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Evaluation of Mindray BC-6800 body fluid mode for automated cerebrospinal fluid cell counting

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Abstract

Background: Cellular analysis in cerebrospinal fluid (CSF) provides important diagnostic information in various medical conditions. The aim of this study was to evaluate the application of Mindray BC-6800 body fluid (BF) mode in cytometric analysis of CSF compared to light microscopy (LM).

Methods: One hundred and twenty-nine consecutive CSF samples were analyzed by BC-6800-BF mode as well as by LM. The study also included limits of blank (LoB), limit of detection (LoD), limit of quantitation (LoQ), carryover and linearity.

Results White blood cells LoQ was 4.0×10^6 cells/L. Linearity was good and carryover was negligible. As for the total and white blood cells, the BC-6800-BF parameters vs. LM showed both bias ranged from –10.28 to 0.06×10^6 cells/L. Polymorphonuclear and mononuclear cells ranged from 6.64 to 10.90%. For white blood cell the diagnostic agreement was 93% at the cut-off $>5.0\times10^6$ cells/L, and for polymorphonuclear and mononuclear at the cut-off >50% was 91% and 92%, respectively.

Conclusions: BC-6800-BF offers rapid and accurate counts in clinically relevant concentration ranges, replacing LM for most samples. However, in samples with abnormal cell counts or with abnormal white blood cell differential scattergrams the need to microscopic review for a correct clinical outcome remains.

Keywords: automated cell counting; cerebrospinal fluid; light microscopy; Mindray BC-6800.

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Introduction

Total and differential cellular counts in cerebrospinal fluid (CSF) provide important diagnostic information in different pathological conditions of the central nervous system (CNS) [1–7].

An elevated number of nucleated cells ($>5.0\times10^6$ cells/L in adults, $>7.0\times10^6$ cells/L in children or $>27.0\times10^6$ cells/L in newborns) is often present in CSF whenever an inflammatory process directly affects the CNS [7]. Therefore, a simple, fast and inexpensive test like the total nucleated cell count and differentiation can be of great help in providing clinicians with useful diagnostic information on CNS conditions [1–9].

The "gold standard" method for determining total cells (TC) in CSF and differentiating them is manual light microscopy (LM) [7]. On the other hand, LM is limited by its high imprecision, with an estimated CV that varies, according to different authors, from 13.8% to 91.3% [1, 2, 10]. This, combined with inter-operator variability, time-consuming sample preparation and examination, the need for highly qualified specialists for interpretation, and finally a higher turn around time (TAT) and costs, make the integration between manual and automated methodologies for CSF analysis the best solution [11]. Cells can be easily detected by modern hematology analyzers used for common routine analysis of peripheral blood samples. The BC-6800 is equipped with a dedicated platform for body fluid (BF) examination, like other hematology analyzer [10–23]. More recently CSF analysis has been performed even on automated urine flow cytometers [23–26] and urine microscopy analyzers [27, 28].

However, automated cell counts in CSF have poor precision and accuracy when the cellularity is less than $<50.0\times10^6$ cells/L [1–3, 12–15, 27]. To reduce these limitations, some analyzers included specific applications for BF analysis [16–23, 26, 28].

The new automated hematology analyzer BC-6800 (Mindray Medical International Ltd., Shenzhen, China) have a dedicated BF analysis module (BC-6800-BF) that has shown good counting performances on ascitic and pleural fluids. Interestingly, no data are currently available on BC-6800-BF performances analysis on CSF [29, 30].

The aim of this study was to evaluate BC-6800-BF mode counting performances on CSF, according to CLSI document H56-A, in 2006 [7] and ICSH guidelines for the verification and performance of automated cell counters for body fluids, in 2014 [31].

Materials and methods

CSF samples

A total of 129 consecutive CSF samples (50 from subjects in follow-up for acute lymphoblastic leukemia admitted to the Hematology Unit, 50 from subjects admitted to the Neurology Unit, 10 from subjects admitted to the Neurosurgery Unit, 15 from subjects admitted to the Infectious Diseases Unit and 14 patients admitted to the Emergency Department), were collected in sterile tubes (Becton Dickinson, Franklin Lakes, NJ, USA). The samples were referred to the laboratory for routine analysis from adult inpatient wards over approximately a 6-month period, were simultaneously assessed by LM and BC-6800-BF. No samples were pre-treated and all analyses were carried out within 1 h from dispatch. The study was approved by the Ethical Committee and was carried out in accordance with the Declaration of Helsinki under the terms of all relevant local legislation.

Manual light microscopy (LM)

Manual LM cell count of CSF samples was performed in Nageotte and in Fuchs-Rosenthal chambers.

Samples were analyzed according to CLSI document H56-A, in 2006 [7] and the ICSH guidelines, in 2014 [31, 32] as described below. In order to ensure the standardization of the procedure for manual counting, appropriate materials were used such as certified pipettes and cleaned chambers. In addition Turk's staining was used to enhance cell recognition.

Finally, cell count was performed by two skilled operators with a light microscope at ×400 magnification. An additional count was performed by a third operator in case of disagreement above 5%.

LM cell counts were performed under standard conditions, which include the incubation time for the cell sedimentation (5 min) and a priori definition of which number and squares included in the counts. In the Nageotte chamber CSF samples were diluted 1:1 with Turk's solution (Carlo Erba, Italy), (50 µL CSF sample +50 µL Turk's solution). Cells were counted in 12 squares, corresponding to 7.5 µL of CSF.

In the Fuchs-Rosenthal chamber CSF samples were diluted 9:1 with Turk's solution (180 µL CSF sample +20 µL Turk's solution). For each sample, cells were counted in the entire chamber, corresponding to 3.2 µL of CSF.

In order to determine the overall imprecision in the Nageotte and Fuchs-Rosenthal counting chambers, we tested three CSF samples with different ranges of cellularity. In each sample, counting was repeated 10 times by two operators as described above, and thus calculating the standard deviation (SD) and coefficient of variation (CV) of the methods, according to the CLSI document EP05-A3, in 2014 [16].

For the differential count, CSF samples were centrifuged (100 *g* for 3 min) on a cytospin (Cytospin2, Thermo Scientific, MA, USA) and stained with May-Grunwald-Giemsa (Carlo Erba Reagents, Italy).

According to the ICSH guidelines, in 2014 [31, 32] and CLSI document H20-A2, in 2010 [33], a differential count was performed on 200 cells in each sample by two experienced examiners and by a third person if the first two results showed about a 5% disagreement; a 400× magnification was used.

BC-6800-BF mode analysis

Using a methodology similar to the one used for routine cell counting on peripheral blood, BC-6800-BF performs the quantification of cells on CSF through fluorescent flow cytometry with hydrodynamic focusing after selective lysis and fluorescent staining of the nucleated elements. Stained cells are then classified by means of laser side scatter (SS), forward scatter (FS) and fluorescence (FL) analysis, into a three-dimensional scattergram (3D) according to their internal complexity (SS axis), size (FS axis) and nucleic acid content (FL axis). The presence of red blood cells (RBC) in CSF can be detected and counted with the impedentiometric channel of the BC-6800-BF.

