Performance Evaluation of Automated Leucocyte Counting in Cerebrospinal Fluid (CSF) by the XE-2100 Compared to Manual Counting

B. SCHROEDER[*1], Dr. F. HAUKAMP[*2], Prof. F.-J SCHMITZ[*1], C. WIENEFOET[*3], and J.LINSSEN[*3]

*[1] *Institute of Laboratory and Transfusion Medicine, Klinikum Minden, Friedrichstraße 17, 32427 Minden, Germany.*
*[2] *Institute Neurological Clinic, Klinikum Minden, Friedrichstraße 17, 32427 Minden, Germany.*
*[3] *Sysmex Europe & Sysmex Deutschland GmbH, Bornbarch 1, 22848 Norderstedt, Germany.*

Objectives: To evaluate the performance of automated leucocyte counting in CSF from neurological patients using the Sysmex XE-2100.

Methods: The number of WBC was determined in 226 fresh (maximum 2h from collection) CSF samples by microscopy (reference method) and by the XE-2100 WBC/BASO and DIFF channels. Validation included measurement of linearity, within-day imprecision, carryover, limit of detection and correlation with reference method. A truth table was made for non-pathologic samples at counts less than 5 WBC/µL and pathologic samples greater than 4 WBC/µL for the XE-2100 and the reference method. Additionally PMN (neutrophils + eosinophils + basophils) and MN cell (monocytes + lymphocytes) differential counts were compared (only n=30) with the microscopy cytopreparations.

Results: Within-day imprecision of the XE-2100 DIFF channel and with the manual method showed nearly the same results, 46 % and 37%, for extremely low WBC counts (2 cells/µL). For higher WBC counts the DIFF channel is superior to the manual method (WBC approximately 50/µL, 9% and 15%; WBC approximately 500/µL, 4% and 9%). The within-day imprecision for the DIFF channel is superior to the WBC/BASO channel. Linearity and carryover showed excellent results for all channels.

The XE-2100 counts showed excellent correlation with the microscopy reference counts in the range 0-3,000 WBC/µL (DIFF channel: R^2=0.985; WBC/BASO channel: R^2=0.883). In the range lower than 50 WBC/µL however, the DIFF channel (R^2=0.870 [n=201]) again showed a good correlation with the microscopy reference method, but, in contrast, the WBC/BASO channel showed no correlation (R^2=0.06 [n=201]).

The truth table showed that the DIFF channel is superior to the WBC/BASO channel particularly at a decision point of > 4 WBC/µL.

The differentiation of WBC into PMN cells and MN cells with the DIFF channel give respectively R^2=0.893 and R^2=0.850, but only with 30 samples.

Conclusions: Determination of the WBC count in CSF using the DIFF channel of the XE-2100 is highly correlated to the microscopic reference method. Especially no false normal (0-4/µL) WBC counts were found, so it is unlikely that a pathologic pleocytosis has been missed. Some false positive results were obtained (3%). It is important for the supervising doctor to be aware of this for interpretation of the data. From a clinical point of view, however, the automated CSF count quality in the DIFF channel of the XE-2100 is absolutely sufficient for competent patient treatment.

The differential count (PMN and MN) appears promising, but more samples must be studied.

(Sysmex J Int 14 : 8-12, 2004)

Key Words Cerebrospinal Fluid, Leukocyte Count, Automated Hematology Analyzer, XE-2100, WBC Count DIFF Channel

Received 22 June, 2004; Accepted 29 June, 2004

INTRODUCTION

All hospitals which treat patients with acute inflammatory neurological diseases require to measure the basic cerebrospinal fluid (CSF) parameters: cell count, total protein and lactate/glucose within 1 hour at any time, day or night. The cell count is the key parameter for correct diagnostic classification of most diseases. Falsely negative (< 5 WBC/µL) reported results could significantly delay diagnosis and commencement of therapy. Falsely elevated counts could lead to unnecessary diagnostic procedures and therapy.

