Performance Evaluation of the Sysmex XE-2100™, Automated Haematology Analyser

Carol BRIGGS, Paul HARRISON, Donna GRANT,
Julie STAVES, Naina CHAVADA and Samuel J. MACHIN

Department of Haematology, University College London Hospital, Gower Street, London WC1E 6AU, U.K.

The performance characteristics of this latest automated haematology analyser were evaluated over a three-month period. The new diagnostic features on the XE-2100 are an immature granulocyte (IG) count, nucleated red blood cell (NRBC) count and the ability to measure platelets in two ways by impedance and also an "optical" platelet count by a new polymethine fluorescence dye and a semiconductor diode laser in the red cell reticulocyte channel.

Our investigation included appraisals of accuracy, precision, linearity, carryover and sample stability, all of which were satisfactory. The correlation study was performed against our routine analyser Sysmex SE-9500™ and included manual differentials performed according to National Committee for Clinical Laboratory Standards (NCCLS) Document H20-A and flow cytometry for a limited number of platelet and of monocyte counts.

The XE-2100 NRBC count was excellent, r^2- value of 0.9916 when compared to the manual count. The "optical" platelet count gave a correlation coefficient of > 0.9 when compared to flow cytometry. The immature granulocyte count compared to a manual differential gives a r^2- value of 0.668. The correlation coefficients for all complete blood count (CBC) and differential parameters obtained by comparison to the SE-9500, manual differentials or flow cytometry were all good.

The flagging on the XE-2100 was also evaluated.

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Key Words Automated Hematology Analyzer, XE-2100, Nucleated Red Blood Cell (NRBC) Counts, "Optical" Platelet Counts, Immature Granulocyte Counts, Fluorescence Flow Cytometry

INTRODUCTION

The XE-2100™ is the latest haematology analyser to be introduced to the market and utilises the technology of fluorescence flow cytometry to quantitate the standard five part differential, immature granulocytes (metamyelocytes, myelocytes and promyelocytes), nucleated red blood cells (NRBC), reticulocyte count, immature reticulocyte fraction and "optical" fluorescent platelet count.

The combination of side scatter (inner complexity of the cell), forward scatter (volume) and fluorescence intensity of nucleated cells gives a concise but precise image of each cell detected in the peripheral blood. A well-defined physical description of the different leucocyte populations (clusters) is obtained. Abnormal and immature cells, with their larger nuclear volume show much higher fluorescence intensity than normal cells, and are easily distinguishable in the DIFF scattergram.

The immature information (IMI) channel of the XE-2100 counts human progenitor cells (HPC). The reagents specifically affect the lipid components of the cell membranes; the membranes of mature cells, with a higher content of lipid are lysed while immature cells retain their membranes. In normal samples no intact cells are seen in the IMI area. The HPC has been shown to be an important parameter in the prediction of the apheresis yields of $CD34^+$ cells in peripheral blood in patients undergoing progenitor cell mobilisation. It has been demonstrated that the use of peripheral blood HPC counts gives a more precise measurement of early cells than visual blast cell counts and allows a more quantitative assessment of the release of progenitor cells into the blood. The use of an HPC screening assay followed by $CD34^+$ cell measurements restricted to those patients with an HPC count of $<10 \times 10^6$/L was found to be highly predictive and more cost effective than the use of $CD34^+$ cell measurements alone[1].

For the NRBC the cell membranes of NRBC and red blood cell (RBC) are lysed, the white blood cell (WBC) are slightly perforated to allow quick influx of the dye, but remain intact. Nuclear material is stained with a polymethine-based fluorescent dye, and cells and nuclei are hydrodynamically focused. The fluorescence intensity and forward scattered light intensity of each cell are electronically analysed. This allows the clear separation of leucocytes and NRBC.