BC-6800-BF provides the following parameters: total cell count (TC-BF) and white blood cell count (WBC-BF) of BFs; a differential cell count for mononuclear cell (MN) and polymorphonuclear cell (PMN) (both in percentage [%] and absolute number values [#]), whereas eosinophil (EO-BF), neutrophil (NE-BF) and cells with high fluorescence (HF-BF) are available in the search parameters (% and #).

Overall, BC-6800-BF analysis can be performed in volumes as small as 150 µL of untreated CSF samples, with high-throughput capabilities (~40 analysis/h). To avoid cross-contamination from blood samples, as well as carryover, BC-6800-BF automatically performs a rinse cycle, followed by a background check after each analysis.

All BC-6800-BF measurements were taken in accordance with the manufacturer's instructions and previous BC-6800-BF calibration control and quality internal control on three levels (R&D Body Fluid Hematology controls, R&D Systems, Inc., USA)

Imprecision

Within-run imprecision of BC-6800-BF was evaluated using 10 replicates of eight fresh CSFs routine samples, assessed according to the CLSI document EP05-A3, in 2014 [16]. Mean samples values ranged from 3.0 to 429.0×10^6 cells/L.

Between-run imprecision was also assessed according to the CLSI document EP05-A3, in 2014 [16], by analyzing body fluids control of three levels (1, 2 and 3) (R&D Body Fluid Hematology controls, R&D Systems, Inc., USA) in duplicate over 40 consecutive working days.

Carryover

Carryover was assessed on three CSF samples with a high cell count (between 996.0 and 2033.0×10^6 cells/L). Each sample was measured three times (A1, A2, A3) followed by three measurements of a blank (physiological saline solution; B1, B2, B3). Percentage of carryover was calculated using the formula [(B1-B3)/(A3-B3)]×100 [7, 30].

Limit of blank (LoB) and limit of detection (LoD)

LoB and LoD for TC-BF and WBC-BF were assessed according to CLSI document EP17-A2, in 2012 [34].

LoB was determined using non parametric analysis, as the 95th percentile value from 60 replicates of BC-6800-BF sample diluent (M-68DS).

LoD was assessed on six CSF samples, diluted with physiological saline solution to obtain very low cell concentrations. Ten replicates of each sample were assayed, for a total of 60 measurements. Mean values calculated for both TC-BF and WBC-BF parameters spanned between 1.0×10^6 cells/L and 8.0×10^6 cells/L, respectively. LoD was determined as the lowest TC-BF and WBC-BF value that could be detected above their respective LoB with 95% probability. LoD was calculated using the formula [LoD]=[LoB+1.645×SD] (where SD is the pooled standard deviation of results obtained on 60 measurements of low value samples above described).

Functional sensitivity (limit of quantitation [LoQ])

Functional sensitivity was assessed on 10 replicates of six native samples with different cell concentrations: TC-BF and WBC-BF from 1.0 to 449.0×10^6 cells/L, PMN from 7.0 to 258.0×10^6 cells/L; MN from 6.0 to 201.0×10^6 cells/L. The mean TC-BF, WBC-BF, PMN and MN count of each sample was plotted against the coefficient of variation (CV). Functional sensitivity was then mathematically estimated from power regression equation at a concentration in which the CV corresponded to 20%. This value was defined as the LoQ [7, 31, 32].

Linearity evaluation

Linear reportable range for cell count on BC-6800-BF was assessed by checking the counting performance of BC-6800-BF throughout, the manufacturer's stated range at varying levels of cell concentration and also considering the range of clinical applicability of cell counts.

For linearity testing, standards samples were obtained through isolation of cells from peripheral blood treated with HetaSep (Stemcell Technologies, Canada), mixed with cell-free CSF pool.

The sample obtained, with TC-BF of 1902.0×10^6 cells/L, was serially diluted with phosphate buffered saline (PBS) to produce 10 values in the low range, respectively (i.e. TC-BF from 1.0 to 1902.0×10^6 cells/L). Each dilution was measured for five consecutive times. Results were plotted against the expected cell counts, and linearity was then evaluated according to the CLSI document EP06-A, in 2003 [35].

Method comparison and Bias estimation using patient samples

BC-6800-BF parameters were compared both vs. the LM Nageotte chamber (129 samples) and LM differential counts on cytospin slides (44 samples).

LM cells differentiation was carried out according to morphological criteria reported in CLSI document H56-A, in 2006 [7]. All cells were hence included in one of the following classes: neutrophils (NE), lymphocytes (LY), monocytes (MO), eosinophils (EO), basophils (BA), macrophages (MA), and other cells (OTH; also including "blast-like" cells). Cells were then clustered in a discrete number of homogeneous cell categories to enable direct comparison between BC-6800-BF parameters to LM cell counts, as follows:

- TC-BF vs. total cell count by LM (TC-LM) #;
- WBC-BF vs. white blood cell count by LM (WBC-LM) (=TC-LM-[OTH]) #;
- MN vs. mononuclear cell count by LM (MN-LM) (=LY+MO+MA) %;
- PMN vs. polymorphonuclear cell count by LM (PMN-LM) (=NE+EO+BA)%;

Agreement between BC-6800-BF parameters and LM cell classification was assessed with Passing-Bablok regression and Bland-Altman plot analysis. Slope and intercept of Passing-Bablok regression were calculated within 95% confidence interval (95% CI) to highlight any significant difference between methods. As for the Bland-Altman plot, absolute differences were plotted against results of Nageotte counts. A significant bias was noted whenever the mean difference 95% CI missed the null-value. The Wilcoxon's test was applied to compare TC-BF vs. WBC-BF parameters and TC-LM vs. WBC-LM.

Diagnostic agreement between BC-6800-BF and LM

Diagnostic agreement of BC-6800-BF compared to LM was evaluated with receiver operating characteristics (ROC) curves along with sensitivity (SN) and specificity (SP) at the best thresholds of cellularity in CSF, as identified with ROC curves analysis at the following cut-offs: TC-BF and WBC-BF $\geq5.0\times10^6$ cells/L; PMN and MN ≥50% [7].

A further estimation of the Youden index and its associated cut-off point enabled the selection of an optimal instrument threshold value for TC-BF, WBC-BF, PMN% and MN% in discriminating samples between positive and negative groups.

Statistical analysis

Statistical analysis was carried out with Analyse-it™ software version 3.90.5 for Microsoft Excel (Analyse-it software Ltd., Leeds, UK).

Results

Comparison methods

The imprecision of LM counts performed in the Nageotte chamber revealed to be always lower than those obtained by Fuchs-Rosenthal. The imprecision in the Nageotte for TC ranged between 38.7% (mean 0.8×10^6 cells/L standards deviation [SD]±0.3), 12.2% (mean 7.8×10^6 cells/L and SD±0.96) and 5.6% (mean 97.6×10^6 cells/L and SD±5.1) in comparison to 50.0%, 17.9% and 6.7% in the same samples with the Fuchs-Rosenthal chamber.

Comparison between BC-6800-BF and gold standard LM was performed using a Nageotte chamber.

TC-LM, in all 129 CSF samples, ranged from 0.0 to 3853×10^6 cells/L (mean 127.0×10^6 cells/L 95% CI 53.0 to 203.0).

