

# Precision and Accuracy of the Leukocyte Differential on the Sysmex XE-2100

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*The automated leukocyte(WBC) 5-part differential count is expected to produce accurate and precise results. Furthermore pathological blood samples need to be detected at a high sensitivity.*

*In this study we evaluated the WBC differential count as well as the new parameters for immature myeloid granulocyte counts of the new automated haematology analyser, the Sysmex XE-2100. We tested the performance in terms of accuracy and precision using normal, pathological and leukopenic samples.*

*Precision profiles showed excellent performance even for leukopenic samples. Investigations of the count parameters for immature cell populations obtained from the IMI-channel (immature cell information) and the IG (immature granulocyte count) obtained from the DIFF-channel also showed a good performance, although the imprecision was found to be higher. We compared the WBC differential to the manual 500 cell diff. The general performance for the differential count was excellent with a correlation coefficient ( $r$ ) of 0.83 for monocytes (MONO), 0.97 for eosinophils (EO), and 0.99 for lymphocytes (LYMPH) and neutrophils (NEUT). For obvious reasons the basophil (BASO) correlation was found at expected level of 0.51, which is caused by the very low total count and the high imprecision of the manual differential for this particular parameter."*

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## Key Words

Automated Hematology Analyzer, XE-2100, WBC Differential, Precision, Accuracy

## INTRODUCTION

The reliable detection of pathological blood samples and the screening of normal blood samples, respectively, are of major importance for the haematology laboratory. Use of modern haematology analysers aims to reduce the need for visual differential leukocyte(WBC) counts whilst retaining optimal diagnostic security and in that respect system precision and accuracy are very important. The modern differential WBC count, as a minimum, is expected to produce an accurate quantitation of the 5 major physiologic WBC subpopulations: neutrophil (NEUT), eosinophil (EO), basophil (BASO) granulocytes (GRAN), monocytes (MONO) and lymphocytes (LYMPH).

Of additional interest is quantitation of immature myeloid and lymphoid cells on one hand and the detection, and, if possible, quantitation of reactive NEUT (band cells) and atypical LYMPH on the other.

The parallel application of different measurement principles, based on flow cytometry, allows comparatively inexpensive and rapid analysis of a remarkable number of blood cells thus delivering the statistical prerequisites for acceptable precision at all count levels.

The purpose of the present study was to evaluate the precision and accuracy of the differential WBC count generated by one of the latest and recently launched haematology systems, the Sysmex, XE-2100<sup>1)</sup>. According to the

manufacturer's claims, the system can be utilised for the regular 5-part differential WBC count, enumeration of nucleated red blood cells (NRBC), reticulocytes (RET) and their precursors, and for quantitation of immature granulocytes (IG) (metamyelocytes, myelocytes and promyelocytes). Additionally flagging algorithms suggest the presence of further pathological cells and elements such as blasts, IG, etc.

## MATERIAL AND METHODS

### WBC Differentiation on the XE-2100

The Sysmex, XE-2100 analyser differentiates WBC into the following 5 sub-populations (**Table 1**).

In the presence of certain abnormalities the XE-2100 can often count specific cell types in a different channel<sup>2)</sup> and correct the original sub-population by an equivalent amount (the corrected parameter will be flagged with "&" - see **table 2**). In case of other abnormalities the abnormal cells are allocated to a sub-population without correction. IG and NRBC are detected separately and are subtracted from NEUT and LYMPH respectively.

Additional morphological abnormalities are reported as suspect messages (flags). These are listed in **Table 3** together with the cell type included in each message.

**Table 1** The regular WBC parameters of XE-2100

Abbreviation	Parameter	Parameter contents, respective blood cells
WBC	Leukocytes	NEUT, LYMPH, MONO, EO, BASO
NEUT	Neutrophils	Band cells and mature neutrophils
LYMPH	Lymphocytes	Lymphocytes, plasma cells
MONO	Monocytes	Monocytes
EO	Eosinophils	Eosinophils
BASO	Basophils	Basophils

**Table 2** XE-2100 parameter when morphologically abnormal cells are present

Abbreviation	Parameter	Parameter contents, allocation of normal and abnormal cells
WBC	Leukocytes	WBC&, NRBC
WBC&	Leukocytes corrected	NEUT, LYMPH&, MONO, EO, BASO
NEUT	Neutrophils	NEUT&, IG
NEUT&	Neutrophils corrected	Band and segmented neutrophils
IG	Immature granulocytes	Promyelocytes, myelocytes, metamyelocytes
IMI	Immature myeloid information	Myeloblasts, promyelocytes, myelocytes, metamyelocytes
LYMPH	Lymphocytes	LYMPH&, nucleated red blood cells (NRBC)
LYMPH&	Lymphocytes corrected	LYMPH, plasma cells, myeloblasts
MONO	Monocytes	Monocytes
EO	Eosinophils	Eosinophils
BASO	Basophils	Basophils
NRBC	NRBC	NRBC

**Table 3** Suspect messages (flags) for morphological abnormalities of the WBC

Abbreviation	Expected cell type
Blasts?	Myeloblasts
Imm Gran?	Promyelocytes, myelocytes, metamyelocytes
Left Shift?	Band cells (stab)
Atypical Lymph?	Atypical lymphocytes, plasma cells
Abn Lymph/L-Blasts?	Abnormal lymphocytes, lymphoid blasts

### Principles and technology used for the WBC differential count

Using flow cytometry technology, the Sysmex, XE-2100 produces a differential WBC count in two distinct channels (WBC/BASO- and DIFF-channel)<sup>1,3)</sup>. When passing through the flow cell, cells are interrogated by the light beam from a semi-conductor laser with a wavelength of 633 nm and are differentiated by two out of three captured signals (forward scatter, side scatter and side fluorescence). Forward scattered light is detected by a photodiode while side scattered light and side fluorescence intensity at an angle of 90° are detected by a photo multiplier after filtering at specific wavelengths ( $\lambda > 640\text{nm}$  and  $\lambda > 660\text{nm}$ , respectively). The intensity of forward scattered light is equivalent to the cell volume while side scattered light yields information about the cellular content (nuclear size and granularity). The side fluorescence intensity is equivalent to the amount of RNA and DNA present in the cell. The XE-2100 is equipped with an additional channel (IMI channel) to generate the flags for the WBC differential. In the IMI channel radio frequency and direct current measurement principles (RF/DC detection method) are used. The direct current (DC) pulse height is equivalent to cell volume. The radio frequency measurement provides information on the internal composition of the cell (nucleus, granules).

### **WBC/BASO-channel: counting WBC and BASO**

In this channel the XE-2100 uses forward and side scattered light signals to quantify total WBC and BASO. An acidic surfactant lyses and shrinks red blood cells (RBC), platelets (PLT) and WBC with the exception of BASO. This results in RBC "ghosts" and PLT, WBC nuclei, and a further population of BASO whose cellular integrity remains intact. The BASO therefore appear on the diagram of forward scatter (y-axis) and side scatter (x-axis) as a distinct population of cells.

### **DIFF-channel counting NEUT, LYMPH, MONO, EO and IG**

In the DIFF-channel, the XE-2100 differentiates the WBC into four sub-populations by means of side fluorescence (x-axis) and side scattered light (y-axis): LYMPH, MONO, EO and NEUT. BASO lie within the NEUT cluster and any NRBC are included in the LYMPH cluster.

$$\text{NEUT} = \text{WBC} - \text{LYMPH} - \text{MONO} - \text{EO} - \text{BASO} \quad (1)$$

The surfactant applied in this channel induces complete lysis and shrinkage of the red cell and PLT membrane while the effect on WBC is only a slight increase in permeability thus permitting a polymethine dye with high affinity for RNA and DNA to enter the cells. When excited by a 633 nm laser beam the stained cells emit a fluorescence proportional to their content of nucleic acid. Blasts, atypical LYMPH, plasma cells and IG show an intense fluorescence. Thus IG are quantified by separating them from the mature NEUT.

$$\text{NEUT\&} = \text{NEUT} - \text{IG} \quad (2)$$

Another reagent (an organic acid) binding specifically to the granules of EO allows their discrimination from the NEUT based on a greater side scatter signal intensity.

