International Journal of Laboratory Hematology
The Official journal of the International Society for Laboratory Hematology

Mindray BC-6800 body fluid mode, performance of nucleated cells, and differential count in ascitic and pleural fluids

S. BUORO*, T. MECCA*, G. AZZARÀ*, M. SEGHEZZI*, E. CANDIAGO†, A. GIANATTI†, A. CRIPPA*, A. LA GIOIA*

*Chimical Chemistry Laboratory, Hospital Papa Giovanni XXIII, Bergamo, Italy
†Pathology Laboratory, Hospital Papa Giovanni XXIII, Bergamo, Italy

Correspondence:
Dr Sabrina Buoro, Clinical Chemistry Laboratory, Hospital Papa Giovanni XXIII – Piazza OMS, 1 -24128 Bergamo, Italy.
Tel.: (039) 035 2674550;
Fax: (039) 035 2674939;
E-mail: sbuoro@hpg23.it

doi:10.1111/ijlh.12446

Received 11 May 2015;
accepted for publication 22 September 2015

Keywords
Ascitic fluid, pleural fluid, automated cell counting, body fluid, Mindray BC-6800

SUMMARY

Introduction: An accurate and rapid analysis of cells in body fluids (BFs) is important for diagnosis and follow-up in many pathological conditions. We evaluated the analytical performance of the module BF Mindray BC-6800 (BC-6800-BF) for cytometric analysis of ascitic and pleural fluids.

Methods: A total of 99 ascitic and 45 pleural samples were collected and assessed with BC-6800-BF and optical microscopy. This study also includes the evaluation of limit blank (LoB), limit detection (LoD), limit quantitation, (LoQ), carryover, linearity, and diagnostic concordance between the two methods.

Results: For TC-BF, LoB was 1×10^6 cells/L, LoD was 3×10^6 cells/L, and LoQ was 4×10^6 cells/L. Linearity was excellent ($r^2 = 0.99$) and carryover was negligible. TC-BF performed with the two methods showed Pearson's correlation of 0.99 ($P < 0.0001$), Passing–Bablok regression $y = 1.04x - 1.17$, and bias 33.7 cells. In ascitic fluids, polymorphonuclear cells (PMN) showed an area under curve (AUC) of 0.98 ($P < 0.0001$). In pleural fluids, mononuclear cells (MN) and PMN % displayed an AUC of 0.79 ($P < 0.0001$) and 0.93 ($P < 0.0001$), respectively.

Conclusions: BC-6800-BF in ascitic and pleural fluids offers rapid and accurate cell and differential counts in clinically relevant concentration ranges. The use of BC-6800-BF may allow to replace routine optical counting, except for samples displaying abnormal cell counts or abnormal DIFF scattergram.

INTRODUCTION

Total and differential counts of nucleated cells in pathological body fluid (BF) as well as ascitic (AF) and pleural fluids (PF) are useful to differentiate malignancies from reactive or infectious diseases [1–3]. Many of the serous cavity effusion in infectious diseases show increased total nucleated cells and leukocytes (WBC).

An accurate evaluation of the number and cellular type in BF is an important diagnostic criteria. For example in AF, the presence of neutrophils (NE)

higher than 250×10^6 cells/L is sufficient for the diagnosis of spontaneous bacterial peritonitis [1, 3], thus allowing a faster start of therapy. Similarly, in AF a total nucleated cells counting $\geq 1000 \times 10^6$ cells/L with the majority of lymphocytes (i.e., >50%) allows for the diagnosis of tuberculous peritonitis [1].

In PF, a cell counting higher than 1000×10^6 cells/L characterizes a pleural exudative effusion [1, 4]. In these cases, if lymphocytosis is higher than 50%, tuberculous pleurisy, lymphoma, metastatic malignancies, sarcoidosis, or chylothorax can be suspected [1]. Also an esinophilic effusion (>10%) may be malignant in etiology or caused by pneumothorax, pulmonary embolism, parasitic infections, or Churg–Strauss syndrome [1].

