Evaluation of the Sysmex XN-550, a Novel Compact Haematology analyser from the XN-L® series, compared to the XN-20 system.

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Introduction
The XN-550 is a new, automated, compact, haematology analyser designed to generate a full blood count with a standard 5-part white blood cell differential and an immature granulocyte count, reticulocyte and optical platelet counts. The aim of the study was to evaluate the performance of the XN-550 and compare it to the established XN-20 system.

Methods
We evaluated the basic parameter and special measurement channels of the XN-550, using the XN-20 (which has a similar operating system), as a reference analyser. Precision, carryover and throughput evaluations were performed. In addition a total of 202 samples including normal controls and various pathological samples were studied for comparability.

Results
Good correlations with the reference analyser were obtained for all parameters expect basophils. The XN-550 offers impedance and optical platelet counts and the latter showed a better correlation and less scatter, than the impedance count and was comparable to the XN-20 fluorescent count at platelet counts $\leq 40 \times 10^9$/L. Precision was good and no significant carryover was detected.

Conclusions
The XN-550 was simple and easy to use, while maintaining the good diagnostic sensitivity seen with high range systems such as the XN-20, making this compact device suitable for near-patient services and smaller satellite laboratories.

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Introduction

Major advances in scientific technology have given rise to sophisticated automated blood cell analysers which are suitable for large and medium sized laboratories as well as smaller, point of care testing (POCT) units. These allow faster turnaround times and permit quicker clinical decisions, aiding patient care. Healthcare professionals welcome the use of POCT analysers within medical practice, but are often concerned about their widespread use as it is essential to ensure that they are safe and efficacious, while remaining economical [1, 2]

The XN-20 (Sysmex Corporation, Kobe, Japan), a high range analyser, was first evaluated in 2012 [3] and a further evaluation with paediatric samples was performed in 2015.[4] A new automated, compact version (Haematology Lite series; XN-L) has recently been launched worldwide. The XN-L series have an integrated image processing unit and a liquid crystal display (LCD) colour touch screen. There are three variants available: the XN-350 offers single sample analysis in open mode; XN-450 providing single sample analysis in closed mode, and the XN-550 which includes a fully automated, continuous loading sampler, as well as rerun and reflex functionality.

In this study we evaluated the XN-550, using the XN-20 as a comparator device. Recent evaluations of the XN-550 (published as Letters) used the XN-9000, XE-2100, or XN-3000 as predicate devices [5, 6]. One of these [5] mainly studied healthy normal subjects with some haematological malignancy patients. The second study [6] had a larger sample number, but little information about the disease states. Both studies presented the data simply in terms of regression and correlation, without indicating the range of values studied for each parameter, or graphical display of the results, making it difficult to assess outliers. Samples with low platelet counts or nucleated red blood cells (NRBC) were not specifically studied. A good correlation was generally observed for all parameters investigated expect for basophils. Our study focuses on the different platelet counting methods, particularly the performance on samples with low platelet counts, and the analysis of samples with low white cell counts, together with the NRBC Q-flag.

The XN-550 is a compact analyser which can perform a full blood count (FBC) and a five part differential (DIFF) analysis as well as optional reticulocyte (RET) testing using an aspiration sample volume of 25µl. Platelet (PLT) count is available by both impedance (PLT-I) and optical (PLT-O) methods (PLT-O is produced in the RET channel, dependent upon the intensity of fluorescence staining. Fluorescent platelet analysis (PLT-F) is not available on the XN-L series analysers). The XN-20, with its dedicated PLT-F channel, provides the most accurate and precise automated platelet count of the three [3] and a publication by Sysmex scientists has shown that it clearly distinguishes between platelets and fragmented erythrocytes [7]. The XN-550 incorporates a set algorithm to specifically evaluate and select the most accurate platelet count (PLT-I or PLT-O) so as to display the best 'reportable' results. Samples need to be run in FBC+DIFF+RET mode to obtain PLT-O, which can be useful in various clinical situations to confirm a low platelet count or to eliminate interferences.

The XN-550 does not offer a dedicated method for quantification of nucleated red blood cells (NRBC), but all samples containing NRBC are indicated by a unique Q-flag, which grades NRBC content between 0-300 with a (default threshold = 100 and values ≥100 suggest NRBC). The XN-550 also provides a NRBC% calculation and a total nucleated count as research parameters (activated by manufacturer on request). Samples with low WBC counts

(<0.5) can trigger a reflex analysis in the Low WBC mode (LW) where the count time is extended by three-fold.