The "optical" platelet count is measured in the reticulocyte channel. A fluorescent dye is used to stain the RNA/DNA of reticulated cells. This technology allows the counting of reticulocytes, erythrocytes and fluores-

cence thrombocytes. In the flow cell each single cell is passed through the beam of a semiconductor diode laser. The XE-2100 also produces a reticulocyte "differential" based on the RNA/DNA content of the cell.
The analyser has a maximum throughput of 150 samples per hour, and uses 130 μL of blood in open mode and 200 μL in closed mode. The analyser can be run in seven different settings, CBC, CBC + DIFF, CBC + NRBC, CBC + RET, CBC + DIFF + NRBC, CBC + DIFF + RET, and CBC + DIFF + RET + NRBC. The workstation has the capacity to store data on 10,000 samples, including graphics. Quality control, L-J and X bar M files are built in. Quality control files for low, normal and high levels for both CBC DIFF and reticulocytes are available, and the new parameters, NRBC, "optical" platelets and immature granulocytes (IG) also have assigned values.
The correlation of the CBC, WBC differential and reticulocyte data were performed against our Sysmex SE-9500™. This is an automated analyser with a 23-parameter haematology analyser with automated sample handling and barcode reader attached to a PC-based workstation. The analyser has an attached automated reticulocyte analysis module RAM-1™. This employs the RNA staining fluorochrome Auramine O, laser light and the principles of flow cytometry.

MATERIALS AND METHODS

The XE-2100 tested at University College London Hospital (UCLH) was a pre-production model; all specimens were sampled in the open mode.
For this evaluation residual samples were taken from the routine workload, approximately 100 normals and 100 haematological malignancies, the rest being made up of various other abnormalities such as HIV, haemoglobinopathies, platelet disorders, malaria and neonates. Samples were processed on the XE-2100 and the laboratorys routine SE-9500. The anticoagulant used was K_3EDTA.
The manufacturer initially checked the calibration status of both the XE-2100 and SE-9500. Quality control was run on both analysers on a daily basis.
Immediately after sampling, four separate blood films were prepared by a manual wedge method. Slides were stained on an automated Hematek® Slide Stainer using a modified Wright's stain. A manual 400 cell WBC differential count was performed according to National Committee for Clinical Laboratory Standards (NCCLS) H20-A protocol[2]. 2 × 200 cell differentials were performed by at least two qualified biomedical scientists.
50 selected samples with low, normal or high monocyte counts were also analysed on the XE-2100, SE-9500 and Epics® XL – MCL Flowcytometer (Beckman Coulter) after whole blood staining by application of monoclonal antibodies CD13, CD14, and CD45[3].
50 samples were also analysed on the SE-9500 for impedance platelet count, and the XE-2100 for impedance and "optical" platelet count. These results were compared to the platelet count measured by our reference flow cytometric RBC ratio method[4]. Whole blood was mixed with Anti-CD61-FITC (Beckton Dickinson) and Isoton (Beckman Coulter) and incubated for 1 minute and further diluted with Isoton to give a final dilution of whole blood of 1:2000. Flow cytometric analysis was performed on an Epics® XL – MCL Flowcytometer. This is a proposed reference method for platelet counting.
The method of Broughton[5], et al. was used, as recommended by the International Council for Standardization in Haematology (ICSH)[6] to assess carryover. Linearity was studied on all cells by diluting a sample with a high count with AB serum to cover the entire analytical range. Precision was measured for all cells using samples with a low, normal and high count. The samples were processed ten times. The means, and CV% calculated on the data obtained.
The stability of all parameters was examined over 72 hours in blood stored at room temperature and at 4°C.

RESULTS

Comparison of the XE-2100 immature granulocyte count to the manual reference count