CSF manual microscopy chamber cell counts are time consuming, labour intensive and frequently imprecise, but remain the gold standard according to NCCLS[1)]. Until now, automated methods have encountered difficulties in performing counts on samples at WBC levels lower than 50 cells /µL[2)] due to carryover, air-bubbles or other contaminating particles. This rendered automated counts unusable since the reference range in health for CSF samples is less than 5 cells /µL. In our laboratory we have recently evaluated the ability of the new Sysmex XE-2100 multiparameter haematology analyser to perform cell counts on CSF.

The Sysmex XE-2100 multiparameter haematology analyser combines impedance and fluorescence flow cytometry for the differentiation of blood cells. The leukocyte count (WBC/BASO channel) is established by measuring the cell volume (forward light scatter [FSC]) and structure (side light scatter [SSC]) after fixation of the WBC. The leukocyte differential count (DIFF channel) is performed by simultaneously measuring fluorescence (side fluorescence, the cellular RNA/DNA content [SFl]) and cell structure [SSC].

MATERIALS AND METHODS

Within a maximum of 2h from specimen collection, 10µL acetic acid was mixed with 90µL CSF. The Fuchs-Rosenthal counting chamber was used and all 16 squares counted after sedimentation of the cells. The following edge rules were applied: cells touching the left and bottom border were added to the count, but not those touching the right and upper border. The unit was WBC count /µL.
The cytological specimens were prepared by cytocentrifugation, re-suspension with cold culture medium, stained by the May Grünwald Giemsa method, and assessed microscopically[3].
130 µL undiluted CSF was analysed on the XE-2100 system[4]. WBC counts (/µL) were analysed in two different channels, the WBC/BASO and DIFF channels. The WBC results were taken from the service screen in counts /µL; the XE-PRO software is needed to ensure consistent results.

Method WBC comparison

Always running a blank prior to the patient samples, all 226 samples were analysed on the XE-2100 in the "open manual mode" (CBC+DIFF profile), and compared with the reference Fuchs-Rosenthal chamber counts.
Agreement between reference methods and two different channels from the XE-2100 was determined using Passing-Bablok regression analysis[5] in three different ranges, A: all data (0-3,000 WBC/µL), B: 0-50 WBC/µL and C: 51-1,000 WBC/µL.

Linearity

The linearity for WBC was determined following the NCCLS EP6 protocol[6].
A CSF sample with a high WBC count (WBC ca. 500/µL) was diluted in normal saline solution (NSS). Dilution ratios of 100%, 80%, 60%, 40 %, 20%, 10%, 5%, and 1% were prepared and analysed in duplicate by the reference chamber method (performed by the same medical technician) and by the XE-2100 DIFF and WBC/BASO channels measured in the "open manual mode".

Within-day imprecision

3 different CSF samples, with a normal value < 5 WBC/µL, a pathologic value (approximately 50 WBC/µL), and a high pathologic value (approximately 500 WBC/µL), were analysed 10 times by the reference chamber method (performed by the same medical technician) and by the XE-2100 DIFF- and WBC/BASO channels measured in the "open manual mode". The CV for each count level and method was calculated.

Carryover

Absolute carryover was determined for the WBC in the XE-2100 DIFF- and WBC/BASO channels on 3 full blood specimens each being followed by 3 blank measurements. Any carryover was recorded as absolute counts.
Proportional carryover for the WBC DIFF channel and WBC/BASO channel was measured using the Broughton method [7]. Using 3 CSF specimens with low (l1-l3) and 3 specimens with high WBC (h1-h3) concentrations, the carryover percentage was calculated as follows:

$$\text{carrryover}\,(\%) = \frac{\text{l1-l3}}{\text{h3-l3}} \times 100$$

Truth table

All patient samples (226) analysed on the XE-2100 (CBC+DIFF profile) and analysed with the reference Fuchs-Rosenthal chamber method were compared in a truth table with a threshold for normal samples < 5 WBC/µL and a threshold for pathologic samples > 4 WBC/µL .