There is a pre-dilution mode available which can be used to obtain a FBC result from low volume blood samples. Body-Fluid mode can also be used for analysis of cerebrospinal, synovial, and serous fluids, but was beyond the scope of this performance evaluation, which focussed on precision and comparability in a wide range of normal and abnormal peripheral blood samples.

Methods

Both analysers were serviced and calibrated using XN-CAL by specialist engineers before the evaluation. QC-Level 2 material was run before each day of work and precision was assessed in accordance with ICSH guidelines [8]. Normal and abnormal patient samples were selected for within run precision, commercial normal QC samples were used for both within and between run precision (n=6 for each estimation).

In comparability studies we tested a total of 202 residual K_2EDTA samples from adults, selected (to give information on each parameter at the extremes of the pathological range) and then anonymised, after all routine testing had been performed, from the University College London Hospitals haematology laboratory workload. The use of remnant samples is approved by the local ethical committee for internal quality control and the evaluation of new technology. Samples were run in FBC+DIFF+RET mode on the routine XN-20 system (as the predicate analyser) followed by the XN-550 analyser, within a 20 minute time period. All work was completed between February and April 2016. 30 samples with a normal FBC and 172 samples from patients with a variety of disorders, including: haematological malignancies, haemoglobinopathies, idiopathic thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP) and other diseases were tested. All samples were run in the closed automated cap piercing mode. Pre-dilute mode was compared to the normal, whole blood mode for FBC, using 12 samples with abnormal and 8 samples with normal counts.

Samples with high and low values for each parameter (WBC, PLT, haemoglobin) were selected for a carryover study; a sample with high values was analysed three times consecutively, followed by a sample with low values, 3 times consecutively. Percentage carryover was calculated by the method of Broughton [9], as recommended by the ICSH [8]. Throughput was evaluated using 80 randomly selected samples from the normal hospital workload, with analysis running continuously for 1 hour, both in FBC+DIFF and in FBC+DIFF+RET modes.

Statistics:
Data was assessed using summary statistics and Pearson correlations; a probability value of $p<0.05$ was considered significant. No outliers were eliminated unless specifically indicated in the Results sections (e.g. where a few data points were widely separated from the main data group and could potentially have distorted the correlation). Platelet, neutrophil and reticulocyte counts were analysed both as the entire data set and also as a separate analysis of samples with low counts. This was performed for two reasons: firstly since the overall data plots make it difficult to see whether the correlation holds true at low counts; and secondly because low counts may be critical in clinical decisions. In the case of platelet counts, the critical count for prophylactic platelet transfusion is generally taken as $10-30\text{x}10^9$/L, depending on the sensitivity and accuracy of the technology available [10]; we therefore analysed samples with counts $<40\text{x}10^9$/L in order to study method comparability in this range.

Results

XN-550 within and between run precision was good for FBC, PLT-I, PLT-O and RET, using normal and abnormal samples, and similar to the XN-20 (Table 1). 57 samples per hour were processed using FBC+DIFF mode, and 35 samples using FBC+DIFF +RET mode. In pre-dilute mode, the XN-550 showed excellent correlation with whole blood (WB) mode for the standard FBC parameters (excluding basophils) (r^2 value range 0.844-0.998). No significant carryover was detected (<0.29%).

There was a good correlation (r^2>0.99) between the two analysers for FBC parameters over the following measurement ranges: WBC 0.01-108.9x10^9/L, Hb 61.0-171.0g/L, MCV 56.1-108.6x10^9/L. There were also good correlations (Fig. 1) for neutrophils (measurement range 0.01-34.4x10^9/L), monocytes (0.01-6.5x10^9/L), lymphocytes (0.01-96.8x10^9/L), eosinophils (0.1-0.79x10^9/L), and reticulocytes (0.1-21.9%; 2.3-431.9x10^9/L). There was a small bias between methods for reticulocytes which did not appear to be clinically significant. Basophils gave a weak correlation. There was one outlier with a neutrophil count of 0.2 by XN-550 and 1.0 by XN-20 in a patient with chronic liver failure. There were four monocyte outliers with counts of 0.89, 1.77, 3.06 and 6.50x10^9/L compared to 2.05, 3.59, 7.26 and 2.15x10^9/L on the XN-550 and XN-20, respectively. Two subjects with very high lymphocyte counts (approximately 91.0 & 97.0x10^9/L), were distinctly separated from the main data cluster, but gave similar results by both analysers. These were excluded from the regression and correlation calculations (Fig. 1) to avoid potential distortion of the statistics.

Both impedance and optical platelet counts produced similar results, with good correlation over the whole measurement range (6-1007x10^9/L) (Fig. 2, upper panels). However, at low platelet counts (≤40x10^9/L), the XN-550 PLT-O count showed a better correlation with the XN-20 PLT-F count, than that seen for the XN-550 PLT-I, with less scatter and no significant bias (Fig. 2, lower panels).