Sixty-six samples (51.2%) were classified as positive by LM reference method ($\geq5.0\times10^6$ cells/L). Ninety-two samples (71.3%) showed a low cell count, ranged from 0.0 to 20.0×10^6 cells/L (mean 4.0×10^6 cells/L, 95% CI 3.0 to 5.0) whereas 20 samples with cellularity did fall into decision threshold, i.e. between 4.0 and 10.0×10^6 cells/L (mean 6.4×10^6 cells/L, 95% CI 5.6 to 7.2). Finally, 42 samples with RBC-BF ranged between 0.001 and 0.318×10^{12} cells/L, whereas TC-LM mean value was 403.4×10^6 cells/L (95% CI 152.5 to 654.3).

Table 1 shows a comparison of cell counts by BC-6800-BF vs. LM (Figure 1).

Comparison between BC-6800-BF and LM counts for TC-BF and WBC-BF parameters showed Passing-Bablok regression slope ranged from 0.97 (95% CI, 0.93 to 1.01) to 1.04 (95% CI, 0.94 to 1.12) and intercept ranged from −0.22 (95% CI, −0.77 to 0.54) to −0.06 (95% CI −0.50 to 1.24) (Figure 1A, B, C and D). Bland-Altman bias was -10.28×10^6 cells/L (95% CI −22.87 to 2.31) and -4.47×10^6 cells/L (95% CI −28.81 to 11.88), respectively, for TC-BF and WBC-BF in all 129 samples (Figure 1E, F).

Agreement between BC-6800-BF and LM counting in the 92 low count samples was better, as for TC-BF and WBC-BF parameters notably the bias was 0.08×10^6 cells/L (95% CI −0.22 to 0.38) for TC-BF and 0.06×10^6 cells/L for WBC-BF (95% CI −0.27 to 0.39) (Table 1) (Figure 1G and H).

Table 1 summarizes correlations between BC-6800-BF parameters and LM for different cell population together, bias between -6.64×10^6 cells/L and 10.90×10^6 cells/L.

Table 1: Passing-Bablok regression and Bland-Altman bias for different cell categories with light microscopy versus BC-6800-BF.

	Passing-Bablok regression (95% CI Slope and Intercept)	**Bias Bland-Altman (95% CI)**
TC-BF# (all 129 samples ranged 0–3853×10^6 cells/L)	y=1.00×−0.13 (Slope: 0.94 to 1.03 Intercept: −0.27 to 0.00)	-10.28×10^6 cells/L (−22.87 to 2.31)
TC-BF# (66 samples ranged from 0 to 20×10^6 cells/L)	y=1.07×−0.15 (Slope: 0.98 to 1.14 Intercept: −0.36 to 1.56)	0.08×10^6 cells/L (−0.22 to 0.38)
WBC-BF# (all 129 samples 0–3853×10^6 cells/L)	y=0.97×−0.13 (Slope: 0.93 to 1.01 Intercept: −0.26 to 0.01)	-4.47×10^6 cells/L (−28.81 to 11.88)
WBC-BF# (66 samples ranged from 0 to 20×10^6 cells/L)	y=1.04×−0.14 (Slope: 0.94 to 1.12 Intercept: −0.29 to 0.00)	0.06×10^6 cells/L (−0.27 to 0.39)
PMN% (63 samples ranged from 20×10^6 cells/L to 3853×10^6 cells/L)	y=0.87×+1.31 (Slope: 0.74 to 1.08 Intercept: −8.59 to 8.00)	−6.64% (−11.63 to −1.64)
MN% (63 samples ranged from 20×10^6 cells/L to 3853×10^6 cells/L)	y=0.95×+10.19 (Slope: 0.73 to 1.31 Intercept: −4.60 to 20.41)	10.90% (4.65 to 17.15)
TC-BF# (43 samples RBC ranged from 0.001 to 0.318×10^{12} cells/L)	y=1.00×−0.06 (Slope: 0.92 to 1.04 Intercept: −0.50 to 1.24)	-24.70×10^6 cells/L (−63.31 to 13.80)
WBC-BF# (43 samples RBC ranged from 0.001 to 0.318×10^{12} cells/L)	y=0.98×−0.22 (Slope: 0.73 to 1.31 Intercept: −0.77 to 0.54)	-2.1×10^6 cells/L (−21.59 to 17.44)
PMN% (43 samples RBC ranged from 0.001 to 0.318×10^{12} cells/L)	y=0.89×+0.81 (Slope: 0.65 to 1.22 Intercept: −16.32 to 16.37)	−2.92% (−9.54 to 3.71)
MN% (43 samples RBC ranged from 0.001 to 0.318×10^{12} cells/L)	y=0.95×+9.30 (Slope:0.60 to 1.48 Intercept: −16.5 to 20.80)	6.71% (−2.0 to 15.42)

Values in absolute (#) and percentage (%) count of total cells (TC), white blood cells (WBC), polymorphonuclear cells (PMN) and mononucleted cells (MN).