Manual counting by optical microscopy (OM) is still the 'Golden Standard' to determine WBC and other nonhematological cells in BF. However, microscopic counting showed high inaccuracy as well as poor standardization and reproducibility. In addition, it requires skilled and well-trained technical people to have high turnaround time (TAT) [5–8]. As a matter of facts, in many clinical laboratories, to avoid the difficulties of microscopic counting, body fluids analysis is performed using hematological analyzers with dedicated modules [2, 8–10]. Not only an hematological analyzer was used, but also instruments for urinalysis as Sysmex UF-series and Iris iQ200 have shown good performance in BF [9–12].

BC-6800 (Mindray, Shenzhen-China) is an hematology analyzer able to perform BF analysis in a dedicated module (BF). BC-6800-BF offers a number of default parameters including total nucleated cells (TC-BF) and leukocyte (WBC-BF), differential cell count for mononuclear cells (MN) and polymorphonuclear cells (PMN). Additional research parameters include esinophils (EO-BF), neutrophils (NE-BF), and cells with a high fluorescence (HF-BF).

The aim of our study was the evaluation of the analytical performance both in ascitic and pleural fluids according to CLSI document H56-A in 2006 (CLSI document H56-A) [1] as well as to the ICSH Guideline for verification and performance of automated cell counters for body fluids in 2014 [13].

MATERIALS AND METHODS

Samples

One hundred and forty-four samples (99 AF and 45 PF) were collected in K_3EDTA tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and studied to evaluate the correlation between BC-6800-BF and OM. The collection and analysis of all BFs were performed according to the CLSI document H56-A [1].

The study was carried out in accordance with the Declaration of Helsinki, under the terms of all relevant local legislation.

Optical microscopy

Manual microscopic cell count was performed in a Nageotte counting chamber to count total nucleated cells (TCs). BF samples were diluted (1 : 20 or 1 : 200) with Turk's solution (Carlo Erba, Milano, Italy). For each sample, cells were counted in 12 squares (7.5 μL of BF samples) at ×400 magnification. TCs differential was performed by two skilled operators with a light microscope at ×400 magnification and by a third person if the first two results disagree [13, 15]. Slides were prepared by cytospin of BF samples at 100 ***g*** for 3 min (Cytospin2 Thermo Scientific, Milano, Italy) followed by May–Grunwald–Giemsa staining.

The differential count was performed according to the standard CLSI document H20-A2 in 2010 [14] and ICSH guidelines for the evaluation of blood cell analyzers including those used for differential leukocyte and reticulocyte counting in 2014 [15], for all sample cells differential was performed by two experienced examiners and a third person if the first two results disagree [13, 15].

Mindray BC6800 BF mode

As for the analysis of BF samples, BC-6800-BF uses fluorescent flow cytometry with hydrodynamic focusing to provide quantification of RBC, WBC, and TC in BF samples. Similarly, as methodology for cellular counting, in peripheral blood, BF mode uses flow cytometry after selective lysis and fluorescence staining. In BF mode, laser side scatter (SS), forward

scatter (FS), and fluorescence analysis (FL) are used to classify all nucleated cells in BF. Cells are clustered in a three-dimensional scattergram (3D) according to their internal complexity (SS axis), size (FS axis), and nucleic acid content (FL axis) as MN, PMN, NE-BF, EOS-BF, and HF-BF (Figure 1).

HF-BFs are nucleated high-fluorescent cells (for research purpose only). These cells are different from WBCs (i.e., mesothelial cells, macrophages, malignant nonhematopoietic cells) and included in the TC count. The RBCs are counted in the impedentiometric channel.

All additional information was examined according to CLSI document H56-A [1] such as the data of DIFF scattergram. The scattergrams were assessed using the same criteria as for complete blood cell count. DIFF scattergram results in all samples were evaluated by two experienced examiners by comparison with respect to the reference DIFF scattergram.

