Performance evaluation of the Sysmex XS-1000*i* automated haematology analyser

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SUMMARY

The Sysmex® XS-1000*i* is a compact new, fully automated haematology analyser, designed to generate complete blood counts with five-part leucocyte differential. In our study, a Sysmex XS-1000*i* instrument was evaluated according to Clinical Laboratory Standards Institute (CLSI) and International Council for Standardization in Haematology (ICSH) guidelines. Precision, carry-over and linearity were determined. Using a total of 700 patient samples, results from the Sysmex XS-1000*i* were compared with those from a Sysmex XE-2100, an Abbott Cell Dyn 4000 and the manual reference leucocyte differential. Using quality control material, total and within-run imprecision was less than 3% except for platelets. The system demonstrated good linearity over the entire reporting range and no carry-over (<0.5%). The Sysmex XS-1000*i* showed good correlation with XE-2100, CD-4000 and the manual reference leucocyte differential. Overall flagging sensitivity and specificity were 91% and 48%, respectively. In conclusion, the Sysmex XS-1000*i* demonstrated good analytical performance, is able to generate a complete blood count with five-part differential on low blood volumes and has considerable back-up capacity.

INTRODUCTION

Automated haematology analysers generate a complete blood count and five-part differential leucocyte count (CBC 5-diff) with a rapid turn around time. The Sysmex XS-1000*i* is a new, fully automated haematology analyser with 5-diff functionality. In our study, a Sysmex XS-1000*i* with auto-sampler was evaluated according to Clinical Laboratory Standards Institute (CLSI) and International Council for Standardization in Haematology (ICSH) guidelines. The purpose of our study was to evaluate performance characteristics of the Sysmex XS-1000*i*.

MATERIALS AND METHODS

Specimens

Evaluation of the instrument was carried out using K_2EDTA (1.2–2 mg/mL blood; Sarstedt, Essen, Belgium) anticoagulated venous blood samples sent to our

laboratory for routine CBC testing. All samples were stored at room temperature and analysed within 4 h.

Instruments

The Sysmex XS-1000*i* (XS-1000*i*, Sysmex Corporation, Kobe, Japan) is a new fully automated haematology analyser capable of reporting a CBC 5-diff using an aspiration volume of 20 µl whole blood. The analyser is available with an optional cap piercing autosampler (20 samples) and therefore suitable as a back-up system. The XS-1000*i* comprises a compact main unit (including sampler WxHxD: 425 mm× 385 mm× 600 mm), analysing patient and control samples and an information processing unit (IPU) which serves as data processor. The IPU is controlled by user friendly software and stores results of 10000 patients. Beside the 24-parameter haemogram, the XS-1000*i* can generate following flags: blasts, abnormal lymphocytes/L-blasts, immature granulocytes, left shift, atypical lymphocytes, nucleated red blood cells, RBC-agglutination, turbidity/haemoglobin interference, iron deficiency, haemoglobin defect, RBC fragments and platelet clumps. The XS-1000*i* uses fluorescence flow cytometry with a laser semiconductor to determine leucocyte differential and hydrodynamic focusing with impedance for RBC and platelet counting. Haemoglobin is determined using the sodium lauryl sulphate methaemoglobin method (Oshiro, Takenaka & Maeda, 1982; Karsan *et al.*, 1993). In our study, a Sysmex XS-1000*i* with autosampler was evaluated in its standard configuration, calibrated by Sysmex Corporation according to the manufacturer's specifications. The Abbott Cell-Dyn 4000 (CD 4000, Abbott Diagnostics, Santa Clara, CA, USA) and the Sysmex XE-2100 (XE-2100, Sysmex Corporation, Kobe, Japan) served as comparison instruments (Grimaldi & Scopacasa, 2000; Ruzicka *et al.*, 2001; Nakul-Aquaronne *et al.*, 2003). All instruments were calibrated and used according to the manufacturer's guidelines; the instruments were controlled using routine quality control measurements. During the entire study, commercial two-level controls were tested to guarantee proper performance.