Immature granulocytes were defined as metamyelocytes, myelocytes and promyelocytes. Blast cells were not included in the comparison, nor were stab cells (band cells), as these are not usually counted separately in the U.K.
The XE-2100 gave an immature granulocyte count on 278 out of 465 samples analysed. The total number of samples showing immature granulocytes by the 400 cell manual reference count was 129 out of 465.
The XE-2100 has a lower limit of detection than the manual count and will report immature granulocytes as low as 0.1%, which is obviously not possible on a manual differential, the lowest count seen on the manual film was 0.5%. The number of samples with a count of 0.5% or above on the XE-2100 and no immature cells seen on the manual count was 25, range of counts 0.5% - 4.4% ($0.01 - 1.22 \times 10^9$/L). There were also 21 samples which showed an immature granulocyte count on the manual count but not on the XE-2100, range of counts 0.5% - 1.5% ($0.04 - 1.05 \times 10^9$/L). There was no consistent morphological features or diagnosis on these samples that would account for this discrepancy.
The number of samples showing immature granulocytes on both the XE-2100 and manual count is 108. The scatterplot for these samples are displayed in ***Fig. 1***.
Moderately good correlation was demonstrated between the XE-2100 and manual counts, considering the low number of cells being analysed and the subjectivity in the manual classification of immature granulocytes, especially at the marked left shift, band cell or metamyelocyte stage.

Comparison of manual NRBC count with the XE-2100

The scatterplot for this parameter for 100 samples processed can be seen in ***Fig. 2***.
Excellent correlation was found; the XE-2100 has a lower limit of detection than the manual observers and reports NRBCs as low as 0.1/100 WBC.
There was no evidence of any interference from RBC

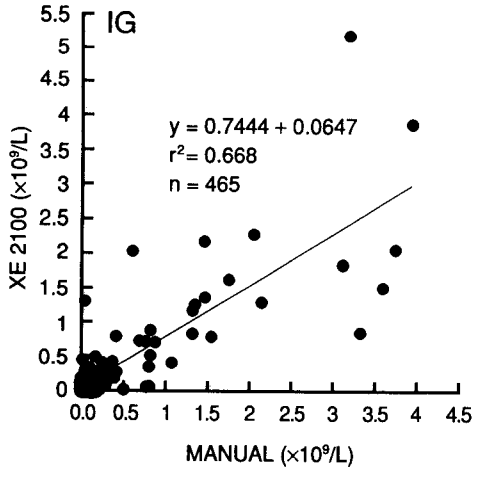

Fig. 1 Correlation of manual and XE-2100 immature granulocyte counts

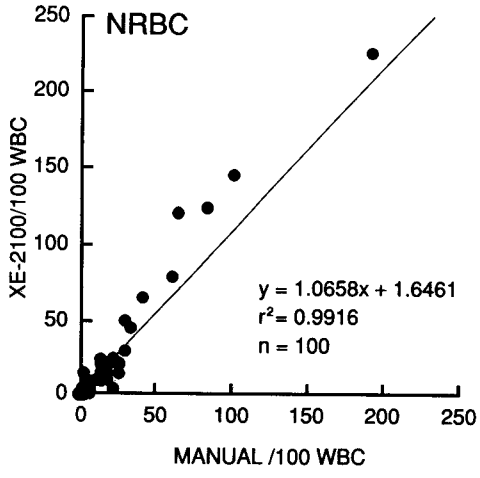

Fig. 2 Correlation of manual and XE-2100 nucleated red blood cell counts

inclusion bodies such as Howell Jolly bodies or malarial parasites. It is most likely that this analyser's NRBC count is more accurate and precise than the manual method, due to the large number of cells counted and the lower limit of detection.

Comparison of XE-2100 impedance and "optical" platelet counts with a flow cytometry

Excellent correlation was found when comparing the SE-9500 and XE-2100 impedance platelet counts (r^2 = 0.9958).

For samples with a platelet count above 100 × 10^9/L the correlation for both impedance and "optical" counts with flow cytometry was excellent (r^2>0.96). For platelet counts below 100 × 10^9/L the correlation of the impedance platelet counts with the flow method were not as good as the XE-2100 "optical" count.

The scatterplots for these platelet comparisons are displayed in ***Fig. 3***.

When the XE-2100 is run in reticulocyte mode either the impedance platelet, or "optical" platelet count is reported, depending on a switching algorithm. From the scatterplots it can be seen that in thrombocytopenic samples the "optical" platelet count is not always reported when by comparison to flow cytometry it is the more accurate count. A refined switching algorithm has been developed and is now being evaluated at UCLH.

Comparison of XE-2100 and SE-9500 CBC parameters and absolute differential results

To make the comparison of the WBC and lymphocyte counts all samples with NRBC were excluded. Due to machine vote-outs there are different numbers of results compared for some parameters.