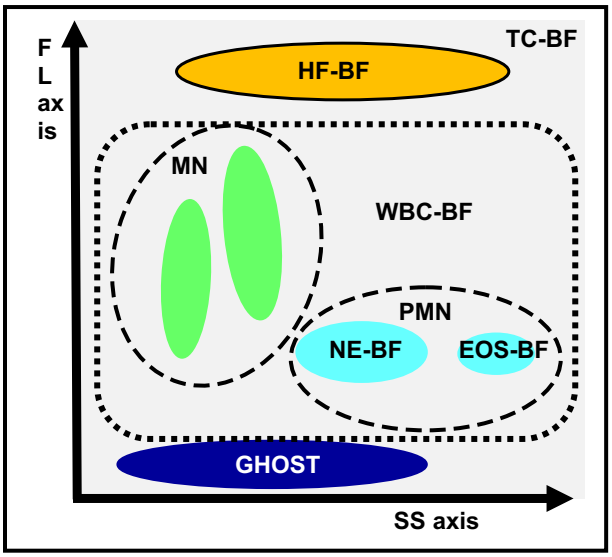


Figure 1. BC-6800-BF DIFF scattergram pattern. All cells are clustered in according to their internal complexity (SS axis), size (FS axis), and nucleic acid content (FL axis). The green clusters are mononuclear cells (MN), azure clusters are polymorphonuclear cells (PMN) cells, yellow cluster is nucleated high-fluorescence cells (HF-BF), and blue cluster is debris cells (ghost). TC-BF, total nucleated cells; WBC-BF, leukocytes; NE-BF, neutrophils; and EO-BF, esinophils.

BC-6800-BF uses 150 μL for the count of a BF sample only, without any preventive manual treatment. Throughput is 40 BF samples/h. Although BC-6800-BF automatically performs a rinse cycle, followed by a background check, to avoid cross-contamination from blood samples and sample carryover in BF mode, an automated rinsing is performed whenever a sample is analyzed. The BC-6800 is calibrated and used according to manufacturer specifications.

Repeatability

The within-run imprecision of BC-6800-BF was evaluated using 10 replicates of 9 fresh BFs routine samples, assessed according to the CLSI document EP05-A3 in 2014 [16]. The mean samples values ranged from 12 to 1666 $\times$ 10^6 cells/L.

Sample carryover

Carryover was assessed on two BF (one AF and one PF) samples with a high cell count (from 1600 to 5000 $\times$ 10^6 cells/L). Each sample was measured three times (H1, H2, H3) followed by three measurements of a blank (physiological saline; B1, B2, B3). Percentage of carryover was calculated using the formula 'carryover = [(B1 − B3)/(H3 − B3)] $\times$ 100' [17].

Limit of blank and limit of detection

The limit of blank (LoB) and limit of detection (LoD) were assessed according to CLSI document EP17-A2 in 2012 [18]. LoB was assessed using nonparametric analysis as the 95th percentile value from 60 replicates of sample diluent of BC-6800-BF (M-68DS). LoD was then assessed on 12 BFs (6 ascitic e 6 pleural) samples diluted with physiological saline solution, to obtain very low concentrations of both TCs. Ten replicates of each sample were assayed, for a total of 60 measurements for each type of BFs. The mean sample values ranged from 1 to 8 $\times$ 10^6 cells/L. The LoD was determined as the lowest TC and WBC concentrations that were detected above their respective LoB with a 95% probability [18].

LoD was calculated using the formula LoD = LoB + 1.645 $\times$ SDs (where SD is the pooled

standard deviation of results on low-level samples).

Functional sensitivity (limit of quantitation)

Functional sensitivity was assessed on ten replicates of nine native samples at different cell concentrations TC from 8 to 1650 × 10^6 cells/L; WBC from 8 to 1639 × 10^6 cells/L; PMN from 8 to 769 × 10^6 cells/L; MN from 3 to 850 × 10^6 cells/L. The mean TC, WBC, PMN, and MN count of each sample was plotted against the CV. Functional sensitivity was mathematically assessed from the power regression equation at a concentration in which the CV equals 20%. This value was defined as limit of quantitation (LoQ) [19].