Precision

Precision was tested according CLSI EP5 (CLSI, 2004) using two levels of control solutions (e-Chek, Level 1 lot number: 63290801; Level 2 lot number: 63290802). Each level was tested two times a run, two runs a day, during 20 days ($n = 80$). Within-run and total imprecision were calculated. Within-run imprecision was also determined using patient samples at three levels ('extreme' low, normal and 'extreme' high level, $n = 20$) and CVs were calculated.

Carry-over

Carry-over was assessed by three consecutive analyses of a patient sample with high analyte concentration (H1, H2 and H3) followed by three consecutive analyses of a patient sample with low analyte concentration (L1, L2 and L3). Carry-over was calculated from the formula: Carry-over (%) = (L1 − L3)/(H3 − L3) × 100 (ICSH, 1994).

Linearity

Linearity was tested using patient samples with high values. These samples were diluted at intervals of 10% using platelet-free autologous plasma and were analysed in triplicate. For WBC and platelets, linearity was also tested starting from patient samples with low WBC and platelet counts. Using the average, graphical dilution curves were plotted and regression equations were calculated (ICSH., 1994).

Comparison studies

For comparison of the XS-1000*i* with the CD4000, 108 patient samples were selected. The specimens used in this comparison comprised 50 samples with CBC results, regarded as 'normal' (within reference ranges) and 58 samples regarded as 'abnormal'. For comparison of the XS-1000*i* with the XE-2100, 592 patient samples (232 'normal', 360 'abnormal') were used. Specimens were selected to span the entire range of concentrations as recommended by the International Council for Standardisation in Haematology (ICSH, 1994). All results (CBC 5-diff) were compared by linear regression and correlation coefficients (r) were calculated.

The XS-1000*i* was also compared with the manual reference leucocyte differential method using a total of 80 samples. All samples included were not flagged by the instrument. Wright–Giemsa stained peripheral blood smear slides were evaluated by two experienced

morphologists (authors TG and JVDB) who each performed a manual differential, counting 200 cells (CLSI, 2007). All results (5-diff) were compared by linear regression and correlation coefficients (*r*) were calculated.

Morphological flagging efficiency was evaluated on 175 samples. Following criteria were used to designate a slide as being abnormal: >0% blasts, >1% immature granulocytes (promyelocytes, myelocytes or metamyelocytes), >5% band cells, >3% atypical lymphocytes and >1% nucleated red blood cells. Beside the overall efficiency, we analysed the individual flagging performance for detection of blasts, nucleated red blood cells, immature granulocytes, left shift and atypical lymphocytes.

Throughput

Throughput of the autosampler was tested using four series of 20 randomly selected patient samples. Time was recorded from pushing the start button till finalisation of the last sample (CBC 5-diff).

Minimal volume

The minimal volume whole blood required to perform a CBC 5-diff was tested using Sarsted Microvette® 200 tubes (Sarstedt, Essen, Belgium). Starting at a volume of 30 µl whole blood and reducing in steps of 1 µl, the minimal volume was determined as the lowest volume level able to generate a CBC 5-diff result five times in a row.

RESULTS

Precision

Precision results from Quality Control (QC) and patient samples are shown in Table 1a and b, respectively. Using QC samples (Table 1a), imprecision was less than 3% for all tests except for platelets. Using patient samples (Table 1b), within-run imprecision was less than 3% except for the 'extreme' low level for WBC, Hb and platelets. All results were within specifications recommended by the manufacturer (criteria for within-run

Table 1. (a) Precision QC (b) precision patient samples

	QC Level 1			QC Level 2		
Parameter (n = 80)	Mean	CV_w	CV_T	Mean	CV_w	CV_T
(a)						
WBC, $\times10^9$/l	2.61	1.91	2.86	7.09	1.71	2.62
RBC, $\times10^{12}$/l	2.32	0.67	1.85	4.36	0.67	1.26
Hb, g/dl	6.08	0.80	1.85	12.54	0.63	1.94
Hct, l/l	0.18	0.67	1.74	0.36	0.82	1.39
MCV, fl	77.67	0.79	1.09	81.52	0.37	0.58
Plt, $\times10^9$/l	56.93	3.55	7.08	222.69	1.87	7.63