Of 202 samples evaluated, 15 (from patients with haematological malignancies or haemoglobinopathy) had a positive NRBC Q-flag 7 samples had NRBC Q-flag ≥150, all having a raised XN-550 NRBC% (>3%). Two of these patients had beta thalassemia major with an increased WBC count (33.2 and 43.9x10^9/L by XN-550, 15.5 and 13.3x10^9/L by XN-20). This apparent discrepancy was due to the presence of large numbers of NRBCs interfering with the uncorrected WBC reported by the XN-550.

Discussion

The XN-550 analyser produced equivalent results to the XN-20 high range system, showing that this is a very capable and sensitive device that can easily be implemented in a small satellite laboratory, or can be used as a secondary analyser for out of hours service or as an emergency system.

Our throughput data was in line with the manufacturer's specification (approximately 60 samples per hour in (FBC+DIFF mode) and 35 samples all per hour in (FBC+DIFF+RET mode) in the (WB) mode). The XN-550 was able to process samples rapidly with the FBC+DIFF mode, while the RET mode can be used for further analysis of certain selected patient samples.

XN-550 PLT-O analysis compared well with the PLT-F results from the XN-20, especially when the count was less than $40x10^9$/L; whereas a weaker correlation and greater scatter was observed between XN-550 PLT-I and XN-20 PLT-F (Figure. 2). The PLT-F method was previously found to be superior to the other platelet counting methods available on the XN-20 [3] in comparisons with the International reference flow cytometric assay [11]). However, the XN-550 does not have a PLT-F count, but PLT-O has the advantage of separating the platelets from small red cell fragments and other interfering debris [7, 12]. The clinical management of patients with malignant haematological disorders, as well as TTP and ITP, is dependent on measurement of the correct platelet count, since this influences the use of platelet transfusion or other specific therapies; hence an accurate, reliable count is crucial [13]. At low platelet levels the impedance count can be misleading in certain circumstances, particularly at a pre-set clinical cut-off count for prophylactic platelet transfusions. The optical count was certainly comparable to the impedance counts and thus gives the new user confidence in applying clinical decisions to a specific low count. Our results indicate that clinicians can rely on the reported XN-550 platelet count (the most valid count is selected automatically) to instigate and regularly support a local protocol guided platelet count threshold within their in-house transfusion policies.

The presence of peripheral blood NRBC is associated with various pathological conditions and usually leads to the overestimation of the total WBC counts in automated haematology analysers [14]. The XN-550 provides a NRBC Q-Flag which alerts the healthcare professional to investigate further. The XN-20 with its dedicated WNR channel, corrects the leucocyte count according to NRBC count, reducing further test requirements. NRBC Q-Flag above 150 was in good agreement with NRBC% on both analysers. However, XN-550 NRBC% is a non-reportable research parameter so users need to rely on the NRBC Q-flag to initiate further clinical investigations or reflex testing on the main analyser.

Occasional discrepant results were seen with the monocyte counts. Identification of monocytes by blood cell counters, flow cytometry, and microscopic means is difficult and controversial, especially when abnormal cells are present [15] (which are not always of a monocytic lineage). The observation of weak/poor correlation for monocyte counts has been observed regularly in previous instrument evaluations [16, 17]. For this set of clinically abnormal samples, discrepant results were also occasionally observed for, neutrophils and lymphocytes. These sample sets were mainly from leukaemia patients and both instruments generated error flags to help further assess the patient samples.

The limitations of this study are that we did not perform linearity studies or use reference morphological and flow-cytometric procedures for WBC differential counts, however, the XN-550 was evaluated against the XN-20, which we have previously studied in detail with full morphology reporting [3].

The simplicity of operation, excellent display design, and the precise results of the XN-550 analyser make it a recommendable haematology instrument for POCT services; including stem cell laboratories, a mobile trolley service for specific outpatient clinics and physician style office laboratories.

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Legends to Figures

Figure 1. Comparison of WBC Differential and Reticulocyte Parameters on the XN-550 and XN-20.
The results for the whole data set for neutrophils, eosinophils and basophils are shown as Pearson scatter regression plots; those for lymphocytes, monocytes and reticulocytes are shown as Bland and Altman plots (dotted line = zero bias; solid line = regression line; broken lines represent +/-2SD range from mean bias). Samples with low neutrophil or low reticulocyte counts are also shown as Bland and Altman plots.