### **Corrected WBC and LYMPH count when NRBC are present**

The Sysmex XE-2100 features a separate channel to quantify NRBC (NRBC-channel). Even though the present study did not focus specifically on NRBC, the data from this channel is important since it allows correction of total WBC and LYMPH counts.

$$\text{WBC\&} = \text{WBC} - \text{NRBC} \quad (3)$$

$$\text{LYMPH\&} = \text{LYMPH} - \text{NRBC} \quad (4)$$

In the following, LYMPH and WBC # are always meant to be the corrected values thus transforming equation (1) into equation (5).

$$\text{NEUT} = \text{WBC\&} - \text{LYMPH\&} - \text{MONO} - \text{EO} - \text{BASO} \quad (5)$$

### **IMI-channel: Presence of myeloid precursors**

The IMI-channel (Immature Myeloid Information) selectively detects immature myeloid cells normally not occurring in peripheral blood of healthy individuals.

The reaction principle in the IMI-channel is based on differences in membrane composition between mature and IG: mature cells contain in their membranes a relatively high concentration of cholesterol and lower concentration of proteins. Addition of the channel-specific reagents (non-ionic surfactant of the polyoxyethylene class, sulphur containing amino acids, cationic surfactant) causes membrane damage (mainly due to the cationic surfactant) in mature cells followed by elution of intracellular components. The membranes of IG are also partly damaged, however, the non-ionic surfactant and the sulphur containing amino acids enter the cell and fix both the membrane and the intracellular components. Since various types of IG react differently to the reagent, they occupy distinct areas in the IMI scattergram. Thus it is possible to distinguish blast cells from less immature WBC and in turn, IG such as myelocytes, metamyelocytes from left shifted NEUT.

The IMI-channel determines the total number of myeloid precursor cells and the information about their positioning is used to generate different suspect messages.

### **Q-flag system: Quantitative interpretative (IP) messages for WBC from DIFF- and IMI-channels**

The XE-2100 replaces the traditional "Yes/No" flags by a series of column-diagrams<sup>4</sup>. The numerical value for the intensity of a flag reflects the probability of this specific flag. The trigger limit can be adjusted and is pre-set to an arbitrary value of 100. If the value is below 100 the information is considered as being "negative". If it is between 100 and 300 (max. level) the respective warning is displayed as a flag. The value of the Q flag is that it provides information on the degree of positivity or negativity of a flag.

The XE-2100 offers the following Q-flags indicating the suspicion for a qualitative (morphological) abnormality of WBC: Blasts?, Imm Gran?, Left Shift?, Abn Lymph/L-Blasts? and Atypical Lymph?. The Blasts? flag indicating the suspicion for myeloblasts is displayed when both, the DIFF-channel (in the Blasts/Atypical Lymph area) and the IMI-channel (in the Blast area) identify cells of the respective cell type, . e.g., the message for IG (theoretically promyelocytes, myelocytes and metamyelocytes = Imm Gran?) appears as soon as in the respective area of either the DIFF scattergram or the IMI scattergram cells are spotted. The intention of this system is the diagnostic interpretability of characteristic patterns of Q-flags leading to selective action messages as well as the adaptation of sensitivity and specificity of the flagging performance to individual laboratory needs<sup>4</sup>.

### **Blood samples and reference values**

For the present study whole blood samples from the routine workload of the Kantonsspital, Aarau, Switzerland collected in 4.5mL K<sub>3</sub>EDTA-Vacutainers (Hemogard,

**Table 4** Reference ranges. Absolute and percentage reference ranges for leukocyte differentiation

	Abbreviation XE-2100	Manual Diff.	Reference range	
			( $\times 10^9/L$ )	(%)
Leukocytes	WBC		4.0 – 10.0	
Neutrophils	NEUT		1.6 – 8.5	40 – 85
Neutrophils corrected	NEUT&		1.6 – 8.5	40 – 85
Segment Gran.		SEG	1.6 – 7.5	40 – 75
Band cells (stab)		STA	< 1.0	< 10
Immature granulocytes	IG		< 0.05	< 0.5
Metamyelocytes		MET		
Myelocytes		MYZ	< 0.05	< 0.5
Promyelocytes		PMZ		
Myeloblasts		MYB	< 0.02	< 0.2
Eosinophils	EO	EOS	< 0.6	< 5
Basophils	BASO	BAS	< 0.2	< 2
Monocytes	MONO	MON	0.1 – 1.0	2 – 10
Lymphocytes	LYMPH	LYZ	0.8 – 4.0	20 – 40
Plasma cells		PLA	< 0.1	< 1
Immature Myeloid Information	IMI		< 0.07	< 0.7

Becton Dickinson), were used after having been processed on one of the routine systems of the haematology laboratory (Sysmex, SE-9500). Both, pathological and normal samples (the latter representing the control/reference groups) were collected and stored at room temperature (18–22°C). Pathological samples for the study were selected when showing a positive flag for WBC on the SE-9500 (Blasts?, Imm Gran?, Left Shift?, Aty/Abn Lymph?, Abn Lymph/Aged?). If no flags were present and percentage and absolute values for all parameters were normal, then the sample was included in the control/reference group.

The reference ranges in this study have been adapted to those established in our laboratory except for Blasts, IG and the IMI count which had to be fixed anew due to the much higher analytical sensitivity of the analyser compared to visual microscopy. Reference ranges as listed in **Table 4** have been used to analyse both the results of the XE-2100 and the microscopic differentiation.

Newly introduced reference ranges are displayed in italics. The reference range for myeloblasts, promyelocytes, myelocytes and metamyelocytes is 0 (routine). The reference range for IMI-count is composed of the sum of the reference ranges for IG and myeloblasts.

### Precision

The precision of the XE-2100 in counting WBC and differentiating the sub-populations has been determined for pathological and for normal samples in two different ways. All measurements were performed within 2 hours of sample collection.

First, the standard deviation (SD) and coefficient of variation (CV) profiles have been determined by 10 measurements each from samples representative of the range encountered in clinical practice. The variations of those different samples were checked for equivalence. The results obtained caused us to fix limits that made CV and SD not equivalent when those limits were undercut. The

average of the values above the fixed limits represents the total precision for each sub-population of the WBC count (total SD, total CV).

Secondly, from the first two measurements of the same samples the total precision has been calculated by analysis of variance (ANOVA). The results then were compared to those of the precision profiles.

### Accuracy of WBC differentiation

The accuracy of WBC differentiation and of detection of immature myeloid precursors on the XE-2100 has been analysed by measuring 153 pathological samples within 2 hours of sample collection and comparing the results with those of the visual differential. Passing / Bablock, linear regression and correlation coefficient have been calculated.

### Microscopic differential WBC count

For the determination of accuracy, from each sample two spun smears (Horizontzer, Hettich) were prepared within two hours of sample collection. The smears were stained by the May-Grünwald-Giemsa (Pappenheim) method. The smears were distributed to several technical assistants of the haematology laboratory who performed 500 cell-differentials on each under routine conditions.

## RESULTS

### Precision

First, the precision profiles, i.e., CV and SD profiles for the absolute WBC counts were determined. For that purpose normal, leukopenic and pathological samples were utilised. **Fig. 1** displays the CV of the absolute results. As expected the CV increased for leukopenic samples. However, even in extremely low WBC # a CV of only

8% was found representing a highly acceptable precision.  $2.5 \times 10^9/L$  has been fixed as the upper limit for a sample to be classified as leukopenic and thus not included in the total CV determination. It should be noted that all leukopenic samples have also been excluded from the calculation of the total precision of the individual WBC sub-populations.

**Fig. 2** shows the SD as function of the absolute values for pathological, normal and leukopenic samples. For the calculation of total SD of WBC the leukopenic samples have been excluded (lower limit for "normal":  $2.5 \times 10^9/L$ ).

A similar study was performed using the absolute NEUT #. **Figs. 3 (CV) and 4 (SD)** show the precision data for NEUT. Here, as well, the total-CV when considering the sample exceeding the fixed cut-off shows a maximum of 4% and on average is as low as 1.3%. The XE-2100 thus performs absolute NEUT # with very high precision.

Since the differentiation of LYMPH and MONO is technically challenging those two cell populations were considered initially as the sum of both and analysed accordingly. **Fig. 5 (CV) and 6 (SD)** display the imprecision above the cut-off level of  $0.5 \times 10^9/L$ . This is slightly higher at 3–4%, however these CV values are very acceptable from a clinical point of view.

**Figs. 7 and 8** show the CV- and SD-profiles respectively for LYMPH at concentrations  $> 0.5 \times 10^9/L$ . A CV of ~5% has been obtained.

**Figs. 9 and 10** reflect the CV and the SD for MONO, respectively. Not unexpectedly the MONO differentiation shows the highest imprecision. This results from, on one hand, the low concentration in the peripheral blood, and, on the other hand, by its well recognised instability over time. Nevertheless, a surprisingly low total impreci-

sion of 8% and 12% is found.