	Patient low		Patient normal		Patient high	
Parameter (n = 20)	Mean	CV_W	Mean	CV_W	Mean	CV_W
(b)						
WBC, $\times10^9$/l	0.02	22.76	4.40	2.39	111.62	2.06
RBC, $\times10^{12}$/l	1.04	2.52	3.89	0.75	10.27	1.50
Hb, g/dl	3.04	3.09	12.53	0.92	24.00	1.49
Hct, l/l	0.09	1.66	0.36	0.76	0.71	1.46
MCV, fl	61.85	0.59	93.30	0.98	99.48	0.59
Plt, $\times10^9$/l	12.20	10.84	343.95	1.98	1163.75	1.19

CV_W, within-run CV; CV_T, total CV; Hct, haematocrit; Plt, platelets; QC, quality control.

Table 2. Carry-over

	WBC, $\times 10^9$/l	RBC, $\times 10^{12}$/l	Hb, g/dl	Hct, l/l	Plt, $\times 10^9$/l
H1	111.19	10.25	23.80	0.70	1128
H2	113.41	10.14	23.80	0.70	1164
H3	109.10	10.10	23.70	0.69	1172
L1	0.24	2.84	6.70	0.21	11
L2	0.23	3.07	7.30	0.23	9
L3	0.21	3.06	7.40	0.22	13
Carry-over, %	0.03	−3.13	−4.29	−2.13	−0.17

See Table 1b for abbreviation definitions.

CV of a normal patient sample: WBC CV $\leq$ 3%; RBC, Hb, Hct, MCV CV $\leq$ 1.5%; platelet CV $\leq$ 4%).

Carry-over

Carry-over data are presented in Table 2. The results from high to low carry-over were less than 0.5% for all tested parameters.

Linearity

Linearity data are shown in Table 3. The XS-1000*i* showed excellent linearity for all tested parameters ($r \geq 0.99$). Both platelets and WBCs also showed excellent linearity ($r \geq 0.99$) in a lower range (Plt < 50×10^9/l, WBC < 1.0×10^9/l).

Table 3. Linearity

Parameter	Range	Correlation coefficient (r)	Intercept	Slope
WBC, $\times 10^9$/l	544.67–60.94	0.99	−10.58	1.00
WBC, $\times 10^9$/l	5.33–0.50	0.99	0.08	0.94
RBC, $\times 10^{12}$/l	7.13–0.68	0.99	0.04	0.99
Hb, g/dl	21.07–2.10	0.99	−0.02	1.00
Hct, l/l	58.83–6.00	0.99	−0.48	1.00
Plt, $\times 10^9$/l	901–90	0.99	−15.23	1.02
Plt, $\times 10^9$/l	55–8	0.99	9.68	0.86

See Table 1 for abbreviation definitions.

Comparison studies

Results for the comparisons of the XS-1000*i* with the CD4000 and the XE-2100 are presented in Table 4. Overall correlation of the XS-1000*i* with CD4000 and XE-2100 was good. All parameters showed r values $\geq$0.95 with exception of MCHC (CD4000, r = 0.65;

Table 4. Comparison of XS-1000*i* (ordinate) with CD4000/XE-2100 (abscissca) for CBC 5-diff

Parameter	n	Correlation coefficient (r)	Intercept	Slope
WBC, $\times 10^9$/l	108/592	0.99/1.00	0.07/0.01	0.96/1.00
RBC, $\times 10^{12}$/l	108/592	0.99/1.00	−0.16/−0.12	1.03/1.02
Hb, g/dl	108/592	0.98/1.00	−0.44/0.15	1.03/0.99
Hct, l/l	108/592	0.98/0.99	0.01/0.72	0.96/0.99
MCV, fl	108/592	0.97/0.99	1.34/0.70	0.98/1.01
MCH, pg	108/592	0.98/0.99	0.52/0.64	0.99/0.99
MCHC, g/dl	108/592	0.65/0.93	8.00/2.95	0.78/ 0.91
RDW-CV, %	108/592	0.94/0.98	0.52/−1.09	1.03/1.05
Plt, $\times 10^9$/l	108/592	0.99/0.99	−6.70/−4.43	0.94/1.05
NEUT, #	108/592	0.99/0.99	0.10/−0.01	0.94/1.01
EO, #	108/592	0.97/0.99	0.01/0.01	0.97/0.99
BASO, #	108/592	0.10/0.30	0.03/0.03	0.11/0.25
LYM, #	108/592	0.99/0.99	−0.04/0.02	0.96/0.97
MONO, #	108/592	0.95/0.98	0.02/−0.05	1.09/1.14

CBC-5diff, complete blood count and five-part differential leucocyte count; RDW-CV, red cell distribution width coefficient of variation; NEUT, neutrophils; EO, eosinophils; BASO, basophils; LYM, lymphocytes; MONO, monocytes; #, absolute numbers. See Table 1b for other abbreviation definitions.
All results are obtained from CD4000/XE-2100.