Linearity

For linearity testing, TCs obtained from native samples were used. A sample with high cell concentration counts was serially diluted with phosphate-buffered saline (PBS) to produce eight concentration levels as follows: TC from 8 to 3965 × 10^6 cells/L, WBC from 8 to 3936 × 10^6 cells/L, PMN from 24 to 3063 × 10^6 cells/L, and MN from 18 to 2279 × 10^6 cells/L.

Each sample was measured five times consecutively. Results were plotted against the expected cell counts, and linearity was evaluated according to the CLSI document EP06-A in 2003 [19].

Comparison of patient samples

Methods comparison was made on 144 BF samples (99 AF and 45 PF). These samples were processed both with BC-6800-BF and with the OM: in Nageotte chamber for total cell counts as well as differential counts on cytospin stained with May–Grunwald–Giemsa (MGG) (Carlo Erba Reagent spa Italy).

Morphological differentiation by OM entailed cell classification in one of the following classes: neutrophils (NE), lymphocytes (LY), monocytes (MO), esinophils (EO), basophils (BASO), macrophages (MACRO), mesothelial cells (MESO), and other cells (OTHER; also including 'blastlike' cells).

Due to different classification and cells designation from BC-6800BF mode and OM, cells were clustered in a discrete number of homogeneous cell categories to enable a direct comparison between the two methods, as follows:

- TC-BF *vs.* OM-TC # (in absolute value);
- WBC-BF *vs.* OM-WBC (= OM-TC − [MACRO + MESO + OTHER CELLS]) #
- MN *vs.* OM-MN1 (= LY + MO) % and # (both as percentage and absolute value);
- MN *vs.* OM-MN2 (= LY + MO + MACRO) % and # (same as above);
- PMN *vs.* OM-PMN (NE + EO + BASO) % and #;
- HF-BF *vs.* OM-NO-WBC1 (= MESO) % and #
- HF-BF *vs.* OM-NO-WBC2 (= MESO + MACRO) % and #
- HF-BF *vs.* OM-NO-WBC3 (= MACRO) % and #
- HF-BF *vs.* OM-NO-WBC4 (= MESO + OTHER CELLS)

The agreement between BC-6800-BF and OM was assessed with Pearson's correlation coefficient (r), Passing–Bablok regression, Bland–Altman plot analysis, and Spearman's rank correlation. Slope and intercept of Passing–Bablok regression were calculated with their 95% confidence interval (95% CI) to check statistical significant proportion or systematic difference between methods. In Bland–Altman plot, absolute differences were plotted against the results of the OM. A significant bias is appreciated when the 95% CI of mean of differences did not contain the value.

Diagnostic concordance

Diagnostic concordance of BC-6800-BF mode compared to OM was evaluated with receiver operating characteristics (ROC) curves.

Using the counting chamber as reference method, sensitivity, specificity, and agreement (proportion of true-negative and true-positive samples correctly identified by BC-6800-BF) were calculated. Youden index (i.e., the maximum value of sensitivity + specificity) was used to determine the optimal cutoff value to discriminate samples in negative and positive groups.

Analysis of sensitivity and specificity was performed also using the diagnostic thresholds of cellularity in PF and AF fluids as defined by the CLSI

document H56-A: for all samples WBC-BF or TC-BF >1000 × 10^6 cells/L; for AFs PMN ≥250 × 10^6 cells/L; for PFs PMN or MN ≥50% [1].

Cytologic examination

Samples were fixed with Cytolyt hemolytic and preservative solution (Hologic, Inc., Marlborough, MA, USA). Cells were spun at 100 ***g*** and the sediment transferred in PreservCyt solution (Cytyc Corporation, Marlborough, MA, USA) to be processed with the T5000 automated processor according to the manufacturer's recommendation (Hologic Co.). The resulting slide was fixed in 95% ethanol and stained with Papanicolaou method. The remaining material was stored in PreservCyt solution for possible later use in the preparation of additional slides for further investigations including immunocytochemistry, molecular diagnosis, and flow cytometry. All specimens were classified as 'adequate' or 'inadequate' cytological material by a cytopathologist. Samples with 'adequate' classification were further categorized as: (i) negative, (ii) atypical, (iii) suspicious, or (iv) malignant [20].