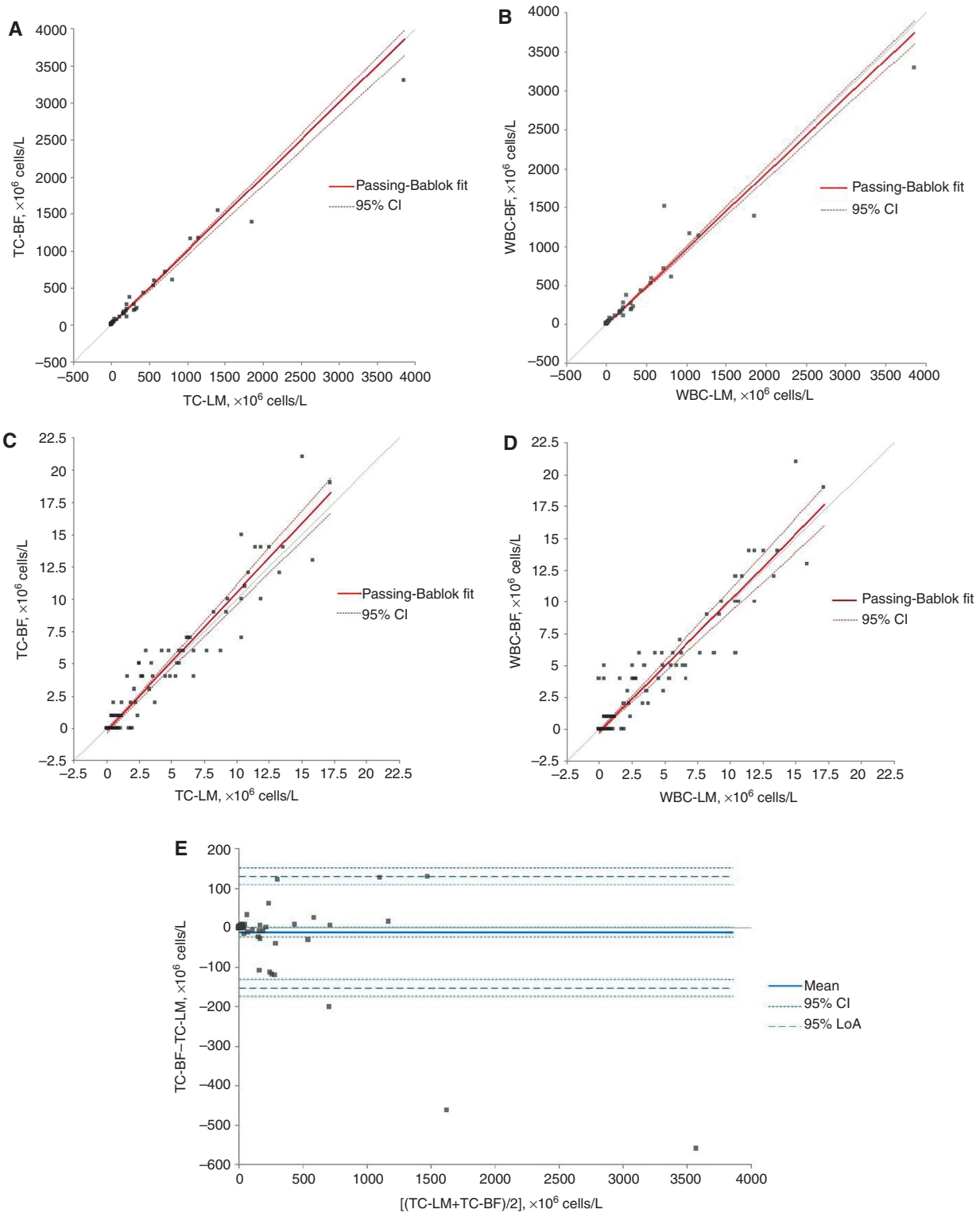
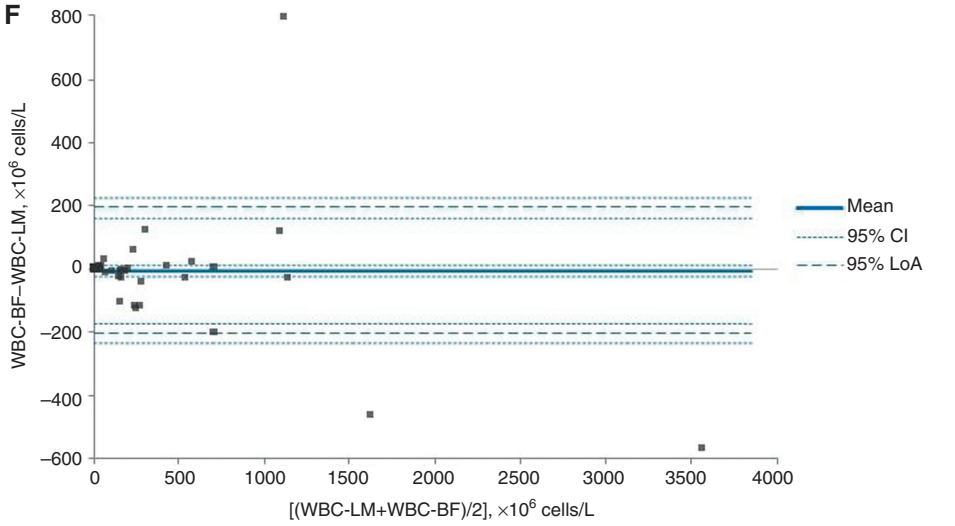
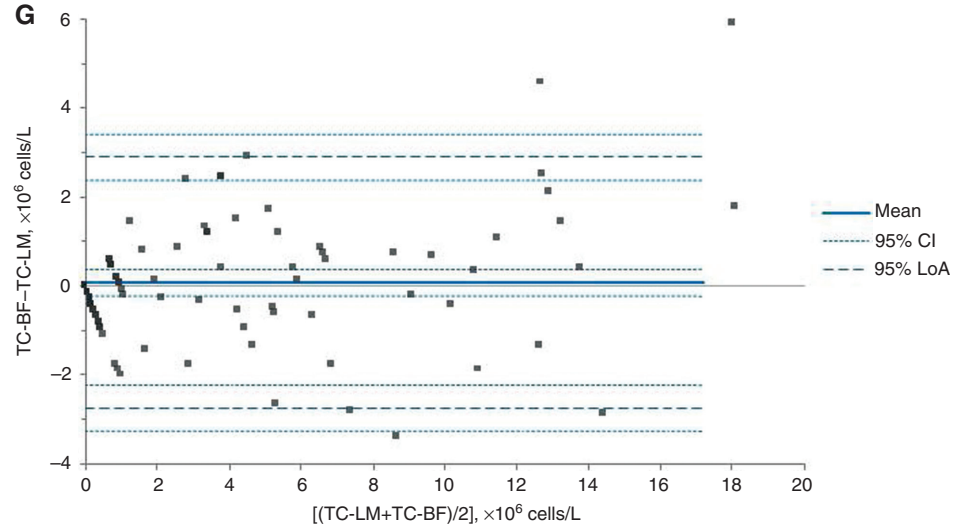


Figure 1: Comparison of cell counts between BC-6800-BF and LM by Passing-Bablok regression and Bland-Altman bias.
Passing-Bablok regression as described for the following comparison: (A) TC-BF versus TC-LM in all CSF samples (n=129): y=1.00×−0.13. (B) WBC-BF versus WBC-LM in all CSF samples (n=129): y=0.97×−0.13. (C) TC-BF versus TC-LM in low cellularity samples (TC <20.0×10⁶ cells/L): y=1.07×−0.15. (D) WBC-BF versus WBC-LM in low cellularity samples (WBC <20.0×10⁶ cells/L): y=1.04×−0.14. Bland Altman as described for the following comparison: (E) TC-BF versus TC-LM in all CSF samples (n=129): Bias equal −10.28×10⁶ cells/L (95% CI −22.87 to 2.31). (F) WBC-BF versus WBC-LM in all CSF samples (n=129): Bias equal to −4.47×10⁶ cells/L (95% CI −28.81 to 11.88). (G) TC-BF versus TC-LM in low cellularity samples (TC <20.0×10⁶ cells/L): Bias equal to 0.08×10⁶ cells/L (95% CI −0.22 to 0.38). (H) WBC-BF versus WBC-LM in low cellularity samples (WBC <20.0×10⁶ cells/L): Bias equal to 0.06×10⁶ cells/L (95% CI −0.27 to 0.39).