Table 5. Comparison of XS-1000i (ordinate) with the manual differential method (abscis) for 5-diff

Parameter	*n*	Correlation coefficient (*r*)	Intercept	Slope
NEUT, #	80	0.99	−0.23	0.97
EO, #	80	0.97	0.02	0.91
BASO, #	80	0.57	0.02	0.32
LYM, #	80	0.98	0.26	0.92
MONO, #	80	0.88	0.16	1.38

See Table 4 for abbreviation definitions.

Table 6. Overall flagging efficiency

	Positive	Negative
Manual differential	74	101
XS-1000*i*	120	55
Sensitivity, % (CI)	91 (83–95)	
Specificity, % (CI)	48 (42–51)	
NPV, % (CI)	87 (78–94)	
PPV, % (CI)	56 (51–59)	
Efficiency, % (CI)	66 (60–70)	

CI, 95% confidence interval; NPV, negative predicitive value; PPV, positive predictive value.

XE-2100, $r = 0.93$), basophils (CD4000, $r = 0.10$; XE-2100, $r = 0.30$) and red cell distribution width coefficient of variation (CD4000, $r = 0.94$). Of all samples compared ($n = 700$), 418 samples were regarded as abnormal. These specimens were selected to determine whether abnormal CBC 5-diff findings could be properly evaluated on the XS-1000*i*.

Results for the comparison of the XS-1000*i* with the manual reference leucocyte differential are presented in Table 5. Overall correlation with the manual differential was good. All parameters showed *r*-values ≥ 0.97, except for the comparison of the basophils ($r = 0.57$) and monocytes ($r = 0.88$).

Morphological flagging efficiency was evaluated on 175 samples using the manual differential as the reference. Abnormal or immature cells were present in 74 of the 175 samples. The XS-1000*i* generated flagging in 67 of these samples. Seven samples were not flagged (blasts, $n = 1$; nucleated red blood cells, $n = 2$; immature granulocytes, $n = 3$; left shift, $n = 1$). No morphological abnormalities were found in the remaining 101 samples. The XS-1000*i* did not generate flagging in 48 of these samples. Fifty-three samples showed false-positive flaggings (blasts, $n = 2$; abnormal lymphocytes/L-blasts, $n = 3$; immature granulocytes, $n = 14$; left shift, $n = 1$; atypical lymphocytes, $n = 9$ more than one positive flagging, $n = 24$). Overall flagging accuracy is presented in Table 6.

Individual analysis of the blast, immature granulocytes, left shift, nucleated red blood cell and atypical lymphocyte flags are presented in Table 7. The blast flag showed the lowest sensitivity. Manual differential counts identified 11 samples positive for blasts. The XS-1000*i* flagged two acute myeloid leukaemias as positive (38% and 93% blasts). The nine false-negative results comprised: one acute myeloid leukaemia (30% blasts, positive abnormal lymphocyte/L-blast flagging), one acute lymphoid leukaemia (28% blasts) and seven samples with regenerative blasts (0.5–12% blasts, six positive for other flaggings that would prompt manual review). All samples positive for blasts were reviewed by a third morphologist who confirmed the presence of blasts in all samples. The XS-1000*i* reported two false-positive blast flaggings. Individual analysis of the immature granulocytes, left shift, nucleated red blood cell and atypical lymphocyte flags showed good efficiency. Manual differential counts identified 54, 16, 18 and 3 samples positive for immature granulocytes, left shift, nucleated red blood cells and atypical lymphocytes, respectively. The XS-1000*i* flagged 44, 12, 15 and 3 samples positive for immature granulocytes, left shift, nucleated red blood cells and atypical lymphocytes, respectively. The XS-1000*i* reported 39, 31, 1 and 49 false-positive flaggings for immature granulocytes, left shift, nucleated red blood cells and atypical lymphocytes, respectively.