Cytological examination was required by the clinician only for 69 samples (63 samples were negative and 6 positive).

Statistical analysis

Statistical analysis was performed using ANALYSE-IT software version 3.80 (Analyse-it software Ltd; Leeds, UK), and CLSI STATIS-PRO software version 3.0 (Clinical and Laboratory Standard Institute, Wayne, PA, USA).

RESULTS

Precision

The within-run imprecision on BC-6800-BF was comprised between 11.9% (mean value 14 × 10^6 cells/L and 95% confidence interval from 1 to 3) and 3.7% (mean value 1.666 × 10^6 cells/L and 95% CI from 41 to 127) for TC-BF#, and between 18.0% (mean value 14 × 10^6 cells/L and 95% CI from 2 to 5) and 3.6% (mean value 1656 × 10^6 cells and 95% CI from 39 to 120) for WBC-BF, respectively. Table 1 shows also the imprecision data for PMN and MN absolute count.

Table 1. Low limit quantification, linearity, and imprecision within run of different BC-6800-BF parameters (total cell [TC], leukocytes [WBC], polymorphonuclear cells [PMN], and mononuclear cells [MN]): mean value, standard deviation (DS), coefficient of variation (CV)

	LoQ Cells (10^6 cells/L)	Linearity Range (10^6 cells/L)	Linearity Coefficient (r^2)	Regression	Imprecision within run			
					Mean ± DS × 10^6 cells/L	Mean ± DS × 10^6 cells/L	Mean ± DS × 10^6 cells/L	Mean ± DS × 10^6 cells/L
					CV	CV	CV	CV
TC-BF	4	8–3965	0.99	$y = 1.03x - 16.2$	14 ± 3	53 ± 4	430 ± 18	1666 ± 62
					11.9%	7.8%	4.1%	3.7%
WBC-BF	3	8–3936	0.99	$y = 1.03x - 18.0$	14 ± 3	53 ± 4	370 ± 16	1656 ± 59
					17.0%	7.1%	4.3%	3.6%
PMN#	22	24–3063	0.99	$y = 1.02x - 3.8$	22 ± 2	40 ± 6	132 ± 8	766 ± 31
					11.7%	15.2%	4.8%	4.0%
MN#	12	18–2279	0.99	$y = 1.04x - 12.7$	12 ± 2	45 ± 4	326 ± 17	884 ± 38
					16.7%	9.4%	5.1%	4.3%

Sample carryover

Carryover was negligible, being 0.00% for TC-BF and WBC-BF counts.

Limit of blank and limit of detection

LoB was 1×10^6 cells/L for both TC-BF and WBC-BF counts. LoD was 3×10^6 cells/L for both TC-BF and WBC-BF counts.

Functional sensitivity (limit of quantitation)

The estimated LoQ was 4×10^6 cells/L for TC-BF count and 3×10^6 cells/L for WBC-BF count. Table 2 shows also the LoQ for PMN and MN absolute count.

Linearity

The best-fitting model was linear regression for both TC-BF count ($y = 1.03x - 16.2$ $r^2 = 0.99$) and WBC-BF count ($y = 1.03x - 18.0$; $r^2 = 0.99$). Table 1 shows also linearity of PMN-BF and MN- BF.

Bias between mean values of WBC-BF or TC-BF and their expected values was within ±10% in the range of 8–3965 $\times 10^6$ cells/L and 8–3936 $\times 10^6$ cells/L, respectively.