Method WBC differentiation comparison

Always running a blank prior to the patient samples, the samples (n=30) were analysed on the XE-2100 DIFF channel (results from research screen) in the "open manual mode" and compared with the reference manual microscopic differentiation from the CSF cytological specimens prepared by cytocentrifugation and stained by the May Grünwald Giemsa method. The differentiation was classified into polymorphonuclear cells /µL (PMN=Granulocytes) and mononuclear cells /µL (MN=Lymphocytes + Monocytes).
Agreement between the methods was determined using Passing-Bablok regression analysis.

RESULTS

Method comparisons

Regression analysis between reference methods and the two different channels from the XE-2100 in three different ranges A, B and C are presented in ***Table 1.*** There was an overall good correlation for microscope count and the XE-2100 DIFF- and WBC/BASO channel count in ranges A (R^2=0.985; R^2=0.883) and C (R^2=0.940; R^2=0.839). In the important range for CSF from 0-50 WBC/µL, however, while the DIFF channel showed a good correlation with the reference method (R^2=0.870), in contrast a poor correlation (R^2=0.064) resulted for the WBC/BASO channel in range B (0-50 /µL) [7].
Figs. 1a-1f show comparisons in graphic display.

Table 1 *Comparability WBC count XE-2100 channels versus reference Fuchs-Rosenthal chamber count at three different levels*

	A: WBC 0-3,000/µL			B: WBC 0-50/µL			C: WBC 51-1,000/µL		
	slope	R^2	n	slope	R^2	n	slope	R^2	n
WBC/BASO channel	1.09	0.883	226	1.93	0.064	201	0.99	0.839	25
DIFF channel	1.11	0.985	226	1.18	0.870	201	0.99	0.940	25

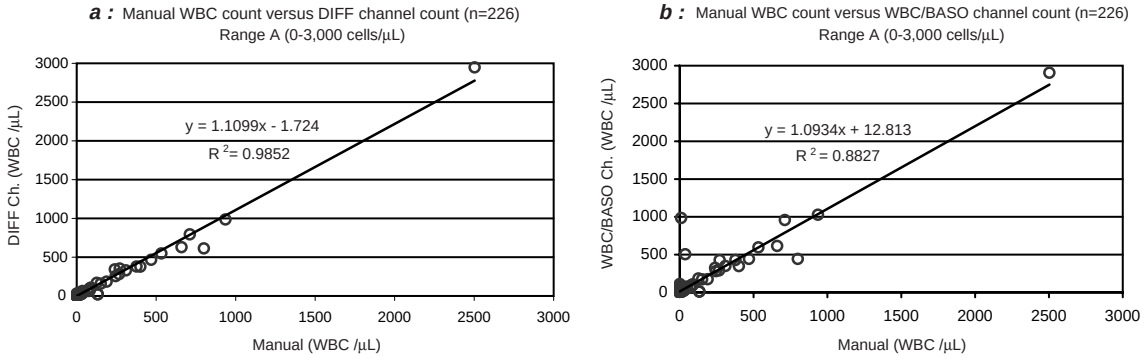

Fig. 1a , b : *Comparison reference WBC count versus DIFF- and WBC/BASO channel from range A (0-3,000 WBC/µL)*