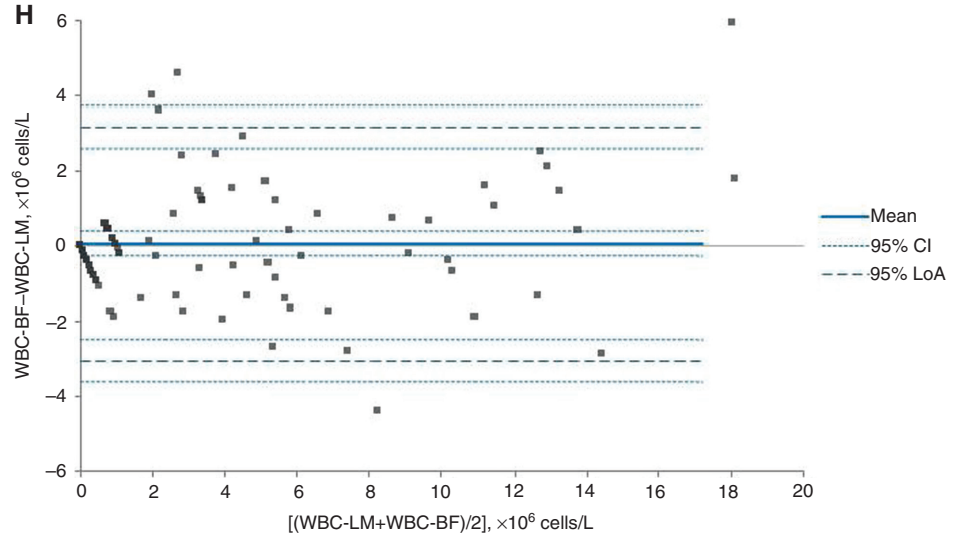


Figure 1 (continued)

We also evaluated the agreement between BC-6800-BF and LM for NE-BF and EO-BF, since these are available in search parameters. NE-BF parameter showed a correlation similar to PMN parameter (Table 1) as their Passing-Bablok regression was identical (data not shown). Unfortunately, no statistical analysis for EO-BF parameter was performed, since the available number of eosinophilic samples was very limited (12/129). It is noteworthy, however, to mention that every sample tested positive for EO-BF parameter by BC-6800-BF was subsequently confirmed by LM (Figure 2).

The agreement between BC-6800-BF parameters (TC-BF, WBC-BF, PMN and MN) and LM was evaluated also for 42 CSF samples showing a high red blood cell (RBC) count (>0.001×10^{12} cells/L), giving results similar to those obtained in all 129 samples CSF (see Table 1).

Finally, six samples showed differences between WBC-BF and TC-BF parameters (1516 vs. 1542, 191 vs. 200, 183 vs. 191, 3284 vs. 3292, 165 vs. 175 and 1130 vs. 1174×10^6 cells/L). Similar differences were found in the same samples between WBC-LM and TC-LM but in both cases the comparison between WBC-BF vs. TC-BF parameters and WBC-LM vs. TC-LM by Wilcoxon's test has not shown any differences with p=1.0.

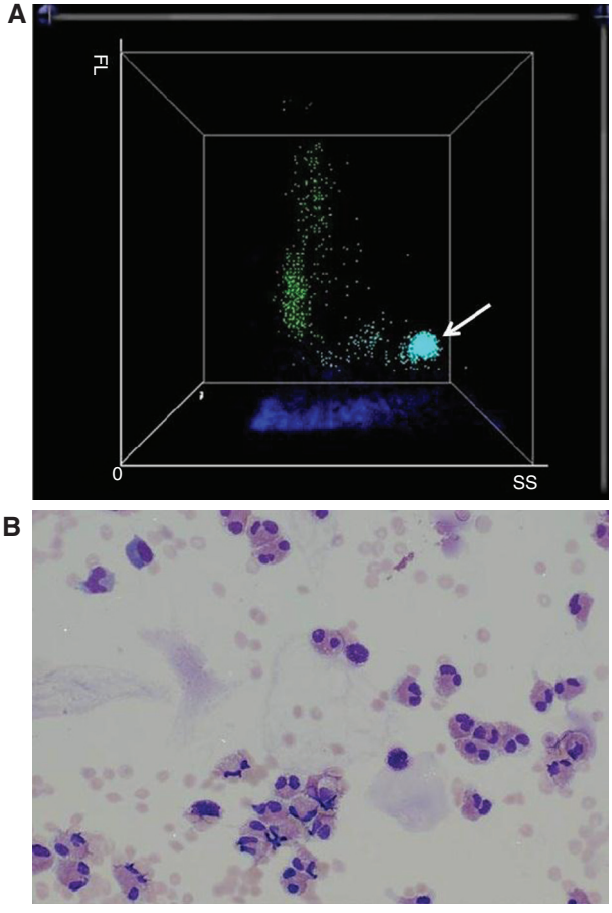


Figure 2: CSF sample with abnormal DIFF-scattergram shown in three dimensional scattergram (3D).
All cells are clustered in according to their internal complexity (SS axis), size (FS axis) and nucleic acid content (FL axis). In the scattergram area green clusters are MN, azure clusters are PMN, blue clusters are debri cells. (A) CSF sample DIFF-scattergram shows in the PMN area the eosinophil cluster (highlighted with arrow) (TC-BF: 608.0×10^6 cells/L; PMN: 72.6%; MN: 27.4%; EO-BF: 402.0×10^6 cells/L by BC-6800-BF). (B) Morphological characteristic of cells showed by LM (400× magnification) on cytospin stained May-Grunwald-Giemsa. Differential count shows: neutrophils 1%; lymphocytes 4%, eosinophils 95% and TC-LM 811.0×10^6 cells/L.

Imprecision

Within-run imprecision on BC-6800-BF for TC-BF was between 32.0% (mean±SD: 2.8±0.8×10^6 cells/L) and 3.6% (mean±SD: 429.1±15.8×10^6 cells/L). For WBC-BF was between 43.9% (mean±SD: 2.6±1.2×10^6 cells/L) and 3.6% (mean±SD: 429.1±15.8×10^6 cells/L). Table 2 shows also the imprecision data for PMN and MN absolute count.

The between-run imprecision on BC-6800-BF was between 2.8% (mean±SD: 989.0±28.0×10^6 cells/L) both for TC-BF and WBC-BF parameters and 39.6% (mean±SD: 4.7±1.7×10^6 cells/L) for MN parameter, respectively. Table 2 shows also the imprecision data for all BC-6800-BF parameters evaluated.

Carryover

As it has never exceeded 0.3%, carryover was considered negligible for all the following parameters: TC-BF, WBC-BF, PMN and MN.

Limit of blank (LoB), limit of detection (LoD) and functional sensitivity (limit of quantitation [LoQ])

LoB was 0.0×10^6 cells/L for both TC-BF and WBC-BF parameters. LoD was 3.0×10^6 cells/L for both TC-BF and WBC-BF parameters. The estimated LoQ for both TC-BF and WBC-BF parameters was 6.0×10^6 cells/L and 4.0×10^6 cells/L, respectively.

Linearity

Linear regression showed to be the best fitting model in both TC-BF and WBC-BF parameters (y=1.85×−0.017; r^2=1.00). Concerning the bias of BC-6800-BF, the

Table 2: Imprecision within-run and between run of different BC-6800-BF parameters (total cells [TC], leukocytes [WBC], polymorphonuclear cells [PMN] and mononuclear cells [MN]): mean value, standard deviation (SD), coefficient of variation (CV).