On this prototype version of the XE-2100 we were able to access both the impedance and "optical" platelet count on every sample, however the analyser will only give one result on the report dependent on the analyser's algorithm. For the platelet comparison between the two analysers, a comparison was made between the two impedance counts, and also the SE-9500 impedance count with the platelet count reported by the XE-2100, which could have been impedance or "optical" depending on the analyser's algorithm.

For ease of comparison the correlation coefficients, slope and intercepts are summarised in ***Table 1***.

Excellent correlation was found between the XE-2100 and SE-9500 for all parameters, except for the reticulocytes. The reticulocyte count correlation coefficient was 0.7977; all outliers were from patients with sickle disease or beta thalassaemia major.

When the two analysers low fluorescence reticulocytes were compared the correlation was 0.84 but the XE-2100 is giving consistently slightly higher results than the SE-9500. The correlation for the high fluorescence reticulocytes is 0.5277.

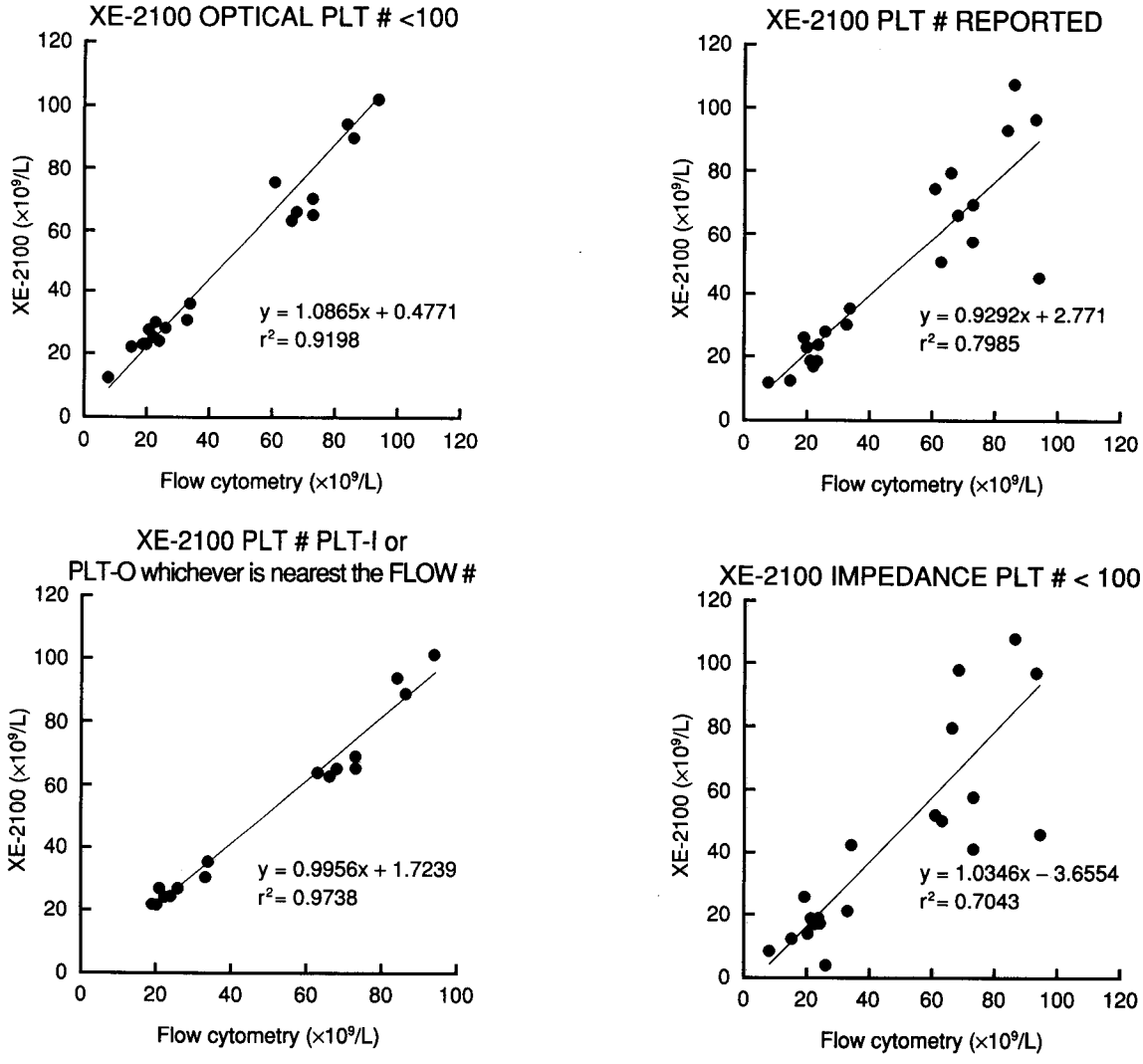