Comparison methods

Cell counting agreement between BC-6800-BF and OM was evaluated on 144 BF samples (99 AF and 45 PF), with a total cellularity ranging from 11 to 7760 $\times 10^6$ cells/L (median: 329 $\times 10^6$ cells/L, 95% CI from 295 to 423).

The total cellularity of AF ranged from 11 and 7760 $\times 10^6$ cells/L (median: 301 $\times 10^6$ cells/L, 95% CI from 267 to 375). The total cellularity of PF was between 54 and 7707 $\times 10^6$ cells/L (median: 616 $\times 10^6$ cells/L, 95% CI from 328 to 1307).

The positive samples according to the CLSI document H56-A [1] was 40/144 (28%) with OM analysis.

Comparison of total cell count and WBC between BC-6800-BF and OM show, respectively, the following: Pearson's correlation $r = 0.99$ ($P < 0.0001$) and $r = 0.97$ ($P < 0.0001$); Passing–Bablok regression $y = 1.04x - 1.17$ (95% CI of slope 1.00–1.09; and intercept −13.30 to 11.49) and $y = 1.16x + 47.97$ (95% CI of slope 1.01–1.24; intercept 28.38–73.53); and finally Bland–Altman Bias 33.7 cells (95% CI from −3.8 to 71.1) and 167.4 cells (95% CI from 110.5 to 224.2) (Table 1).

Table 1 lists correlations between BC-6800-BF and OM for the different cell populations, with Pearson's correlation coefficients always within 0.62 and 0.99 (all $P < 0.0001$).

In all comparison tests, a linearity of correlation by Kolmogorov–Smirnov CUSUM test was confirmed.

The number of HF-BF was correlated with the number of mesothelial and/or cancer cells identified with OM. The resulting Spearman's correlation was $r = 0.60$ ($P < 0.0001$) for both absolute values or percentage.

Diagnostic concordance

As regards the diagnostic concordance of BC-6800-BF for all 144 samples, the AUC was 0.99 (95% CI 0.99–1.00; $P < 0.0001$) for TC-BF, 0.99 (95% CI 0.98–1.00; $P < 0.0001$) for WBC-BF.

PMN# in the 99 AFs showed the AUC of 0.98 (95% CI 0.96–1.00; $P < 0.0001$). When a cutoff of 250 $\times 10^6$ cells/L [1] was used, the diagnostic agreement compared to OM was 94% (sensitivity 0.79 and specificity 0.97). More specifically, 93 of 99 samples were correctly classified (3 false-positive samples and 3 false-negative samples) (Table 3).

An instrument-specific threshold of 170 $\times 10^6$ cells/L could hence be identified with ROC analysis for PMN#. When this threshold was used, the parameter exhibited diagnostic concordance of 92%, while specificity decreases from 0.97 to 0.92 but sensitivity improves from 0.79 to 0.93 (Table 3).

Concerning diagnostic concordance of BC-6800-BF in the 45 PFs, the AUC was 0.79 (95% CI 0.65–0.92; $P < 0.0001$) for MN% and 0.93 (95% CI 0.86–1.01; $P < 0.0001$) for PMN%. Table 3 shows diagnostic concordance, sensitivity, and specificity compared to OM.

DIFF scattergram evaluation

Six samples (three AFs and three PFs) showed abnormal DIFF scattergram (Figure 2) and HF-BF always >50 $\times 10^6$ cells/L. Therefore, the observation of DIFF scattergram morphology itself made necessary a reassessment of samples by OM. All these samples

Table 2. Pearson's Correlation, Passing–Bablok regression, and Bland–Altman Bias for different cell categories with optical microscopy *vs.* BC-6800-BF