Figure 2. Comparison of Platelet counts.
The two upper panels show the comparability between PLT-I and PLT-O methods on the XN-550 and XN-20 for the total data set. The two lower panels show the comparability for samples with low platelet counts (≤ 40 x10^9/L) on by PLT-I or PLT-O on the XN-550 vs PLT-F on the XN-20, using Bland and Altman difference plots.

Figure 1.

Neutrophils

Neutrophils ≤1.0x10^9/L

Lymphocytes

Monocytes

Eosinophils
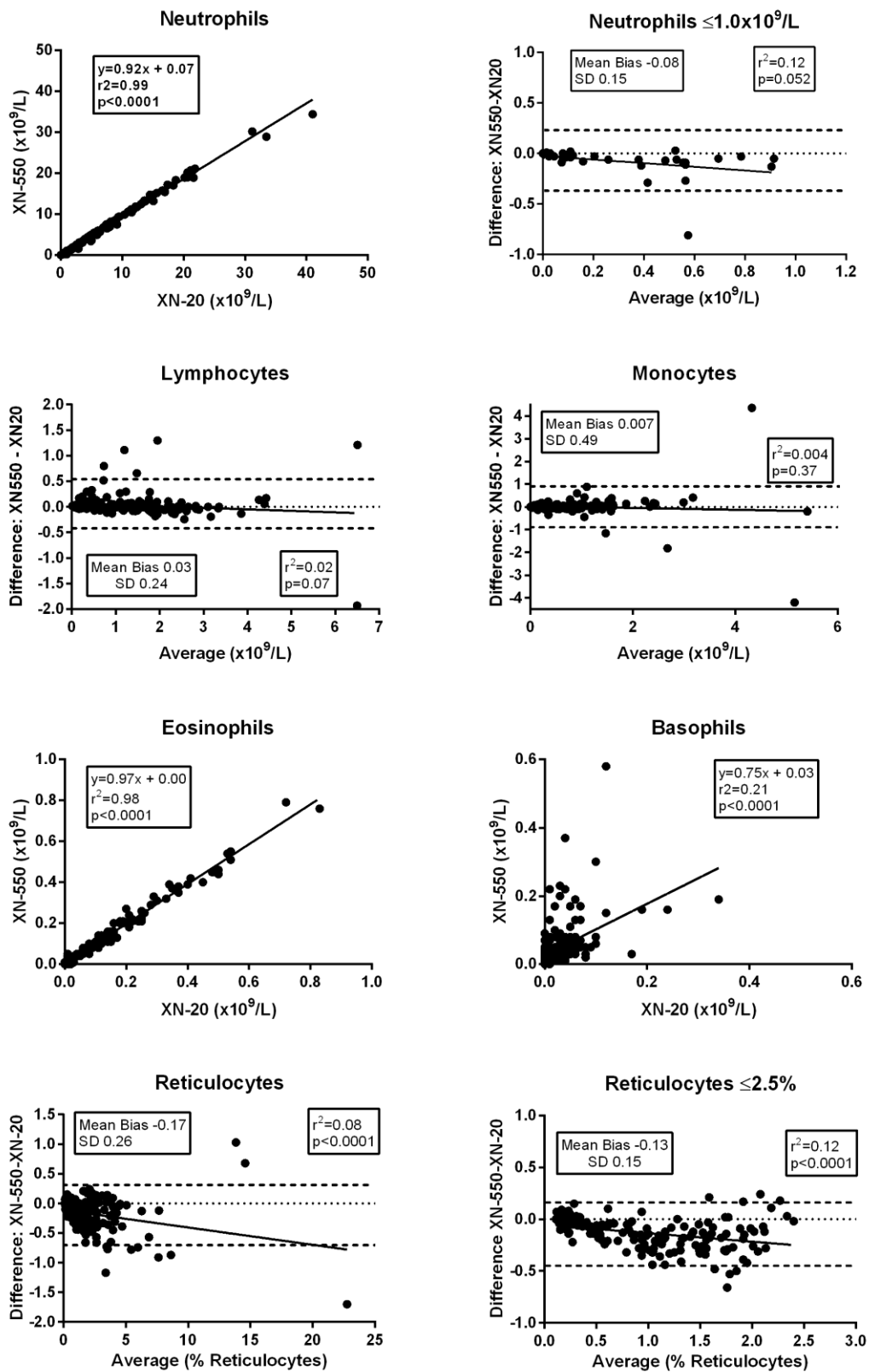
Basophils

Reticulocytes
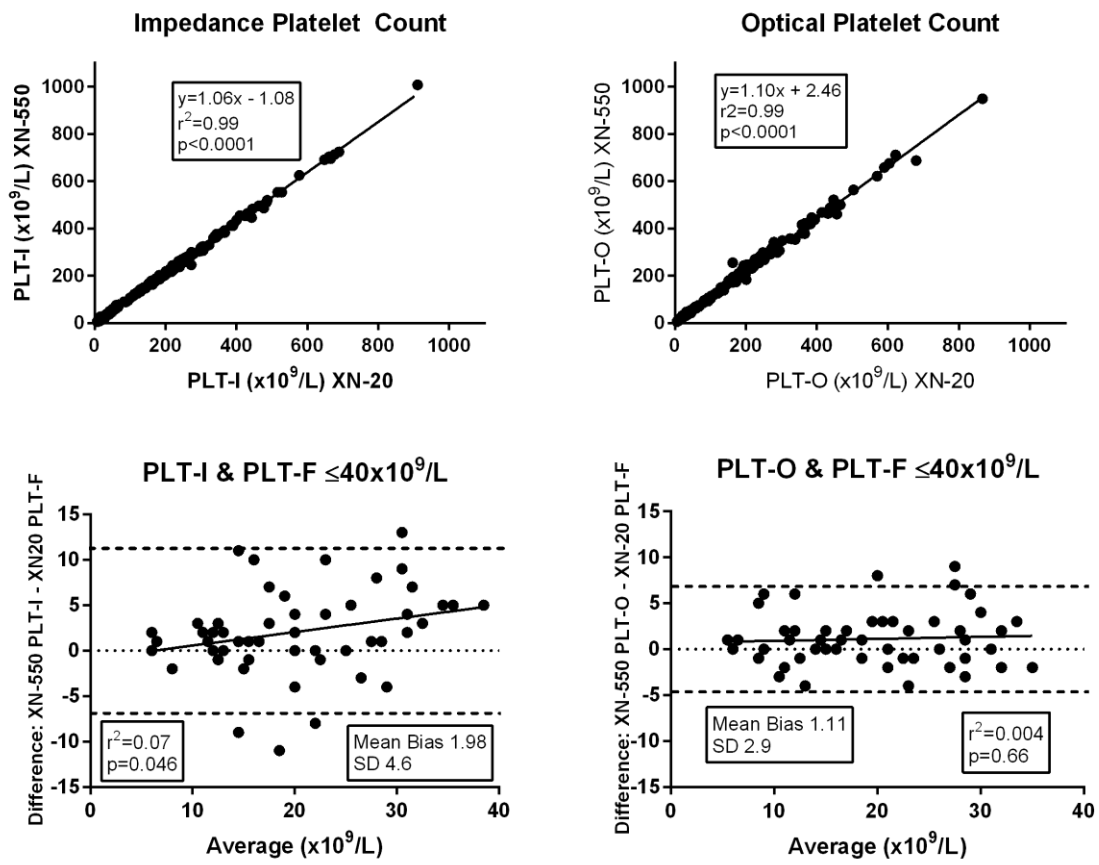
Reticulocytes ≤2.5%

Figure 2.

Table 1. Within and between run Precision
Precision was assessed using clinical samples with appropriate values for each parameter, or commercial quality control (QC) materials

Parameters	WBC (10^9/L)		Hb (g/L)		PLT -I (10^9/L)		PLT-O (10^9/L)		RET (10^9/L)	