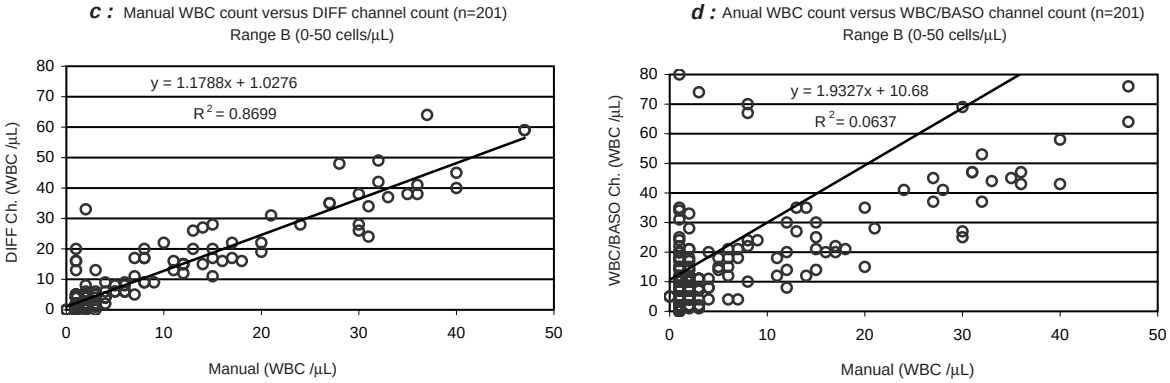

Fig. 1c , d : *Comparison reference WBC count versus DIFF- and WBC/BASO channel from range B (0-50 WBC/µL)*

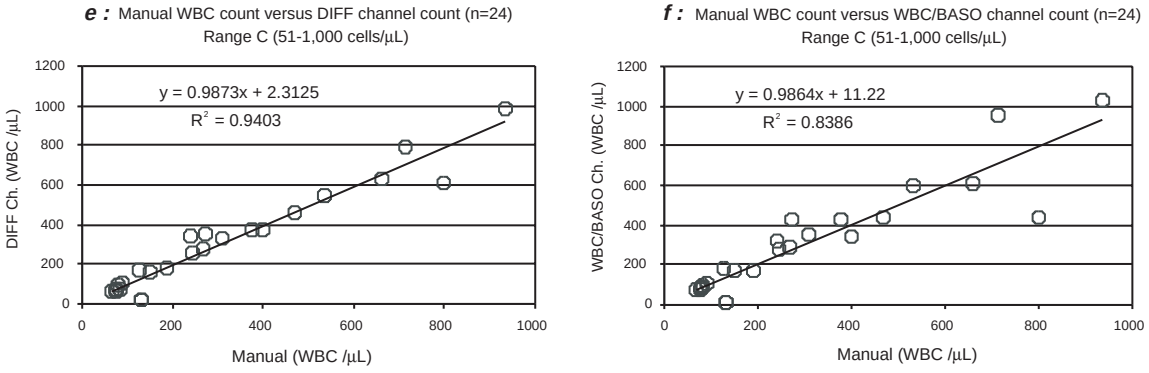

Fig. 1e , f : *Comparison reference WBC count versus DIFF- and WBC/BASO channel from range C (51-1,000 WBC/µL)*

Linearity studies

Linearity data for the samples measured by the microscope chamber method and both XE-2100 channels showed good correlation for all methods (reference method R^2=0.97, WBC/BASO channel R^2=0.98 and DIFF channel R^2=0.99).

Within-day imprecision studies

The within-day analytical imprecisions (CV) of the WBC count of the reference method and the two different channels from the XE-2100 are presented in ***Table 2.*** The CV of the XE-2100 was smaller for DIFF channel counts > 50 WBC/µL (9% and 4%) than the reference method (15% and 9%).
The CV in the range of < 5 WBC/µL was smaller for the reference method (performed by the same medical technician) than for the XE-2100 DIFF channel and the WBC/BASO channel (37% versus 46% and 59 %, respectively).

Carryover studies

The absolute carryover data for WBC on XE-2100 DIFF- and WBC/BASO channels, on 3 full blood specimens (in duplication) each of which was followed by 3 blanks, are presented in ***Table 3.*** The results showed the superiority of the DIFF channel. By the Broughton method, the proportional carryover for both channels was 0.0 % (CSF samples: high = 180 cells/µL and low = 1 cell/µL).