	Imprecision within run						Imprecision between run					
	Mean±SD ×10⁶ cells/L	Mean±SD ×10⁶ cells/L	Mean±SD ×10⁶ cells/L	Mean±SD ×10⁶ cells/L	Mean±SD ×10⁶ cells/L	Mean±SD ×10⁶ cells/L	Level 1 Mean±SD ×10⁶ cells/L	Level 2 Mean±SD ×10⁶ cells/L	Level3 Mean±SD ×10⁶ cells/L
	CV	CV	CV	CV	CV	CV	CV	CV	CV
TC-BF#	2.8±0.8	6.1±0.8	22.2±3.6	42.3±2.9	108.8±5.7	429.1±15.8	7.9±1.5	204.8±18.0	989.0±28.0
	32.0%	13.6%	16.1%	6.8%	5.3%	3.6%	19.0%	8.8%	2.8%
WBC-BF#	2.6±1.2	6.1±0.8	22.2±4.0	42.3±2.9	108.8±5.7	429.1±15.8	7.8±1.5	204.8±18.0	989.0±27.0
	43.9%	13.6%	16.1%	6.8%	5.3%	3.6%	19.9%	8.8%	2.8%
PMN#	//	//	12.4±2.0	26.4±3.9	60.7±4.9	244.6±9.1	//	199.7±17.6	965.0±26.0
			18.8%	14.9%	9.2%	3.7%		8.8%	2.7%
MN#	//	//	10.2±2.1	16.0±1.6	48.1±3.2	184.5±10.2	//	4.7±1.7	23.5±3.6
			15.9%	10.2%	6.6%	5.6%		39.6%	15.5%

Values in absolute (#) count.

comparison between the mean value of TC-BF and WBC-BF parameters and their expected ones was always within±10% (i.e. TC-BF and WBC-BF range: from 4.0 to 1902.0×10⁶ cells/L).

Diagnostic agreement

The diagnostic agreement between BC-6800-BF parameters and LM reference method in the 129 CSF samples was evaluated through ROC curve analysis. The generated area under curve (AUC) was 0.99 (95% CI, 0.98 to 1.00; p<0.0001) for TC-BF and WBC-BF parameters (Table 3).When the standard 5.0×10⁶ cells/L cut-off [7] was chosen, the diagnostic agreement of TC-BF and WBC-BF parameters compared to LM showed an identical value of 93% (sensitivity of 0.97, specificity of 0.89). In detail, 120 samples were correctly classified, with only nine samples erroneously classified (seven false positives and two false negatives) (Table 3).

An optimal instrument specific threshold of 4.0×10⁶ cells/L was identified with ROC analysis for TC-BF and WBC-BF parameters. When this threshold was used, parameters exhibited diagnostic agreement of 89%, specificity decreased from 0.89 to 0.77, but sensitivity improved from 0.97 to 1.00 (Table 3).

Diagnostic agreement between BC-6800-BF parameters and LM differential count for both PMN% and MN%, has been evaluated through ROC curve analysis generating an AUC of 1.00 (95% CI 0.99 to 1.01; p<0.0001) for both of them. Conversely, the diagnostic agreement of BC-6800-BF parameters vs. LM for PMN and MN, at the standard cut-off (i.e. PMN>50%, MN>50%), showed a diagnostic agreement slightly lower than those obtained in TC-BF and WBC-BF parameters, equal to 91% (sensitivity of 0.81, specificity of 1.00) for PMN and 92% (sensitivity of 1.00, specificity of 0.82) for MN parameters.

Finally, using an optimal instrument specific threshold of 45% for PMN parameter, both diagnostic agreement and sensitivity improved to 97% and 1.00, respectively, with only one sample misclassified as false positive (Table 3).

Discussion

Results of our study show that instrumental performances and linearity range of BC-6800-BF on CSF analysis are broadly similar to those declared by the manufacturer, described as follows: the background and blank count for TC-BF and WBC-BF are both equal to 0.0×10⁶ cells/L and for RBC-BF are equal to 0.000×10¹² cells/L, respectively. Carryover for TC-BF, WBC-BF and RBC-BF is ≤0.3%.

Table 3: ROC analysis of different BC-6800-BF parameters in cerebrospinal fluid: total cell count (TC-BF), white blood cell count (WBC-BF), polymorphonuclear cell count (PMN) and mononuclear cell count (MN).

	AUC (95% CI) p-value	Cut-off	Diagnostic agreement	Sensitivity	Specificity
TC-BF	0.99 (0.98 to 1.00) p<0.0001	≥5.0×10⁶ cells/L[a]	93% (2 false negative samples and five false positive samples)	0.97	0.92
		≥4.0×10⁶ cells/L[b]	89% (11 false positive samples)	1.00	0.82
WBC-BF	0.99 (0.98 to 1.00) p<0.0001	≥5.0×10⁶ cells/L[a]	93% (2 false negative samples and five false positive samples)	0.97	0.92
		≥4.0×10⁶ cells/L[b]	89% (13 false positive samples)	1.00	0.77
PMN	1.00 (0.99 to 1.01) p<0.0001	≥50%[a]	91% (3 false negative samples)	0.81	1.00
		≥45%[b]	97% (1 false positive sample)	1.00	0.94
MN	1.00 (0.99 to 1.01) p<0.0001	≥50%[a]	92% (3 false positive samples)	1.00	0.82

[a]cut-off suggested in CLSI reference document H56-A (7); [b]instrumental specific cut-off by ROC analysis.

Linearity was equal to r^2=0.99 both for TC-BF and WBC-BF (range: 0.00–10.00×10⁶ cells/L) and for RBC-BF r^2=0.99 (range: 0.000–5.46×10¹² cells /L). Repeatability for TC-BC, WBC-BF and RBC-BF the CV are equal to 12.9% (mean 56.3×10⁶ cells/L, 95% CI 0.1 to 1.5), to 12.6% (mean 56.6×10⁶ cells/L, 95% CI 0.1 to 1.5) and 12.5% (0.0059×10¹² cells/L), respectively.

Functional sensitivity (LoQ) of BC-6800-BF proved to be aligned with those obtained by different analyzers (i.e. Sysmex UF1000i-BF, XE-5000-BF and XN-BF) tested in previously published papers [16, 21, 26], thus confirming the significant validity of LoQ for WBC-BF (4.0×10⁶ cells/L) as suitable in clinical settings.

LM imprecision shows better results in Nageotte than Fuchs-Rosenthal chambers counting (Table 1). The difference between the two methods can easily be explained by the different volume of CSF analyzed on each chamber (7.5 µL in Nageotte versus 4.3 µL in Fuchs-Rosental chambers). Therefore, Nageotte chamber was preferred and considered the reference method for LM counting, in our study.

A comparison between TC-BF and WBC-BF parameters versus LM cell counts displayed for both a good agreement, even if a slight positive bias was observed against BC-6800-BF parameters in all CSF samples. However, this doesn't affect the ability of BC-6800-BF parameters in properly identifying any abnormal cell count in samples, with a sensitivity of 0.97 for both TC-BF and WBC-BF parameters, at the standard cut-off (i.e. 5.0×10⁶ cells/L).

At the above-mentioned cut-off, there were only two false negative samples coming from two patients who were in follow-up for acute lymphoblastic leukemia. In these samples, the LM count was 5.0 and 7.0×10⁶ cells/L, respectively, whereas the BC-6800-BF count showed 4.0×10⁶ cells/L for both. These patients are devoid of clinical neurological symptoms and without other biochemical alteration like CSF glucose and proteins. Into the five false positive samples the BC-6800-BF ranged from 5.0 to 7.0×10⁶ cells/L, whereas at LM ranged between 3.1 to 4.0×10⁶ cells/L, these patients too were in follow-up for neoplastic hematologic disorders, they don't show any alteration in CSF proteins, glucose and neurological clinical signs. These data could be explained by the high CV shown in LM count.