**Figs. 11 (CV) and 12 (SD)** show the precision profiles for EO.

In addition we have studied the innovative sub-populations, IG and IMI counts.

Precision studies for IG are illustrated in **Figs. 13 (CV) and 14 (SD)**. At a concentration of 0.025 IG per  $10^9/L$  a CV of around 20% was observed however this rapidly increases not surprisingly at still lower concentrations.

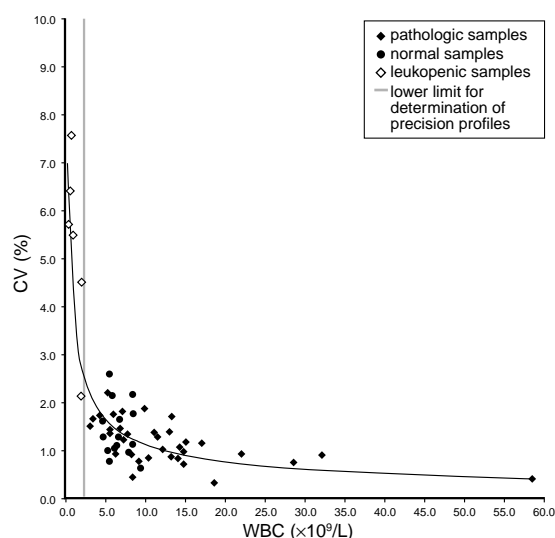
Finally the precision of the IMI-count has been analysed and is displayed in **Figs. 15 (CV) and 16 (SD)**.

**Figs. 17 (CV) and 18 (SD)** display the precision profiles for proportional (percentage) measurement values for NEUT. At first glance these appear unsatisfactory in leukopenic samples, but in reality only serve to highlight the fallacy of statistical treatment on proportional (relative or percentage) numbers. As a result it was decided not to perform profiles on proportional numbers for other cell types. Unfortunately, in doctors' offices, in clinics and on the wards particularly of smaller hospitals it is still common practice to report the differential WBC count in proportional values (percentages) even though such practice can be clinically misleading.

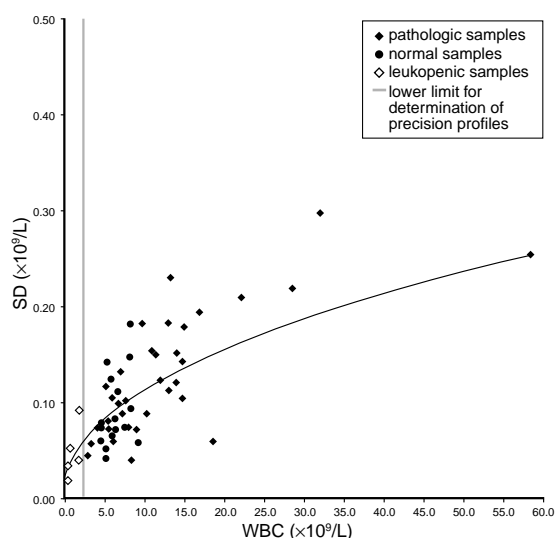
**Table 5** indicates the measurement range of absolute and percentage values for determination of total precision.

**Tables 6 and 7** comprehensively display the calculated SD and CV of our study and the variance analyses of both the absolute and the percentage measurement values. The CV for the variance analysis of the different WBC subtypes varies from 1% to 8%. Only in case of BASO and IG are higher CV values around 20% found.

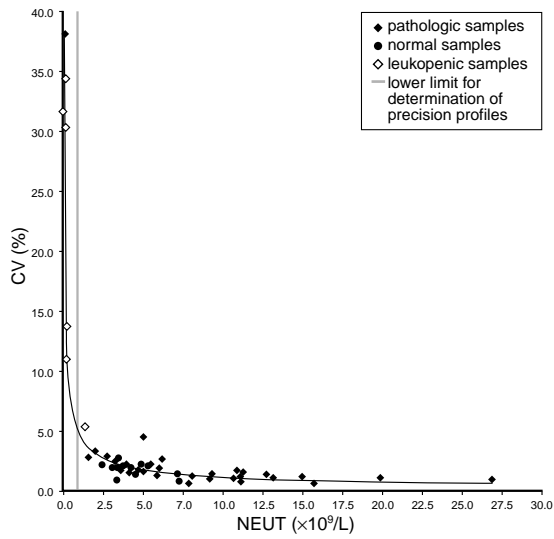
All our experimental results are within the specifications claimed by the manufacturer (**Table 8**).



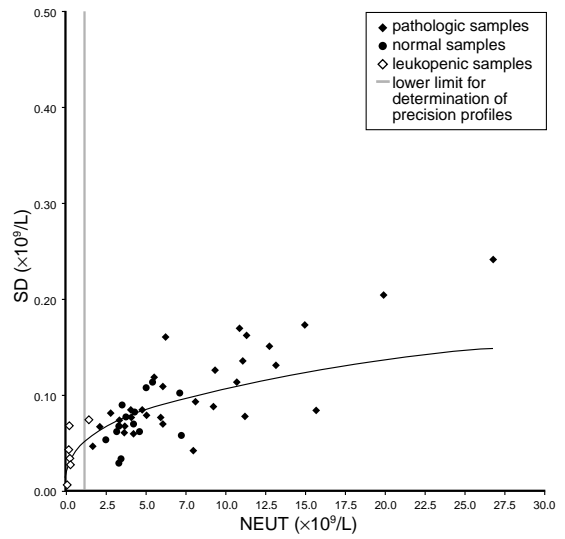
**Fig. 1** Precision profile of WBC-count-CV as function of absolute values



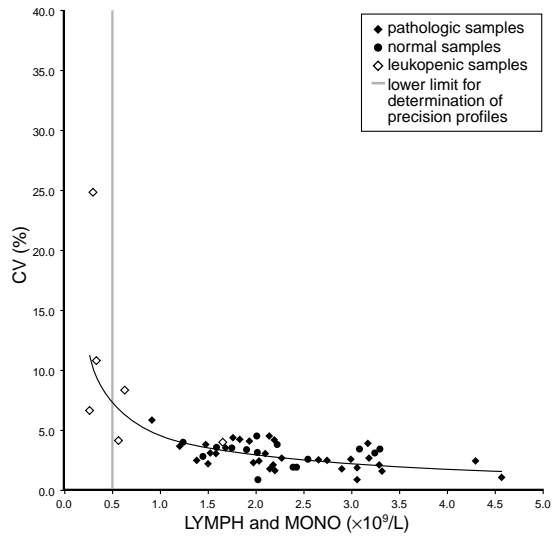
**Fig. 2** Precision profile of WBC-count-SD as function of absolute values



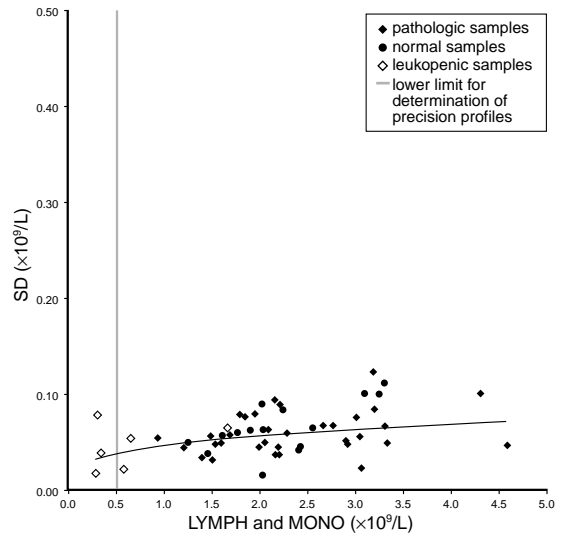
**Fig. 3** Precision profile for NEUT-count-CV as function of absolute values



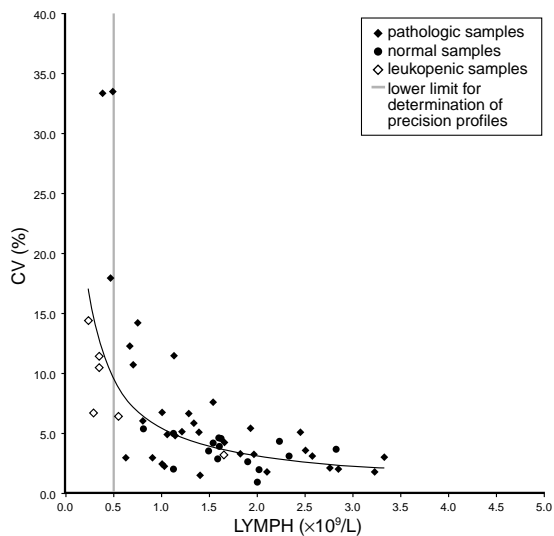
**Fig. 4** Precision profile for NEUT-count-SD as function of absolute values