Fig. 3 Comparison of flow cytometry and XE-2100 platelet counts on samples with counts < 100 × 10⁹/L

Table 1 *Comparison of XE-2100 and SE-9500 CBC and differential*

Parameter	Correlation coefficient	Slope	Intercept	N=
WBC	0.9998	0.9849	0.0939	315
RBC	0.9973	0.9893	−0.0137	415
HGB	0.998	1.0158	−0.11	415
HCT	0.9971	0.9807	−0.0028	415
MCV	0.9949	0.9614	2.3027	415
MCH	0.98	0.9932	0.8148	415
MCHC	0.8847	0.9293	3.4027	415
RDW-SD	0.9958	0.9531	0.8156	411
RDW-CV	0.995	0.9751	0.1375	412
PLT	0.9956	0.9755	1.8672	415
REPORTED PLT	0.9927	0.9798	1.6403	415
PDW	0.8823	0.9417	1.0169	337
P-LCR	0.9465	0.988	3.7674	337
MPV	0.9124	0.9434	1.0709	337
RET #	0.7977	0.932	0.0057	407
IRF	0.84	0.7724	−0.6825	407
LFR	0.84	0.7726	23.437	407
MFR	0.7746	0.8186	−0.7212	407
HFR	0.5277	0.4219	0.5887	407
NEUT#	0.9838	0.9963	0.0889	344
LYMPH #	0.9414	0.9964	0.135	365
MONO #	0.7359	0.7816	0.098	379
EO #	0.9217	0.9837	−0.0075	397
BASO#	0.1395	0.8719	0.0231	338

Table 2 Comparison of XE-2100 and manual reference method (manual 400 cell differential counts)

Parameter	Correlation coefficient	Slope	Intercept	N=
NEUT	0.9812	0.9378	0.094	379
LYMPH	0.9887	0.961	0.1085	381
MONO	0.647	0.9364	0.1569	389
EO	0.8096	0.909	0.0461	398
BASO	0.1983	0.94	0.0415	403