CLINICAL SAMPLES	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
XN-550 MEAN	5.8	1.6	140.8	89.2	185.0	23.0	171.0	22.3	35.6	861.3
XN-550 CV	**1.39**	**3.74**	**0.26**	**1.99**	**1.24**	**5.88**	**1.95**	**4.22**	**3.54**	**4.04**
XN-20 MEAN	5.6	1.5	133.2	84.8	215.2	17.2	184.0	18.3	52.2	933.2
XN-20 CV	**0.80**	**0.98**	**0.52**	**0.81**	**2.20**	**2.33**	**2.13**	**5.14**	**3.12**	**0.90**
QC MATERIALS	Within	Between	Within	Between	Within	Between	Within	Between	Within	Between
XN-550 MEAN	7.4	7.4	125.7	124.8	247.0	251.0	260.0	265.0	109.6	112.0
XN-550 CV	**0.36**	**0.36**	**0.38**	**0.30**	**1.67**	**2.53**	**1.30**	**1.89**	**3.02**	**3.45**
XN-20 MEAN	6.9	7.1	123.5	125.5	232.0	234.0	222.0	225.8	100.8	98.2
XN-20 CV	**1.23**	**0.85**	**1.02**	**1.10**	**3.10**	**2.20**	**0.78**	**1.61**	**2.36**	**5.11**