	All samples			Ascitic fluids			Pleural fluids		
	Pearson correlation (r); P value	Passing–Bablok regression (95% CI slope and intercept)	Bias Bland–Altman (95% CI)	Pearson correlation (r); P value	Passing–Bablok regression (95% CI slope and intercept)	Bias Bland–Altman (95% CI)	Pearson correlation (r); P value	Passing–Bablok regression (95% CI slope and intercept)	Bias Bland–Altman (95% CI)
TC-BF	0.99; <0.0001	$y = 1.04x - 1.17$ (Slope: 1.00 to 1.09, Intercept: −13.30 to 11.49)	33.7 (−3.8 to 71.1)	0.99; <0.0001	$y = 1.04x + 0.35$ (Slope: 0.96 to 1.10, Intercept: −14.55 to 16.99)	28.0 (−14.2 to 70.2)	0.99; <0.0001	$y = 1.03x - 0.88$ (Slope: 0.98 to 1.13, Intercept: −30.15 to 29.37)	16.4 (−8.3 to 53.7)
WBC-BF	0.97; <0.0001	$y = 1.16x + 47.97$ (Slope: 1.01 to 1.24, Intercept: 28.38 to 73.53)	167.4 (110.5 to 224.2)	0.97; <0.0001	$y = 1.17x + 53.35$ (Slope: 1.04 to 1.30, Intercept: 29.71 to 80.61)	148.7 (87.0 to 210.3)	0.98; <0.0001	$y = 1.16x + 29.87$ (Slope: 1.01 to 1.29, Intercept: −15.02 to 84.35)	208.5 (83.8 to 333.1)
PMN#	0.98; <0.0001	$y = 1.05x + 14.37$ (Slope: 0.90 to 1.22, Intercept: 8.76 to 21.14)	11.5 (−20.4 to 43.3)	0.99; <0.0001	$y = 1.11x + 10.40$ (Slope: 0.96 to 1.41, Intercept: 7.24 to 18.77)	20.9 (−9.2 to 51.0)	0.98; <0.0001	$y = 0.86x + 26.00$ (Slope: 0.72 to 1.22, Intercept: 6.34 to 36.09)	−9.2 (−88.7 to 70.3)
PMN%	0.84; <0.0001	$y = 0.87x + 5.96$ (Slope: 0.78 to 1.01, Intercept: 3.93 to 7.33)	4.6 (2.2 to 6.9)	0.87; <0.0001	$y = 0.92x + 6.58$ (Slope: 0.79 to 1.29, Intercept: 3.73 to 8.98)	5.3 (2.9 to 7.6)	0.75; <0.0001	$y = 0.81x + 4.47$ (Slope: 0.68 to 0.98, Intercept: 2.74 to 7.76)	3.1 (−2.4 to 8.7)
MN1#	0.98; <0.0001	$y = 1.27x + 22.67$ (Slope: 1.21 to 1.44, Intercept: 5.0 to 50.79)	161.7 (114.5 to 209.0)	0.95; <0.0001	$y = 1.27x + 30.66$ (Slope: 1.17 to 1.51, Intercept: 6.09 to 54.75)	128.9 (78.3 to 179.4)	0.98; <0.0001	$y = 1.25x + 19.25$ (Slope: 1.13 to 1.56, Intercept: −22.48 to 94.73)	234.1 (131.4 to 336.7)
MN1%	0.62; <0.0001	$y = 0.68x + 40.15$ (Slope: 0.51 to 0.87, Intercept: 28.24 to 52.99)	24.4 (21.1 to 27.7)	0.54; <0.0001	$y = 0.51x + 53.66$ (Slope: 0.34 to 0.78, Intercept: 35.48 to 66.06)	27.1 (23.0 to 31.2)	0.78; <0.0001	$y = 0.83x + 25.77$ (Slope: 0.67 to 1.02, Intercept: 16.40 to 37.35)	18.4 (13.4 to 23.4)

(continued)

Table 2. (Continued)