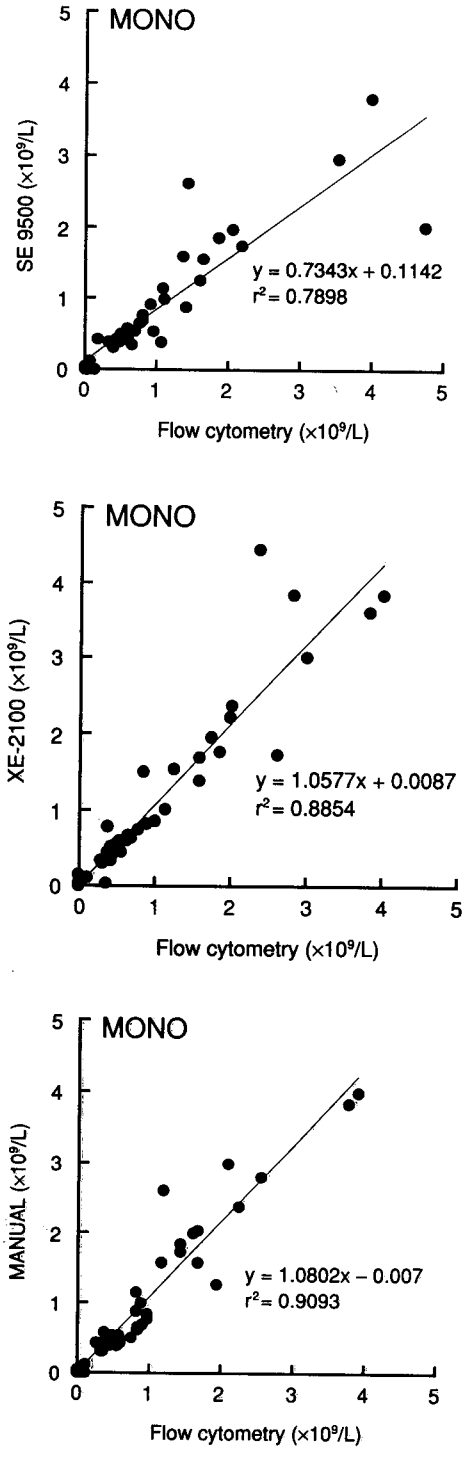

Fig. 4 Correlation of monocyte count of SE-9500, XE-2100 and manual method to flow cytometry

Comparison of XE-2100 with manual differential reference method

To make the comparison of the analyser differentials with the manual counts the following steps were taken:

1) Immature granulocytes (metamyelocytes, myelocytes, promyelocytes, and myeloblasts) were added to the neutrophil count of the manual differential.
2) Prolymphocytes and lymphoblasts were added to the lymphocyte count of the manual differential.
3) The WBC was corrected for any NRBC present.

Due to machine vote-outs different numbers of results are compared to the manual differential for each of the WBC differential.

The correlation coefficients, slope, intercept point and number of results in the comparison are shown in ***Table 2.***

The comparisons with manual counts are good for neutrophils, lymphocytes and eosinophils.

As the correlation of the manual and XE-2100 monocyte count was less good ($r^2 = 0.647$), and SE-9500 with XE-2100 ($r^2 = 0.7359$). A further study of 50 samples with a wide range of monocyte counts ($0 - 4.07 \times 10^9$/L) were analysed by the XE-2100, manual differential and flow cytometry using combinations of CD13, CD14 and CD45 monoclonal antibodies. The comparisons with flow cytometry scatterplots are presented in ***Fig 4.***

Both the XE-2100 and manual differentials showed good correlation with the flow cytometry.

Comparison of XE-2100 and SE-9500 abnormal cell flagging capabilities

The XE-2100 flags generated were compared to those of the SE-9500 and the manual observation of the blood film.

The sensitivity, specificity, predictive value of a positive or negative result and overall efficiency were determined using the Galen and Gambino formula, as recommended by the ICSH[5].

The flagging performance of the two machines is similar, apart from the atypical lymphocyte flag and platelet clump flag which both show far fewer false positives and improved overall efficiency on the XE-2100. The atypical lymphocyte flag showed no false positives on the XE-2100, but 23.1% on the SE-9500. The platelet clump flag gave 11.5% false positives on the XE-2100 and 28.3% on the SE-9500. The RBC fragment flag on the XE-2100 shows more false positives (7.2%) than the SE-9500 (none).

The XE-2100 has an abnormal lymphocyte/lymphoblast flag in addition to the blast flag, but this was not found to be very sensitive, giving many false positives.

Carryover

The method of Broughton, et al. was used, as recommended by the ICSH. A high sample is processed three times (i_1, i_2, i_3), followed by a sample with a low concentration three times (j_1, j_2, j_3). Carryover percentage is calculated by:

$$\text{Carryover (\%)} = \frac{j_1 - j_3}{i_3 - j_3} \times 100$$

Each parameter was tested on three occasions.
Carryover was shown to be insignificant. Results can be seen in ***Table 3.***

Reproducibility

Reproducibility was quantified by performing ten consecutive measurements on 3 different blood samples; samples were selected with low, normal or high values. Coefficients of variation (CV) demonstrated good results for all parameters. The results for the new parameters on the XE-2100 can be seen in ***Table 4.***