Notably, when the optimal instrumental cut-off equal to 4.0×10⁶ cells/L was applied, CSF analysis on BC-6800-BF showed a sensitivity of 1.00. Some data in literature have highlighted the problem of the range normal cellularity in CSF with the automation count. Definition of instrumental optimal specific cut-off was useful in this respect too [36, 37].

Diagnostic performance of BC-6800-BF parameters was satisfactory even at low cell counts ranges (below 20.0×10⁶ cells/L), with negligible bias (0.08×10⁶ cells/L).

This is often the case with routine CSF samples, as it was also for our study.

Moreover TC-BF and WBC-BF parameters were not influenced by the presence of red blood cells (RBC) in CSF samples, as demonstrated by the performance of BC-6800-BF parameters in analysis of CSF sample containing several RBC (see Table 1).

Diagnostic performance of PMN and MN show AUC and sensitivity of 1.00 at both standards (i.e. MN >50%) and instrumental (i.e. PMN> 45%) cut-offs, with no false negatives.

Despite EO-BF parameter is available only as search parameter in BC-6800-BF, the presence of eosinophils can always be easily detected in any CSF sample Figure 2 shows the CSF DIFF-scattergram of a patient with astrocytoma. It highlights the cluster of eosinophils, then confirmed by LM, despite this evidence for the EO-BF parameter, further studies are needed.

In many different circumstances, the presence of pathogenic microorganisms in CSF can be frequently associated with bacterial meningitis. As the presence of microorganisms in blood and body fluids may often cause interference issues in automated analyzers (overestimation of WCB count and abnormal DIFF-scattergram being the most common ones) [38, 39], we were interested also in evaluating BC-6800-BF performance under these conditions. In two case studies the application of BC-6800-BF analysis displayed abnormal DIFF-scattergram, and the presence of bacteria was further revealed by LM and microbiologically confirmed *Staphylococcus* coagulase negative.

The main anomaly evident in the two DIFF-scattergrams was an increased background noise that surprisingly had no effect on the overall TC-BF and WBC-BF parameters, indeed cell counts were confirmed by LM. Results were practically identical (TC-BF: 37.7×10^6 cells/L vs. TC-LM: 37.7×10^6 cells/L in the first sample, and TC-BF: 11.0×10^6 cells/L vs. TC-LM: 10.7×10^6 cells/L in the second sample). Therefore, despite the abnormal DIFF-scattergram, no instrumental flag was highlighted.

Finally, in the six samples that showed differences between WBC-BF and TC-BF parameters, the microscopic review showed a presence of some macrophages or ventricular lining cells. This could be a reason for the slight different between TC-BF and WBC-BF parameters and such discrepancy could represent a useful hint in defining new validation rules or the need to perform a reflex testing (e.g. microscopic review) whenever the results given by BC-6800-BF should not be accepted by default. As a described in previous studies on XE-5000 and XN-9000 analyzers [40, 41].

Conclusions

Results of our study suggest that the use of BC-6800-BF in cell counting and differentiation on CSF can provide an effective and automated alternative to LM in routine screening analysis of CSF, also enhancing laboratory workflow.

In our proposed workflow we suggest the use of WBC-BF parameter per se in CSF analysis, since there are no significant differences between TC-BF and WBC-BF parameters. Also, whenever the WBC-BF cellularity ranges between 4.0 to 7.0×10^6 cells/L in a CSF sample, the microscopic review should always be recommended. The same procedure should be adopted in the presence of abnormal DIFF-scattergram or discrepancy between WBC-BF and TC-BF count and consequent increase of HF-BF [29].

This BC-6800-BF system proved to be more efficient, rapid, and accurate than standard LM, thanks to its ability to perform direct analysis on untreated CSF samples and in separating the RBC, cell and differential counts in a single run. Rapid visualization of instrumental DIFF-scattergram and data counts provided by BC-6800-BF appeared to be particularly useful for the operator, as they can give important hints on the prevalent cell population whenever a high cell count is present, thus being an effective way to make timely therapeutic decisions and save time and manual labor.

Overall, sensitivity and specificity of BC-6800-BF are equal to or greater than other BF platforms [16, 21, 26]. Finally, WBC-BF count is satisfactory even at low ranges (i.e. 20×10^6 cells/L, see Table 1). However, despite some instrumental limits in CSF samples with PMN or MN parameters >50%, BC-6800-BF proved to be a good alternative to LM count, although the latter still remains the gold standard for analysis of CSF. Thanks to these premises and with a good knowledge of instrumental characteristics, together with the careful evaluation of DIFF-scattergram by a skilled operator, we can conclude that BC-6800-BF automated analyzer represents, together with LM, an important auxiliary tool which significantly enhances overall diagnostic information in CSF analysis.