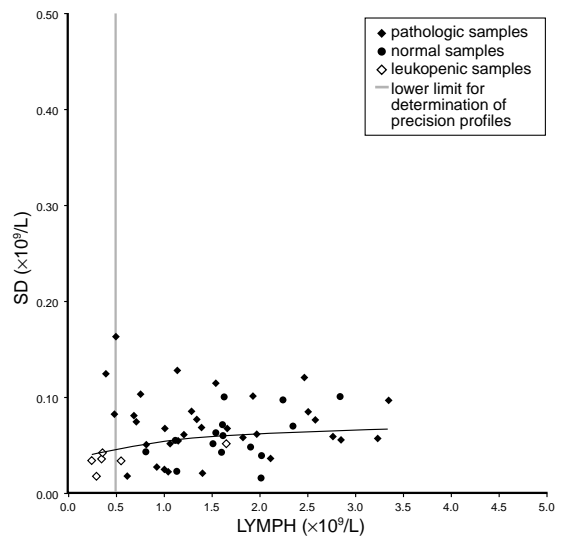
**Fig. 5** Precision profile of LYMPH+MONO-count-CV as function of absolute values



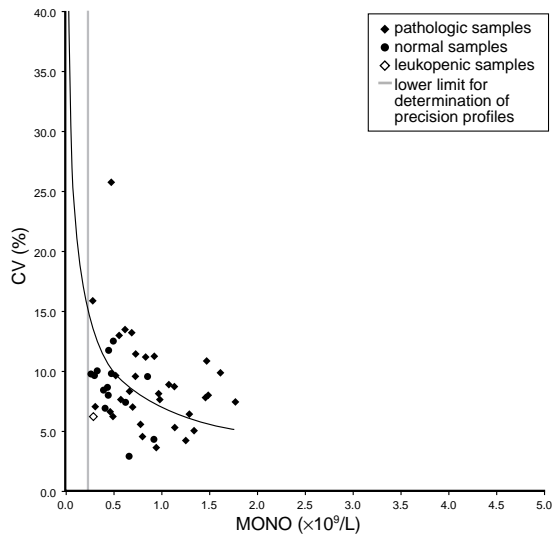
**Fig. 6** Precision profile of LYMPH+MONO-count-SD as function of absolute values



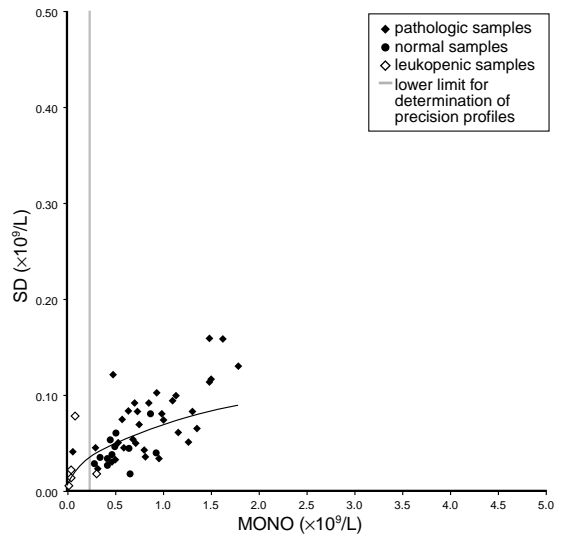
**Fig. 7** Precision profile of LYMPH-count-CV as function of absolute values



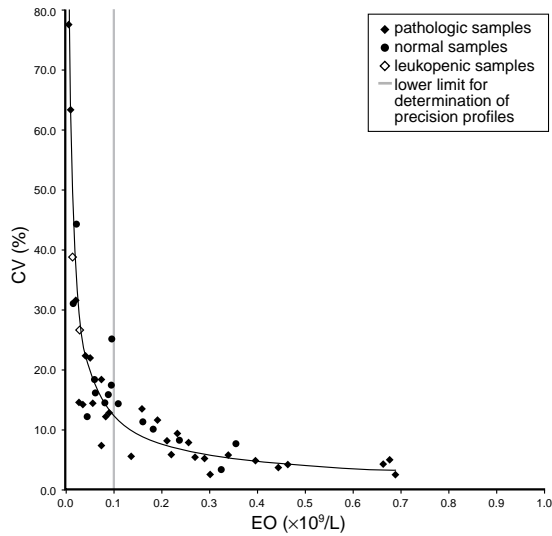
**Fig. 8** Precision profile of LYMPH-count-SD as function of absolute values



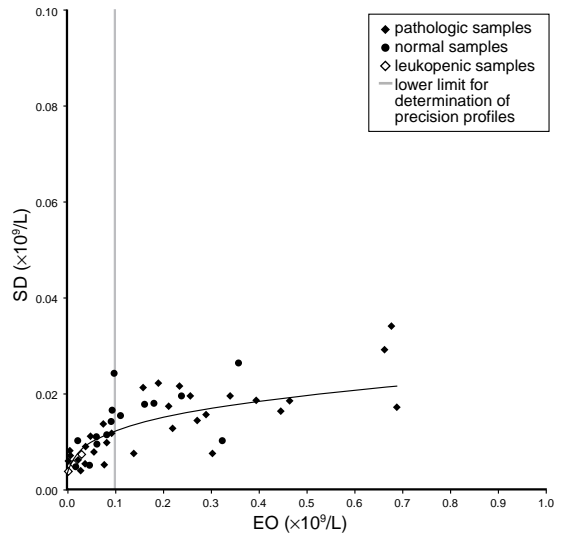
**Fig. 9** Precision profile of MONO-count-CV as function of absolute values



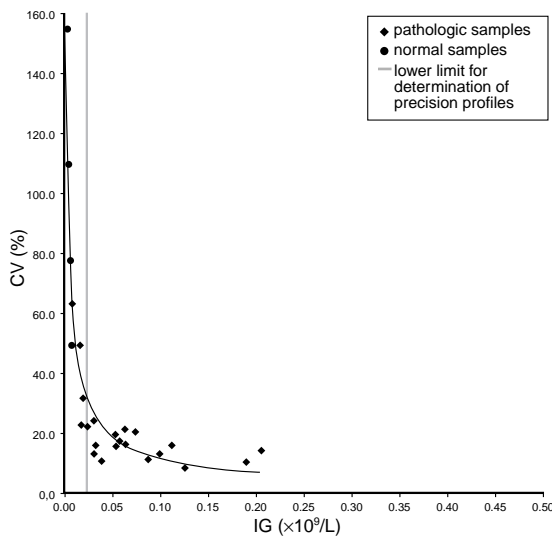
**Fig. 10** Precision profile of MONO-count-SD as function of absolute values



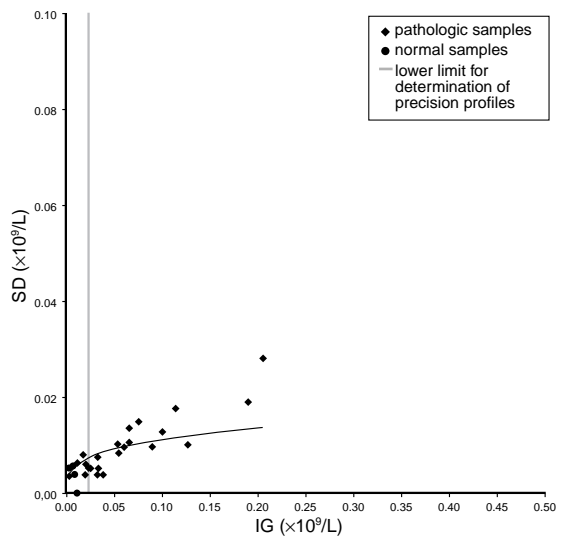
**Fig. 11** Precision profile of EO-count-CV as function of absolute values