Throughput and minimal volume

Average throughput of the sampler was 48 samples/h. Using Sarstedt Microvette® 200 tubes, the minimal volume whole blood required to acquire a CBC 5-diff result was 24 µl.

DISCUSSION

The Sysmex XS-1000*i* is a compact new haematology analyser with 5-diff functionality that is easy to use

Table 7. Individual analysis of flagging efficiency

Flagging	*n*	TP	TN	FP	FN	Sensitivity (%)	Specificity (%)
Blasts	11	2	162	2	9	18	99
Immature granulocytes	54	44	82	39	10	81	68
Left shift	16	12	128	31	4	75	81
Nucleated red blood cells	18	15	156	1	3	83	99
Atypical lymphocytes	3	3	123	49	0	100	72

TP, true positive; TN, true negative; FP, false positive; FN, false negative.

and suitable as a back-up system. Despite its rapid and easy performance, each new haematology analyser should be validated technically before its implementation in daily practice. In our study, a Sysmex XS-1000*i* instrument was evaluated.

The XS-1000*i* showed good imprecision (within-run and total CV) results, comparable with other haematology analysers (Langford *et al.*, 2003; Grimaldi & Scopacasa, 2000; Aulesa and Prieto, 2006; Lehto & Hebdberg, 2007). Overall, platelet counts in the thrombocytopenic range showed worst imprecision. The inaccuracy and imprecision of automated platelet counts in the thrombocytopenic range are a known problem (Kunz, 2001; Segal *et al.*, 2005; Briggs, Harrison & Machin, 2007). In our study, the XS-1000*i* demonstrated no carry-over and good linearity was obtained over a broad range.

Despite the use of different technology, the XS-1000*i* showed excellent correlation with the CD4000 instrument. The XS-1000*i* also correlated well with the XE-2100 instrument using the same technology. Moreover, the fluorescent flow cytometry 5-diff results showed excellent correlation with the manual reference method. In all comparisons (XS-1000*i* *vs.* CD4000, XE-2100 and manual reference method), basophils showed a poor correlation. As already described by others (Grimaldi & Scopacasa, 2000), the cause of the poor correlation for basophils is the low basophil count in the samples used for comparison rather than a poor performance of the analysers. In the comparison with the manual differential count, monocytes also showed a somewhat lower correlation for the same reason (Fuentes-Arderiu, García-Panyella & Dot-Bach, 2007). Monocyte linear regression results, however, are perfectly comparable with those reported by Langford *et al.* (2003) for the Sysmex XT-2000*i*. The differences observed for MCHC measurements are comparable with those found in the determination of reference values for CD4000 and a Sysmex SE 9500 instrument (Van den Bossche *et al.*, 2002).

Overall flagging sensitivity and specificity were 91% and 48%, respectively. Of the samples included for flagging accuracy, 66 (38%) were from children (<18 years of age). The lower specificity is mainly because of the atypical lymphocyte flagging, frequently triggered by the presence of plasma cells in these paediatric samples. Given the low prevalence of atypical lymphocytes, the atypical lymphocytes flagging should be investigated in further studies. Flagging accuracy, especially for detection of blasts is somewhat lower than reported elsewhere (Langford *et al.*, 2003; Kang *et al.*, 2007). This is probably because of the low prevalence of blasts in our study and the different experiment setup (blasts >1% *vs.* blasts >0%). Moreover, five of the nine false-negatives showed low WBC ($<4 \times 10^9$/l) which has been documented to decrease flagging sensitivity (Ruzicka *et al.*, 2001). The blast flagging should therefore be investigated in further studies. Using the sampler, the XS-1000*i* showed a considerable throughput of 48 samples/h, making it a thorough back-up instrument. Using Sarstedt Microvette® 200 tubes, only 24 µl whole blood is required to acquire a CBC 5-diff result. In conclusion, the Sysmex XS-1000*i* demonstrated good analytical performance, is able to generate CBC 5-diff on low blood volumes and has considerable back-up capacity.

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