count was even linear to extreme values, in line with a previous report[6]. In contrast to the NE-8000, carryover is negligible with the XE-2100 so that currently no "blank" samples have to be measured between high and low WBC patient specimens. Low carryover has also been observed in other studies evaluating the performance of the XE-2100 [6, 7]. The lower detection limits for WBC, RBC, PLT, and HGB were very good. Inter-instrument correlation between the XE-2100 and the NE-8000/R-3000 was excellent. Based on the initial calibration settings only RET count was about 20% lower on both XE-2100 compared to the R-3000, in contrast to a previous report[5], which made adjustment of the calibration of the two systems necessary. Since at that time Sysmex Europe also increased the assay target values for XE CHECK (R) (previous control material for XE-2100, used before August 2000) by 5%, Calibration factors were readjusted by +15% (XE-2100 (1)) and +13% (XE-2100 (2)) respectively. After these readjustments, 1) regression data between the R-3000 and the XE-2100 (1) ($y = 0.994x - 0.89$) and XE-2100 (2) ($y = 0.961x - 1.82$) were good and 2) the mean value of the RET control material (XE CHECK (R)) (previous control material for XE-2100, used before August 2000) was within range (but slightly greater than the target value). Correlation between optical and impedance PLT counts and between closed sampler and open sampler modes are good. In conclusion, the XE-2100 is a reliable and accurate hematology analyzer.

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