**Fig. 12** Precision profile of EO-count-SD as function of absolute values



**Fig. 13** Precision profile of IG-count-CV as function of absolute values



**Fig. 14** Precision profile of IG-count-SD as function of absolute values

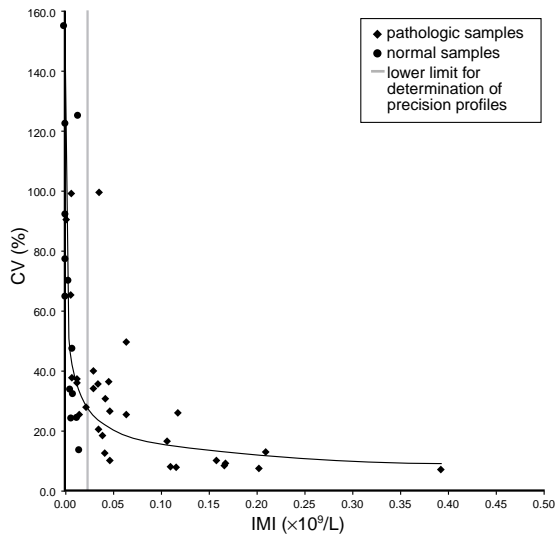


Fig. 15 Precision profile of IMI-count-CV as function of absolute values

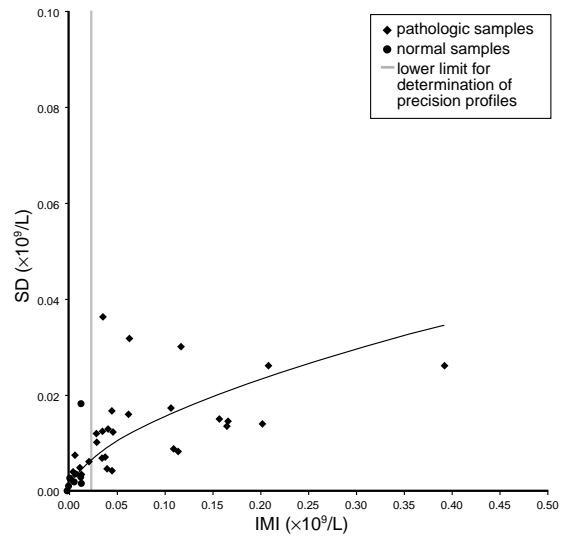


Fig. 16 Precision profile of IMI-count-SD as function of absolute values

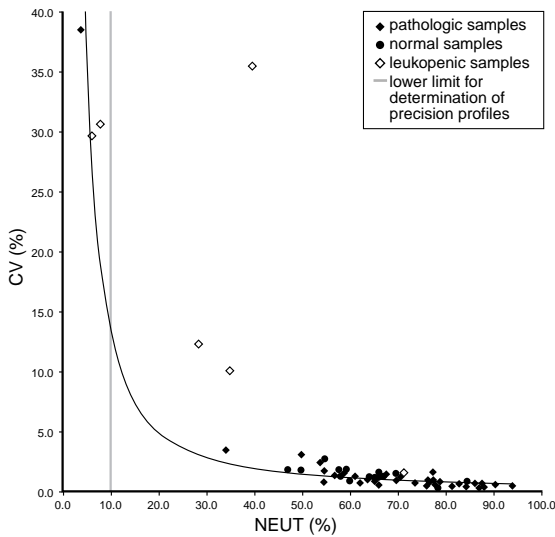


Fig. 17 Precision profile of NEUT-count-CV as function of percentage values

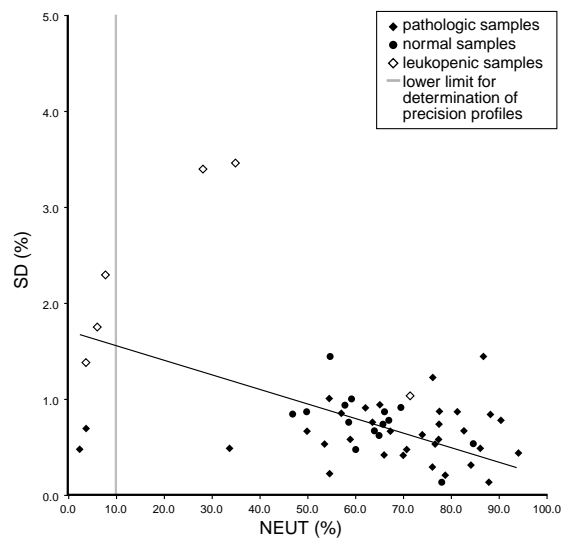


Fig. 18 Precision profile of NEUT-count-SD as function of percentage values

Table 5 Measurement range of absolute and percentage values for determination of total precision

Absolute measurement values					Percentage measurement values				
Parameter	Range				Parameter	Range			
	Limit ( $\times 10^9/L$ )	Mean	Max	Min		Limit (%)	Mean	Max	Min
WBC#	>2.50	9.69	28.60	4.24	NEUT%	>10.0	68.92	93.67	33.75
NEUT#	>1.00	7.11	26.79	2.03	NEUT&#	>10.0	68.57	93.58	32.08
NEUT&#	>1.00	7.08	26.77	1.93	LY+MO%	> 5.0	28.99	70.77	6.26
LY+MO#	>0.50	2.30	4.57	0.65	LYMPH%	> 5.0	21.70	42.28	5.58
LYMPH#	>0.50	1.62	3.33	0.61	MONO%	> 2.5	9.22	32.46	3.45
MONO#	>0.25	0.79	1.77	0.27	EO%	> 1.0	3.28	11.25	1.07
EO#	>0.10	0.32	0.69	0.11	BASO%	> 0.25	0.86	2.52	1.25
BASO#	>0.025	0.072	0.358	0.028	IG%	> 0.25	0.80	1.66	0.26
IG#	>0.025	0.083	0.205	0.032	IMI%	> 0.25	1.26	3.04	0.25
IMI#	>0.025	0.103	0.392	0.030					



**Table 6** Total-SD and -CV from precision profiles and from variance analysis of absolute measurement values  
Included in the values from the profiles are the highest and the lowest measurement values for SD and CV.

Parameter	n	SD from			CV from				
		Precision profiles			Variance analysis	Precision profiles			Variance analysis
		SD ( $\times 10^9/L$ )	Max	Min	SD	CV (%)	Max	Min	CV
WBC#	46	0.114	0.230	0.039	0.105	1.29	2.60	0.33	1.09
NEUT#	46	0.096	0.241	0.031	0.090	1.61	3.35	0.54	1.27
NEUT&#	46	0.097	0.244	0.031	0.091	1.63	3.49	0.55	1.29
LY+MO#	46	0.062	0.122	0.016	0.056	2.94	8.26	0.76	2.44
LYMPH#	43	0.064	0.128	0.017	0.074	4.48	14.01	0.84	4.59
MONO#	46	0.063	0.159	0.018	0.070	8.25	13.37	2.85	8.89
EO#	23	0.018	0.034	0.008	0.018	6.91	14.08	2.50	5.78
BASO#	35	0.013	0.033	0.004	0.011	20.34	41.26	8.43	14.89
IG#	16	0.012	0.028	0.004	0.017	15.18	23.77	8.15	20.10
IMI#	22	0.015	0.032	0.005	0.020	20.20	49.35	6.65	17.97

**Table 7** Total-SD and CV from precision profiles and variance analysis of the relative measurement values  
The values of the profiles also include the highest and lowest SD- and CV-values measured.

Parameter	n	SD from			CV from				
		Precision profiles			Variance analysis	Precision profiles			Variance analysis
		SD (%)	Max	Min	SD	CV (%)	Max	Min	CV
NEUT%	46	0.64	1.46	0.14	0.66	1.03	3.36	0.18	0.96
NEUT&%	46	0.65	1.46	0.10	0.69	1.05	3.75	0.13	1.00
LY+MO%	46	0.66	2.73	0.14	0.66	2.40	5.55	0.52	2.26
LYMPH%	43	0.80	2.36	0.15	0.95	4.27	17.81	1.03	4.38
MONO%	46	0.71	1.71	0.17	0.81	8.00	13.06	2.23	8.77
EO%	23	0.22	0.53	0.06	0.21	8.94	24.94	1.56	6.47
BASO%	35	0.17	0.64	0.06	0.16	22.44	59.57	8.13	19.25
IG%	16	0.12	0.24	0.04	0.16	17.20	49.48	5.33	19.76
IMI%	22	0.21	0.58	0.05	0.35	20.26	48.99	6.15	25.57

**Table 8** Manufacturer's precision specifications

Precision of blood cell differentiation with normal fresh samples is measured 10 times or more. Listed are the maximum acceptable CV% and the validity range of the measurements. If no range is indicated then, in case the CV% is exceeded between two measurement values the maximum deviation as indicated is supposed to occur. The relative measurements are only valid if the WBC count is  $> 4.00 \times 10^9/L$ .