Linearity

Linearity data for specimens run on the XE-2100 showed good correlation for all cells. Samples with a high count for each of the cells were diluted in AB serum to give concentrations to cover the entire analytical range. The results can be seen in ***Table 5.***

Stability studies

The effect of time and incubation temperature for all cells and parameters was examined. Samples were analysed immediately after venesection and at varying intervals over a 72 hour period. The samples were divided into two; one set of samples was stored at room temperature and the other at 4°C.
All cells and parameters were well maintained over 72 hours at 4°C.
At room temperature there was an increase in the MCV, and "optical" platelet count after 24 hours. The immature granulocyte count was stable at room temperature for 48 hours but then began to fall. The reticulocyte count fell slightly over time at room temperature. The NRBC count was well maintained at both temperatures.

DISCUSSION

Our evaluation has shown that the XE-2100 and SE-9500 have excellent correlation on the CBC parameters and differential counts. The use of fluorescence flow cytometry for the WBC differential gives good correlation with manual reference counts and the reference flow cytometry for the monocytes, even when highly abnormal or aged samples are analysed. Good correlation is seen between the XE-2100 immature granulocyte and manual counts considering the low number of cells being analysed and the subjectivity of the manual classification of immature granulocytes.
An evaluation of the HPC count was not undertaken in this study, however the principles for detecting and counting HPCs is the same on the XE-2100 as the SE-9500.
The NRBC count from the XE-2100 gives excellent correlation with the manual reference counts, and is most likely to be more accurate and precise than the manual count due to the larger number of cells counted and the lower limit of detection. The total WBC count and lymphocyte counts are automatically corrected on the report produced by the analyser. The introduction of this new parameter would greatly reduce the number of manual differential counts in the laboratory.
The "optical" platelet count shows excellent correlation with the reference flow cytometry, the correlation is particularly impressive on platelet counts below 100×10^9/L, and shows far better results than the impedance count at this level. In abnormal states such as TTP, ITP or myelodysplastic conditions where large platelets are often present the impedance count may exclude these on the basis of their abnormal size and gives a falsely low count. Where RBC fragments are present the impedance

Table 3 XE-2100 carryover

Parameter	Carryover 1	Carryover 2	Carryover 3
WBC	0.06	0	0.05
HGB	0	0	0
RBC	0	0.24	1.02
PLT	0	0	0
PLT–O	0.27	0.15	0.17
RET	0	0.63	0.39
NRBC	0	0	0

Table 4 XE-2100 reproducibility

Parameter	Unit	Mean	SD	CV%
Immature GRANS	$\times 10^9$/L	0.57	0.034	5.99
NRBC	/100 WBC	63.91	14.35	2.25
PLT–O	$\times 10^9$/L	193.6	3.69	1.91

Table 5 XE-2100 linearity

Parameter	r^2
WBC	0.997
RBC	0.999
HGB	0.999
PLT-IMP	0.997
PLT-OPT	0.987
RETIC	0.997
NRBC	0.999
Immature GRAN	0.991

count may include these in the platelet count on the basis of their size and gives a falsely high count. This accurate "optical" platelet count is very important in aplastic states especially in platelet counts below 10×10^9/L, where prophylactic platelet transfusions may be needed[7].
The abnormal cell flagging capabilities of the XE-2100 were mostly comparable to the SE-9500 except for the atypical lymphocyte flag and platelet clump flag. Both these flags showed far fewer false positives than the SE-9500, however the RBC fragment flag gave more false positives on the XE-2100 than the SE-9500.
Experiments to establish carryover, reproducibility, linearity and sample stability yielded excellent results.
The XE-2100 shows good comparison for all parameters with the SE-9500, and with the new parameters should result in a lower number of manual differentials performed in the laboratory, and confidence in an accurate platelet count in thrombocytopenic samples.
In addition the XE-2100 compared to the SE-9500 has a faster throughput of samples, uses a smaller sample volume of blood for analysis and has minimal machine maintenance, all of which are important advantages to a busy laboratory.

ACKNOWLEDGEMENTS

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