Comparison of cut-off value in a truth table

The true normal and true pathologic results from the reference method against the DIFF- and WBC/BASO channels from in total 226 CSF samples are presented in ***Table 4.***
The reference method showed 84 of 226 samples with counts higher than the cut-off value of > 4 cells/µL. The DIFF channel showed no (zero) underestimated samples, i.e. no false normal results, a concordance of 100 % for pathologic samples. The WBC/BASO channel showed 2 false normal samples and therefore a concordance of 97% for pathologic samples.
The reference method produced 142 normal samples with a cut-off value of < 5 cells /µL. There were 24 falsely ele-

Table 2 *Within-day imprecision WBC count XE-2100 channels versus reference Fuchs-Rosenthal chamber count at three different levels*

	Reference	DIFF channel	WBC/BASO channel
Sample 1			
Mean (WBC/µL)	1.3	1.9	4.2
SD	0.48	0.9	2.5
CV (%)	37.0	46.0	59.0
Sample 2			
Mean (WBC/µL)	55.9	75.9	71.6
SD	8.49	6.7	10.3
CV (%)	15.0	9.0	14.0
Sample 3			
Mean (WBC/µL)	481.9	514.2	503.9
SD	43.5	22.9	32.3
CV (%)	9.0	4.0	6.0

Table 3 *Absolute carryover results for WBC count from XE-2100 DIFF- and WBC/BASO channel*

	DIFF channel (cells/µL)					WBC/BASO channel (cells/µL)				
	Sample	Sample	Blank1	Blank2	Blank3	Sample	Sample	Blank1	Blank2	Blank3
High	189.992	190.034	74	0	0	188.128	187.086	74	1	3
Normal	5.008	4.995	1	0	0	5.198	5.222	5	5	4
Low	1.826	1.801	0	0	1	1.897	1.778	1	11	5

Table 4 *Truth table; true normal and true pathologic results (n=226)*

	DIFF channel				WBC/BASO channel			
WBC reference count	WBC > 4 cells /µL		WBC < 5 cells /µL		WBC > 4 cells /µL		WBC < 5 cells /µL	
WBC > 4 cells /µL	Samples	Agreement	Samples	Disagreement	Samples	Agreement	Samples	Disagreement
	84/226	100%	0/226	0%	82/226	97%	2/226	3%
WBC < 5 cells /µL	Samples	Disagreement	Samples	Agreement	Samples	Disagreement	Samples	Agreement
	24/226	17%	118/226	83%	101/226	71%	41/226	29%

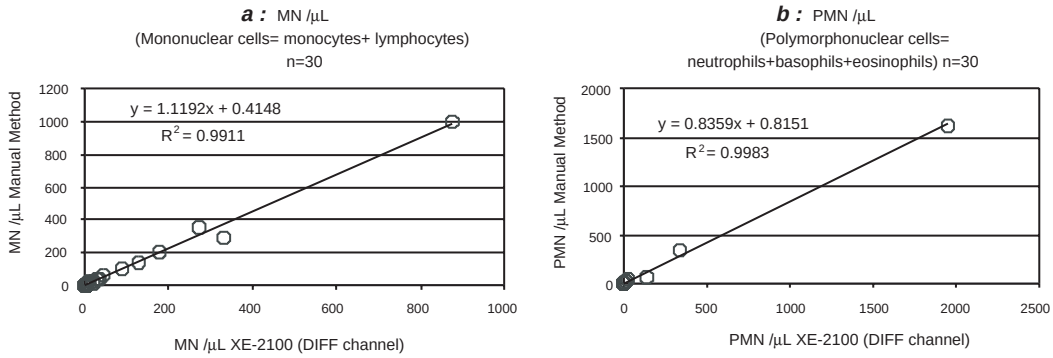

Fig. 2a , b : *Regression analysis in MN cells/µl and PMN cells/µL*

vated counts by the DIFF channel (20 of which were in the range 5-9 cells /µL) and therefore concordance in 83 % of true normal results (118/142 samples). On the other hand, the WBC/BASO channel produced 101 falsely elevated counts (in the range 5-111 cells/µL) and therefore concordance in only 29 % of normal results (41/142 samples).

WBC differentiation comparison studies

Regression analysis (n=30) of PMN cells/µL and MN cells/µL between the reference microscope differential and the differential from the XE-2100 DIFF channel are shown in ***Fig. 2***. There was an excellent correlation for PMN and MN (both R^2=0.99).