Parameter	Absolute measurement values			Parameter	Relative measurement values		
	CV (%)	Limit ( $\times 10^9/L$ )	Tolerable deviation		CV (%)	Limit (%)	Tolerable deviation
WBC#	< 3.0	>4.00		NEUT%	< 8.0	>30.0	
NEUT#	< 8.0	>1.20		LYMPH%	< 8.0	>15.0	
LYMPH#	< 8.0	>0.60		MONO%	<20.0	> 5.0	
MONO#	<20.0	>0.20		EO%	<25.0		$\pm 1.5$
EO#	<25.0		$\pm 0.12$	BASO%	<40.0		$\pm 1.0$
BASO#	<40.0		$\pm 0.06$				

### Accuracy of WBC differential

The accuracy of WBC sub-population enumeration and that of their myeloid precursors has been studied by comparison with a reference method based on visual microscopy. For that purpose samples with normal, reduced and increased WBC counts and pathological samples with marked left shift have been analysed. Comparison of the different cell types was done by calculation of Passing / Bablock and CV. **Fig. 19** displays the correlation for the absolute NEUT #. As NEUT the system classifies segmented NEUT, band cells (stab), and IG. With a slope of 0.999 and an intercept of only 0.105 the correlation of the results of both methods is outstanding. Equally impressive is the correlation coefficient of 0.9986. This can be explained by the very low total imprecision of the NEUT # of the analyser (CV% = 1.27%) and, in case of high cell concentrations, the acceptable precision also for the visual differential.

**Fig. 20** illustrates the comparison of the percentage NEUT # with visual microscopy. As expected linear regression and intercept are less good than for absolute counts. This is due to the high percentage of NEUT in the samples.

The results obtained for the corrected neutrophil counts (NEUT&) are almost identical to the results of the neutrophil count itself (NEUT). This is due to the relatively low percentage of IG as compared to the high percentage of segmented NEUT and band cells. This comparison is not illustrated however linear regression and correlation coefficient are listed in **Table 9**.

**Figs. 21 and 22** display the method comparison for LYMPH. For LYMPH, despite the technically challenging differentiation from MONO, an excellent linear regression between the two methods has been obtained. Also, the increase in imprecision of the visual method in case of low cell concentrations had only little impact on the correlation: the correlation coefficients for the absolute counts and relative counts were 0.9865 and 0.9866, respectively.

**Figs. 23 and 24** illustrate the correlations for absolute and percentage MONO counts. Not unexpectedly the MONO differentiation shows a worse correlation than the

LYMPH. This is due to the higher imprecision of the measurement for both the visual and the instrument rated count. Nevertheless, surprisingly good correlation coefficients have been obtained: 0.8360 for the absolute counts and 0.8629 for the percentage counts. The linear regression reveals a systematic difference between the two MONO detection methods since it is positioned significantly above the ideal function of  $y = x$ . This is for both, the absolute and the proportional count. At a concentration of  $1.0 \times 10^9/L$  or 10% MONO, respectively, the analyser detects 10–13% more MONO as does the microscopy differential count.

**Figs. 25 and 26** illustrate the absolute and percentage comparisons for EO.

Considering the very low EO concentration of the blood samples the measurement values of the two methods show an excellent correlation.

**Figs. 27 and 28** show the respective comparisons for BASO. The XE-2100 showed a higher sensitivity compared to the visual differential. As anticipated the correlation coefficients are poor due to the high imprecision of both methods (particularly the visual differential).

Subsequently we focussed on the myeloid precursors. The system is capable of detecting myeloid precursors by the simultaneous and parallel application of different measurement principles (see methods): in the DIFF-channel the IG are quantified while the IMI-channel generates the supportive qualitative parameter of the IMI-count.

### Immature Granulocytes (IG)

Since the detection of IG is of great clinical importance and has to be carried out quite frequently but hitherto only possible by visual microscopy, the ability of the XE-2100 in that respect has been analysed with great interest. The accuracy of the newly launched IG count was examined.

Initially while the correlation between the XE-2100 and the visual differential was good, the slope of the linear regression was  $< 0.5$  according to Passing/Bablock. A possible cause for this low slope may have been misadjustment of standard sensitivity which would impact on

**Table 9** Summary of comparability studies

For the IG# also the results excluding the promyelocytes are listed (IG#\*). IG data before re-adjustment of sensitivity. For the IMI# also the results excluding the outliers are shown (IMI#\*).

Parameter	Absolute measurement values			Parameter	Proportional measurement values		
	Slope	y-axis intercept	Corr.-coeff. (r)		Slope	y-axis intercept	Corr.-coeff. (r)
NEUT#	0.999	-0.105	0.9986	NEUT%	1.049	-5.001	0.9874
NEUT&#	0.997	-0.043	0.9985	NEUT&%	1.017	-1.915	0.9875
LYMPH#	0.975	0.034	0.9865	LYMPH%	1.010	0.045	0.9866
MONO#	1.121	0.009	0.8360	MONO%	1.044	0.610	0.8629
EO#	0.963	0.000	0.9658	EO%	0.987	0.000	0.9542
BASO#	1.625	0.010	0.5098	BASO%	1.633	0.058	0.5798
IG#	0.438	0.013	0.8509				
IG# *	0.467	0.017	0.8473				
IMI#	1.626	0.011	0.7279				
IMI# *	1.393	0.015	0.9428				

the detection of IG. Following readjustment, a much improved correlation and slope were found when comparing the two methods (Fig. 29). These results are also in agreement with results as found in another study<sup>6</sup>.

**IMI Count**

Even though the XE-2100 only utilizes the IMI-information qualitatively and the results are not regularly displayed, it is nevertheless an important measure for total myeloid precursors (myeloblasts, promyelocytes, myelocytes, metamyelocytes) (see methods below). Figs. 30, 31 and 32 illustrate a number of experimental comparisons.

In Fig. 30 straight comparison of the IMI-count with the visual differential is illustrated. Here the steep slope (1.626) indicates the detection of additional sub-populations in the IMI-channel which are omitted from the IMI definition. This assumption is confirmed in Fig. 30 in which two sub-populations of paired values are present: one is located around the Passing / Bablock linear regression while the second is aligned to the y-axis and consists of badly correlating values (outliers in circles). Those

paired values are from samples with left shift exclusively limited to the stage of band cells (stab). In Fig. 31 the comparison has been repeated but this time excluding those blood samples which showed an absolute band cell count > 0.3 on microscopy but only a few more immature forms. The slope of the linear regression decreases from 1.626 to 1.393 and the correlation coefficient improves to a very good value of 0.9428.

Fig. 32 finally illustrates the comparison between IG-count from the DIFF-channel and the IMI-count from the IMI-channel.

As already observed in Fig. 30 and now also in Fig. 32, the paired values aligned with the y-axis are observed. Yet again they derive almost exclusively from blood samples with a marked left shift but without other immature cells.

In Table 9 the results of our comparability analyses are displayed comprehensively in total. Table 10 shows the results of an UK study<sup>5</sup> for comparison. It can be seen that the results for IG from the UK study correspond well with our results obtained after re-adjustment of the standard sensitivity. All results satisfy the manufacturer performance claims which are displayed in Table 11.