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References

1. Fleming C, Russcher H, Lindemans J, de Jonge R. Clinical relevance and contemporary methods for counting blood cells in body fluids suspected of inflammatory disease. Clin Chem Lab Med 2015;53:1689–706.
2. Bignardi GE. Flow cytometry for the microscopy of body fluids in patients with suspected infection. J Clin Pathol 2015;68:870–8.
3. Sandhaus LM. Body fluid cell counts by automated methods. Clin Lab Med 2015;35:93–103.
4. De Luca GC, Bartleson JD. When and how to investigate the patient with headache. Semin Neurol 2010;30:131–44.
5. Fitch MT, Abrahamian FM, Moran GJ, Talan DA. Emergency department management of meningitis and encephalitis. Infect Dis Clin North Am 2008;22:33–52.
6. de Graaf MT, de Jongste AH, Kraan J, Boonstra JG, Sillevis Smitt PA, Gratama JW. Flow cytometric characterization of cerebrospinal fluid cells. Cytometry Part B Clin Cytom 2011;80:271–81.
7. Clinical and Laboratory Standard Institute. Body fluid analysis for cellular composition; approved guidelines. CLSI document H56-A. Wayne, PA: Clinical and Laboratory Standard Institute, 2006.
8. Zimmermann M, Otto C, Gonzalez JB, Prokop S, Ruprecht K. Cellular origin and diagnostic significance of high-fluorescent cells in cerebrospinal fluid detected by the XE-5000 hematology analyzer. Int J Lab Hematol 2013;35:580–8.
9. Cho YU, Chi HS, Park SH, Jang S, Kim YJ, Park CJ. Body fluid cellular analysis using the Sysmex XN-2000 automatic hematology analyzer: focusing on malignant samples. Int J Lab Hematol 2015;37:346–56.
10. Mahieu S, Vertessen F, Van der Planken M. Evaluation of ADVIA 120 CSF assay (Bayer) vs. chamber counting of cerebrospinal fluid specimens. Clin Lab Haematol 2004;26:195–9.
11. Zimmermann M, Weimann A. Automated vs. manual cerebrospinal fluid cell counts: a work and cost analysis comparing the Sysmex XE-5000 and the Fuchs-Rosenthal manual counting chamber. Int J Lab Hematol 2011;33:629–37.
12. Kresie L, Benavides D, Bollinger P, Walters J, Pierson D, Richmond T, et al. Perfomance evaluation of body fluids on the Sysmex XE-2100 series automated hematology analyzer. Lab Hematol 2005;11:24–30.
13. Brown W, Keeney M, Chin-Yee I, Johnson K, Lantis K, Finn W et al. Validation of body fluid analysis on the Coulter LH 750. Lab Hematol 2003;9:155–9.
14. Hoffmann JJ, Jansen WC. Automated counting of cells in cerebrospinal fluid using the Celldyn-4000 haematology analyzer. Clin Chem Lab Med 2002;44:1168–73.
15. De Smet D, Van Moer G, Martens GA, Nanos N, Smet L, Jochmans K, et al. Use of the Cell-Dyn Sapphire hematology analyzer for automated counting of blood cells in body fluids. Am J Clin Pathol 2010;133:291–99.
16. Boer K, Deufel T, Reinhofer M. Evaluation of the XE-5000 for the automated analysis of blood cells in cerebrospinal fluid. Clin Biochem 2009;42:684–91.
17. de Jong R, Brower R, de Graaf MT, Luitwieler RL, Fleming C, de Frankrijker-Merkestijn M, et al. Evaluation of the new body fluid mode on the Sysmex XE-5000 for counting leukocytes and erythrocytes in cerebrospinal fluid and other body fluids. Clin Chem Lab Med 2010;48:665–75.
18. Pernè A, Hainfellner JA, Womastek I, Haushofer A, Szekeres T, Schwarzinger I. Performance evaluation of the Sysmex XE-5000 hematology analyzer for white blood cell analysis in cerebrospinal fluid. Arch Pathol Lab Med 2012;19:194–8.
19. Li A, Gronlund E, Brattsand G. Autoamted white blood cell counts in cerebrospinal fluid using the body fluid mode on the platform Sysmex XE-5000. Scand J Clin Lab Invest 2014;74:673–80.
20. Letho TM, Leskinen P, Hedberg P, Vaskivuo TE. Evaluation of the Sysmex XT-4000i for the automated body fluid analysis. Int J Lab Hematol 2014;36:114–23.
21. Fleming C, Brouwer R, Lindemans J, de Jong R. Validation of the body fluid module on the new Sysmex XN-1000 for counting blood cells in cerebrospinal fluid and other body fluids. Clin Chem Lab Med 2012;50:1791–8.
22. Zur B, Eichhorn L, Albers E, Stoffel-Wagner B. Evaluation of 2 hematology analyzers in body fluid mode versus flow cytometry immunophenotyping of mainly neurosurgical cerebrospinal fluid samples. J Neurol Surg A Cent Eur Neurosurg 2012;73:93–8.
23. Strik H, Luthe H, Nagel I, Ehrilch B, Bahr M. Automated cerebrospinal fluid cytology. Limitation and reasonable applications. Anal Quant Cytol Histol 2005;27:167–73.
24. Nanos NE, Delanghe JR. Evaluation of Sysmex UF-1000i for use in cerebrospinal fluid analysis. Clinica Chimica Acta 2008;392:30–3.
25. Buoro S, Ottomano C, Appassiti S, Gherardi P, Alessio MG, Crippa A, et al. Analytical and clinical evaluation of Sysmex UF1000i for automated screening of cerebrospinal fluids. J Med Biochem 2013;32:1–6.
26. Buoro S, Apassiti Esposito S, Alessio M, Crippa A, Ottomano C, Lippi G. Automated cerebrospinal fluid cell counts using the new body fluid mode of Sysmex UF-1000i. J Clin Lab Anal 2015; doi: 10.1002/jcla.21866.
27. Glasser L, Murphy CA, Machan JT. The clinical reliability of automated cerebrospinal fluid cell counts on the Beckman-Coulter LH750 and Iris iQ200. Am J Clin Pathol 2009;131:58–63.
28. Walker TJ, Nelson LD, Dunphy BW, Anderson DM, Kickler TS. Comparative evaluation of the Iris iQ200 body fluid module with manual hemacytometer count. Am J Clin Pathol 2009;131:333–8.
29. Buoro S, Mecca T, Azzarà G, Seghezzi M, Vavassori M, Crippa A et al. Mindray BC-6800 automated body fluid mode cell counts on pleural and ascitic fluids. Int J Lab Hematol 2016;38:90–101.
30. Lippi G, Cattabiani C, Benegiamo A, Gennari D, Pavesi F, Caleffi A, et al. Evaluation of white blood cell count in peritoneal fluid with five different hemocytometers. Clin Biochem 2013;46:173–6.
31. Bourner G, De La Salle B, George T, Tabe Y, Baum H, Culp N, et al. ICSH guidelines for the verification and performance of automated cell counters for body fluids. Int J Lab Hematol 2014;36:598–612.
32. Briggs C, Culp N, Davis B, D'onofrio G, Zini G, Machin SJ. ICSH guidelines for the evaluation of blood cell analysers including those used for differential leucocyte and reticulocyte counting Int. Jnl Lab Hematol 2014;36:613–27.
33. Clinical and Laboratory Standards Institute. Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of instrumental Methods; Approved Guideline – second edition. CLSI document H20-A2. Wayne, PA: Clinical and Laboratory Standards Institute, 2010.
34. Clinical and Laboratory Standards Institute. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures;

Approved guideline – second edition. CLSI document EP17-A2. Wayne, PA: Clinical Laboratory Standards Institute, 2012.
35. Clinical and Laboratory Standard Institute. Evaluation of the linearity of quantitative measurement procedures: a statistical approach; Approved Guideline. CLSI document EP06-A. Wayne, PA: Clinical and Laboratory Standard Institute, 2003.
36. Bremell D, Mattsson N, Wallin F, Henriksson J, Wall M, Blennow K, et al. Automated cerebrospinal fluid cell count-new reference ranges and evaluation of its clinical use in central nervous system infections. Clin Biochem 2014;47:25–30.
37. Sandhaus LM, Ciarlini P, Kidric D, Dillman C, O'Riordan M. Automated cerebrospinal fluid cell counts using the Sysmex XE-5000: is it time for new reference ranges? Am J Clin Pathol 2010;134:734–8.
38. Omuse G, Makau P. Interference of cerebrospinal fluid white blood cell counts performed on the Sysmex XT-4000i by yeast and bacteria. Int J Lab Hematol 2013;35:e5–7.
39. Kim HR, Park BR, Lee MK. Effects of bacteria and yeast on WBC counting in three automated hematology counters. Ann Hematol 2008;87:557–62.
40. Buoro S, Appassiti Esposito S, Vavassori M, Mecca T, Ottomano C, Dominoni P, et al. Reflex testing rules for cell count and differentiation of nucleated elements in pleural and ascitic fluids on Sysmex XE-5000. J Lab Autom 2016;21:297–304.
41. Buoro S, Mecca T, Azzarà G, Seghezzi M, Dominoni P, Crippa A, et al. Cell Population Data and reflex testing rules of cell analysis in pleural and ascitic fluids using body fluid mode on Sysmex XN-9000. Clin Chim Acta 2016;452:92–8.