DISCUSSION AND CONCLUSIONS

Cerebrospinal fluid (CSF) manual microscopy chamber cell counts are time consuming, labour intensive and frequently imprecise, but remain the gold standard according to NCCLS. Today, fully automated analysers, which meet time and quality requirements, are the objective. Until now, however, specific automated systems for CSF cell counting have not been available. The following requirements, among others, would be necessary for such a system: to count precisely particularly at the decision level of 4 cells/µL and to differentiate leukocytes into PMN and MN. On the basis of these requirements, in this study we have evaluated the use of the Sysmex XE-2100 with its different WBC counting channels, the WBC/BASO channel (forward scatter [volume] and side scatter [structure]), and the DIFF channel (RNA/DNA content and side scatter) in the routine analysis of CSF.

Some 226 CSF samples, representative of our routine CSF measurement practice, measured on the DIFF and WBC/BASO channels of the XE-2100 were compared with manual Fuchs-Rosenthal chamber counts as reference. Overall comparison of DIFF channel results with the reference method were superior to that of WBC/BASO channel counts at all three count levels studied (0-3,000 /µL; 0-50 /µL; 51-1,000 /µL) but particularly in the clinically important range 0-50 /µL. The method comparison presented a poor correlation between the WBC count generated by the WBC/BASO channel and the reference method at this count range, and often falsely high results were reported by the analyser.

Linearity, imprecision and carryover studies were also performed. Linearity, imprecision and carryover showed that the DIFF channel from the XE-2100 is superior to the WBC/BASO channel and can be described as satisfactory to excellent. These data showed that the DIFF channel has no interference with e.g. air bubbles, dirt, etc. For this reason our standard procedure is to use the DIFF channel for CSF WBC counts, preceding the patient count by one or two blanks.

The truth table (the true normal and true pathologic results) showed superior results from the DIFF channel for counts > 4 /µL. There were no false normal WBC count results and no examples of pathologic pleocytosis missed. For the decision limit < 5 /µL there were 24 (17 %) samples over-estimated in the DIFF channel. The situation is very much worse in the WBC/BASO channel. With both methods, therefore, there is the possibility of erroneous, elevated counts at a decision limit < 5 /µL. This must be considered by the clinician in the interpretation of the data.

The WBC differentiation into PMN and MN from the DIFF channel showed an excellent regression analysis with the reference microscopic differentiation. These data appear promising, however, the number of samples is small (n=30). This study will be extended.

From a clinical point of view the XE-2100 DIFF channel produces counts on CSF which are of sufficient quality to support satisfactory patient treatment.

References

1) *National Committee for Clinical Laboratory Standards (NCCLS): Reference leukocyte count in CSF: Approved standard NCCLS document H20-A, Villanova, PA, 1992.*
2) *Sysmex Announces FDA Clearance of Body Fluids Application on Its Automated Hematology Analyzer XE-2100. PR Newswire: 03/26/04, 10:00a (Copyright (c) 2004, PR Newswire)*
3) *Worofka B, Lassmann J, Bauer K, Kristoferitsch W. Praktische Liquordiagnostik. Springer, Wien 1997.*
4) *Stamminger G, Auch D, Diem H, Sinha P: Performance of the XE-2100 leucocyte differential. Clin Lab Haem, 24: 271-280, 2002.*
5) *Passing H, Bablock W: A new biometrical procedure for testing the equality of measurements from two different analytical methods. Clin Chem Clin Biochem, 21: 709-720, 1983.*
6) *National Committee for Clinical Laboratory Standards (NCCLS):Evaluation of the Linearity of Quantitative Measurement Procedures:A Statistical Approach:Approved Guideline EP6-A, Wayne, PA, USA, 2003.*
7) *Guidelines for the evaluation of blood cell analysers including those used for differential leucocyte and reticulocyte counting and cell marker applications. International Committee for Standardization in Haematology (ICSH), 16:173,1994.*