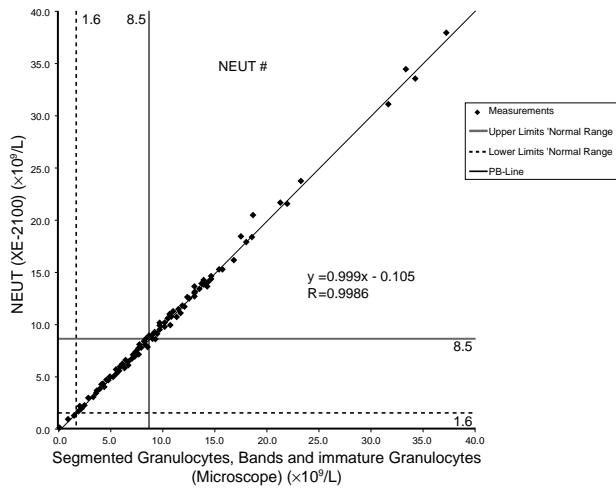


Fig. 19 Absolute NEUT-count: comparison with visual microscopy

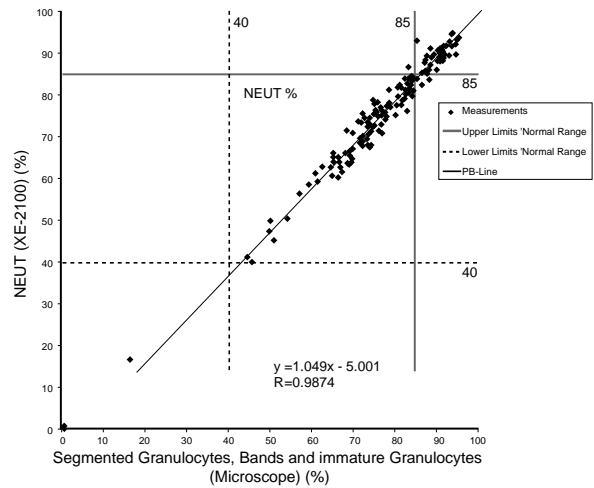


Fig. 20 Percentage NEUT-count: comparison with visual microscopy

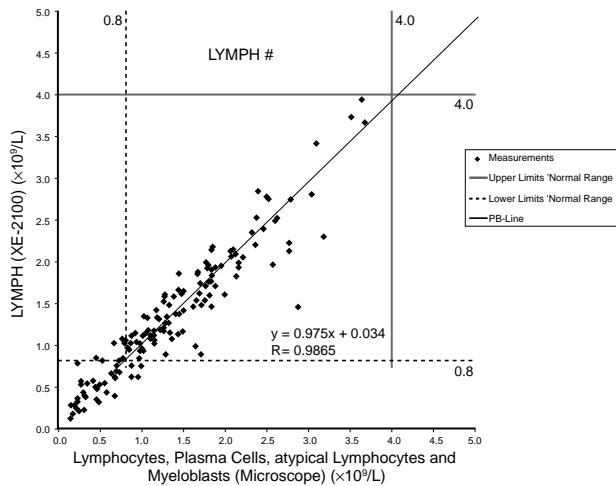


Fig. 21 Absolute LYMPH-count: comparison with visual microscopy

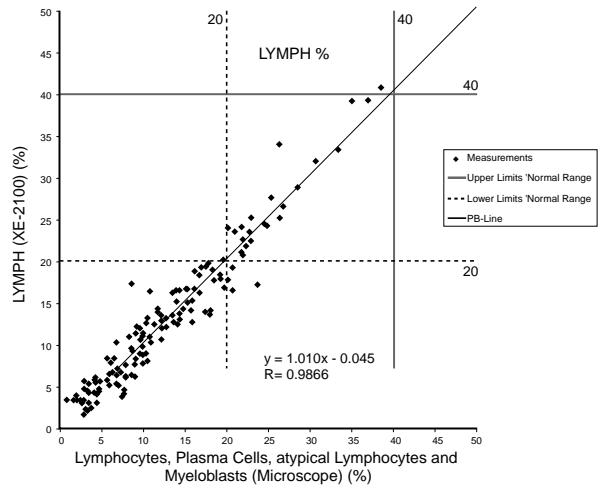


Fig. 22 Percentage LYMPH-count: comparison with visual microscopy

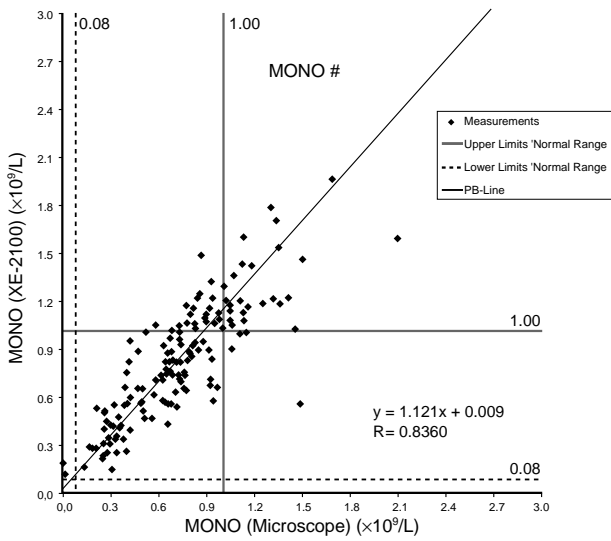


Fig. 23 Absolute MONO-count: comparison with visual microscopy

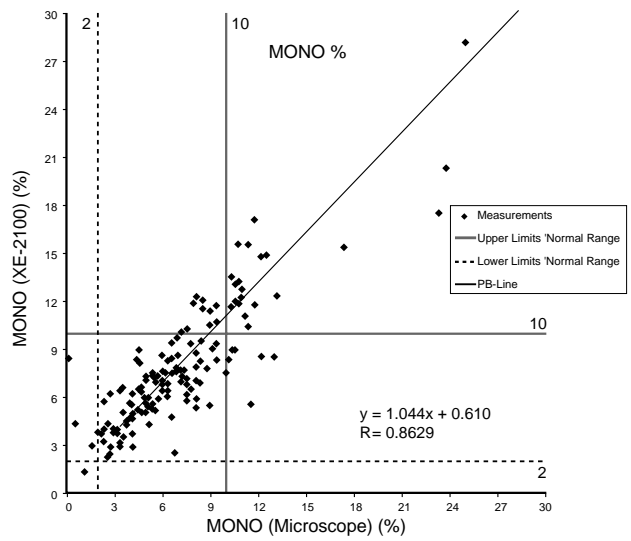


Fig. 24 Percentage MONO-count: comparison with visual microscopy

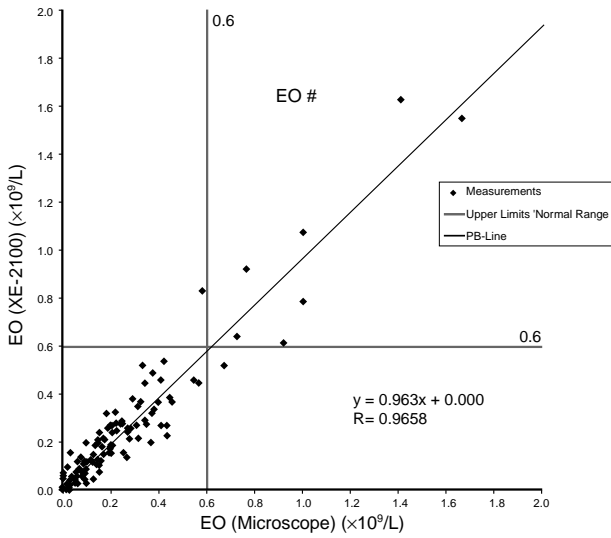


Fig. 25 Absolute EO-count: comparison with visual microscopy

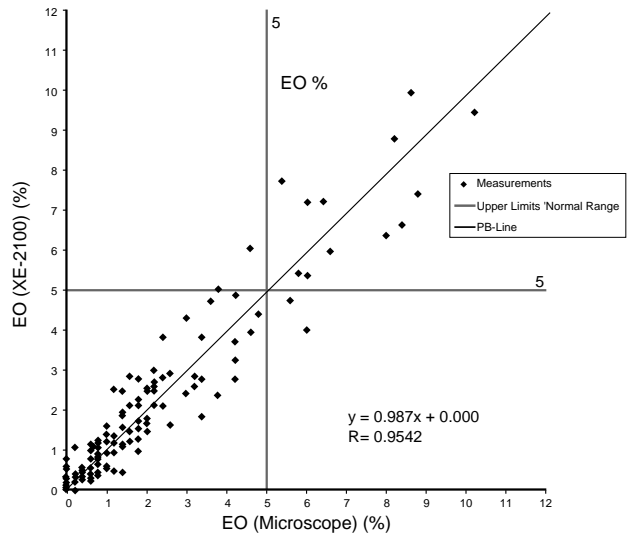


Fig. 26 Percentage EO-count: comparison with visual microscopy

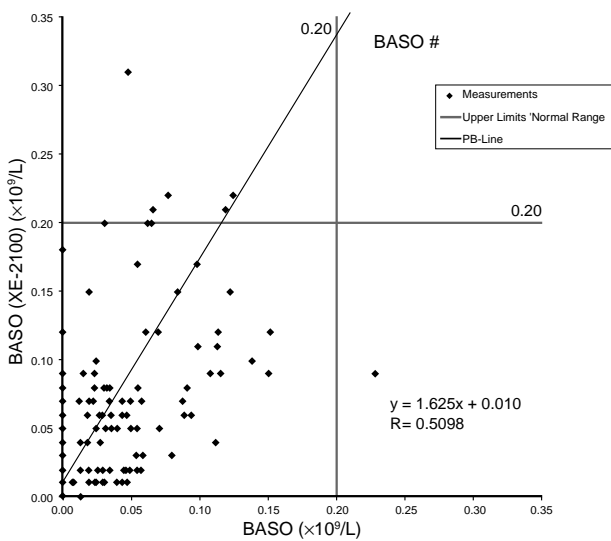


Fig. 27 Absolute BASO-count: comparison with visual microscopy

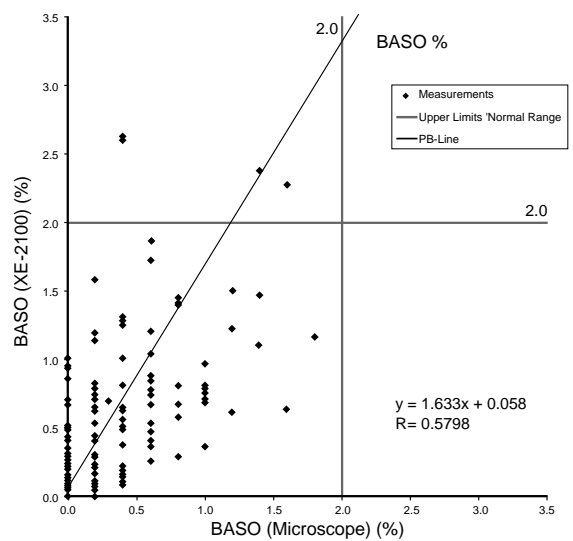


Fig. 28 Percentage BASO-count: comparison with visual microscopy

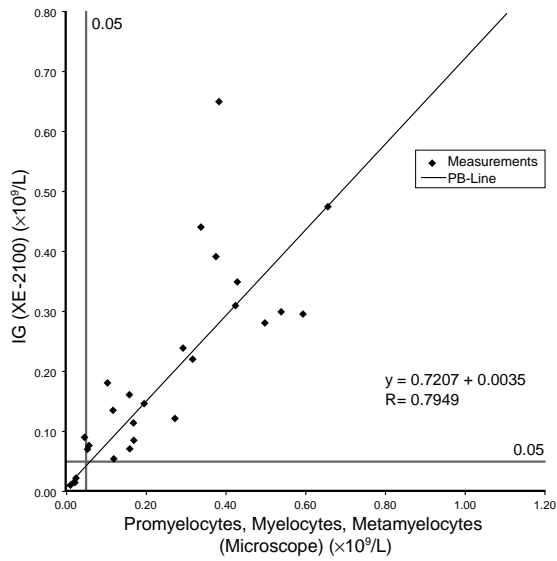


Fig. 29 Absolute IG count: comparison with visual microscopy

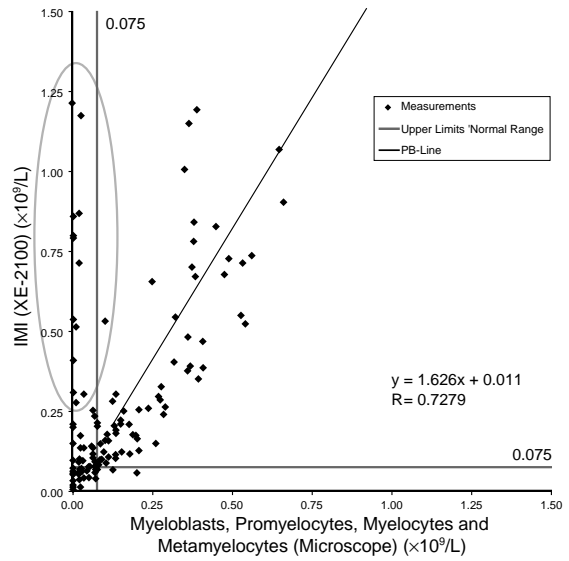


Fig. 30 Absolute IMI-count: comparison with visual microscopy differential which counted band cells and other immature forms

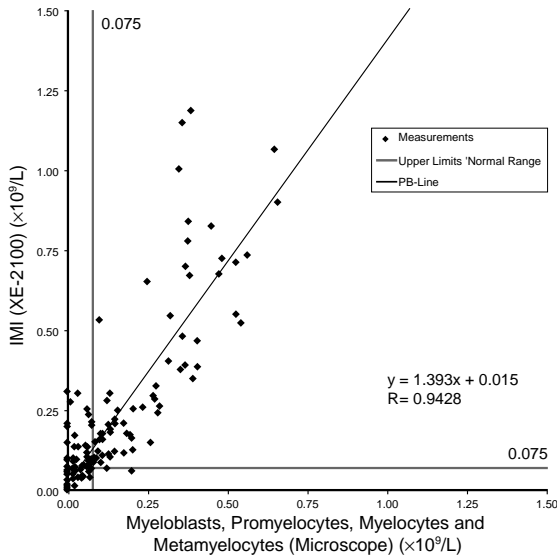


Fig. 31 Absolute IMI-count: comparison with visual microscopy differential. Outliers consisting of samples with band cells > 0.3 excluded.

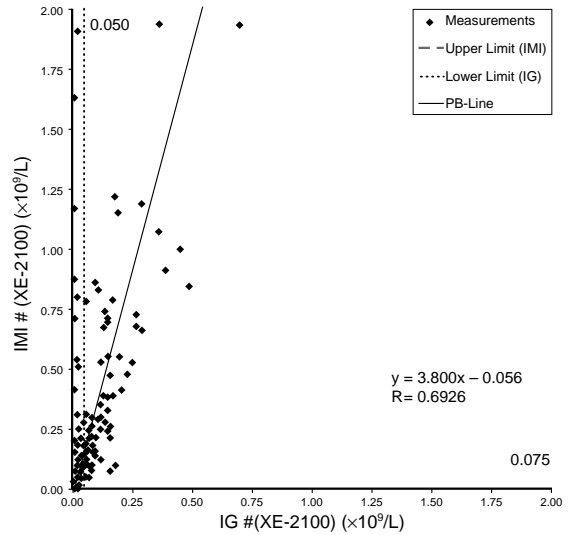


Fig. 32 Comparison of absolute IG-count and absolute IMI-count

Table 10 Results of the comparability study performed in University College London Hospital, London<sup>5)</sup>

Parameter	Relative measurement values		
	Slope	y-axis intercept	Corr.-coeff. (r)
NEUT%	0.938	0.094	0.9812
LYMPH%	0.961	0.108	0.9887
MONO%	0.936	0.157	0.6470
EO%	0.909	0.046	0.8096
BASO%	0.940	0.041	0.1983
IG%	0.744	0.065	0.8173

Table 11 Accuracy of blood cell differentiation Manufacturer's specifications. Displayed is the maximum tolerable mean value of the differences from the result of the standard system and the correlation coefficient with the reference method if 100 or more normal samples are analysed.

Parameter	Relative measurement values	
	Maximum tolerable deviation (%)	Corr.-coeff. (r)
NEUT%	±3.0%	>0.90
LYMPH%	±3.0%	>0.90
MONO%	±2.0%	>0.75
EO%	±1.0%	>0.80
BASO%	±1.0%	>0.50

## DISCUSSION

As shown by our data the XE-2100 possesses outstanding precision. The performance of the instrument far exceeds the precision claims made by the manufacturer which. Only in cases of low absolute and proportional values not unexpectedly does the imprecision increase. For severely leukopenic patients it does not matter that much if the values show greater imprecision. In such circumstances the appearance of blast cells has clinical relevance. But in such case the purely binary statement (yes/no = pathological/non-pathological) is absolutely sufficient. Subsequently, additional diagnostic procedures such as bone marrow puncture (morphology/ flow cytometry, immunophenotyping and special stains) are required anyway. In addition, the highly precise counts of immature myeloid cells will prove extremely valuable. It might be anticipated that the rapid identification and precise enumeration of immature myeloid cells in the peripheral blood will contribute significantly to the diagnosis of infectious diseases. Should this become evident it would then be possible to come to important clinical decisions by just performing a simple automated differential. Additionally, we would expect that therapeutic monitoring would be of great clinical benefit. Such studies are currently underway in our institution.

The present study also raises the question of reference ranges in health. The fact that some samples from apparently healthy individuals show a low concentration of immature cells in rare instances necessitates a re-evaluation of reference ranges in health. Such cells are not detected in the frequently performed routine clinical dif-

ferential of  $2 \times 50$  or  $2 \times 100$  WBC. That leads to a false reference value of "0". The experience and the insight gained with new technology analysers such as the Sysmex, XE-2100 prompts such studies.

In summary, the Sysmex, XE-2100 impresses the user with its outstanding precision for the 5-part differential. In addition, the system generates precise quantitative data on immature myeloid precursors and other immature elements. With sophisticated "flagging" hitherto unrecognisable pathological conditions are certainly detectable. However, in the present study this has not been evaluated any further. Since this technical evaluation resulted in an outstanding performance, clinical studies are highly recommended.

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