	All samples			Ascitic fluids			Pleural fluids		
	Pearson correlation (r); P value	Passing–Bablok regression (95% CI slope and intercept)	Bias Bland–Altman (95% CI)	Pearson correlation (r); P value	Passing–Bablok regression (95% CI slope and intercept)	Bias Bland–Altman (95% CI)	Pearson correlation (r); P value	Passing–Bablok regression (95% CI slope and intercept)	Bias Bland–Altman (95% CI)
MN2#	0.98; <0.0001	$y = 1.23x - 8.80$ (Slope: 1.12 to 1.33, Intercept: −25.71 to 7.15)	98.2 (52.6 to 143.8)	0.96; <0.0001	$y = 1.19x - 0.72$ (Slope: 1.07 to 1.33, Intercept: −19.22 to 18.15)	76.3 (33.4 to 119.3)	0.98; <0.0001	$y = 1.23x - 24.79$ (Slope: 1.04 to 1.50, Intercept: −43.41 to 71.65)	146.3 (32.8 to 259.9)
MN2%	0.64; <0.0001	$y = 0.72x + 27.71$ (Slope: 0.60 to 0.83, Intercept: 18.64 to 38.10)	9.2 (6.0 to 12.5)	0.64; <0.0001	$y = 0.62x + 36.58$ (Slope: 0.40 to 0.78, Intercept: 22.17 to 54.89)	11.0 (7.2 to 14.8)	0.66; <0.0001	$y = 0.79x + 22.35$ (Slope: 0.66 to 0.97, Intercept: 7.45 to 32.78)	5.3 (−1.1 to 11.8)

Values of in percentage (%) and absolute (#) count of total cell (TC), leukocytes (WBC), polymorphonuclear cells (PMN), and mononuclear cells (MN).

Table 3. ROC Analysis of different BC-6800-BF parameters in body fluid

	AUC (95% CI); *P*-value	CUTOFF # (10^6cells/L)	Diagnostic agreement	Sensitivity	Specificity
TC-BF All fluid	0.99 (0.99–1.00); <0.0001	≥1000*	97% (2 false-positive samples and 2 false-negative samples)	0.94	0.98
WBC-BF all fluid	0.99 (0.98–1.00); <0.0001	≥1000*	96% (6 false-positive samples)	1.00	0.95
PMNAscitic fluid	0.98 (0.96–1.00); <0.0001	≥250*	94% (3 false-positive samples and 3 false-negative samples)	0.79	0.97
		≥170†	92% (7 false-positive samples and 1 false-negative sample)	0.93	0.92
	AUC (95% CI); *P*-value	CUTOFF %	Diagnostic agreement	Sensitivity	Specificity
MN pleural fluid	0.79 (0.65–0.92); <0.0001	≥50%*	53% (21 false-positive samples)	1.00	0.30
		≥70%†	73% (11 false-positive samples and 1 false-negative sample)	0.93	0.63
PMN pleural fluid	0.93 (0.86–1.01); <0.0001	≥50%*	91% (2 false-positive samples and 2 false-negative samples)	0.78	0.94
		≥33%†	82% (8 false-positive samples)	1.00	0.78

TC, total cell; WBC, leukocytes; PMN, polymorphonuclear cells; MN, mononuclear cells.
*Cutoff suggested in CLSI document H56-A [1].
†Instrumental specific cutoff by ROC Analysis and Youden index.

showed the presence of neoplastic cells confirmed by cytologic examination.

Sample A (Figure 2a,b) was from a patient with a marginal lymphoma. Cytological examination showed the presence of atypical lymphocytes (B lymphocytes: CD19+, CD20+, CD5−, CD10−, CD23−, and restriction to the surface lambda light chain), according to a neoplastic lymphoproliferative disease.

Sample B (Figure 2c,d) was positive for epithelial malignant cells which were cytokeratin (CK) 7 positive as well as calretinin, Wilms tumor protein-1 (WT-1), and MOC3 negative.

Sample C (Figure 2e,f) was positive for metastatic cells from an undifferentiated ovarian carcinoma (Antigen Clone Ber-EP4 epithelial-related and antigen clone MOC31 positive and calretinin negative).

Samples D (Figure 2g,h) and F (data no showed) had the same subject with diagnosis of epithelioid mesothelioma. Cytological examination showed mesothelial proliferation with atypical cells: WT-1 positive and thyroid transcription factor (TTF-1) negative.

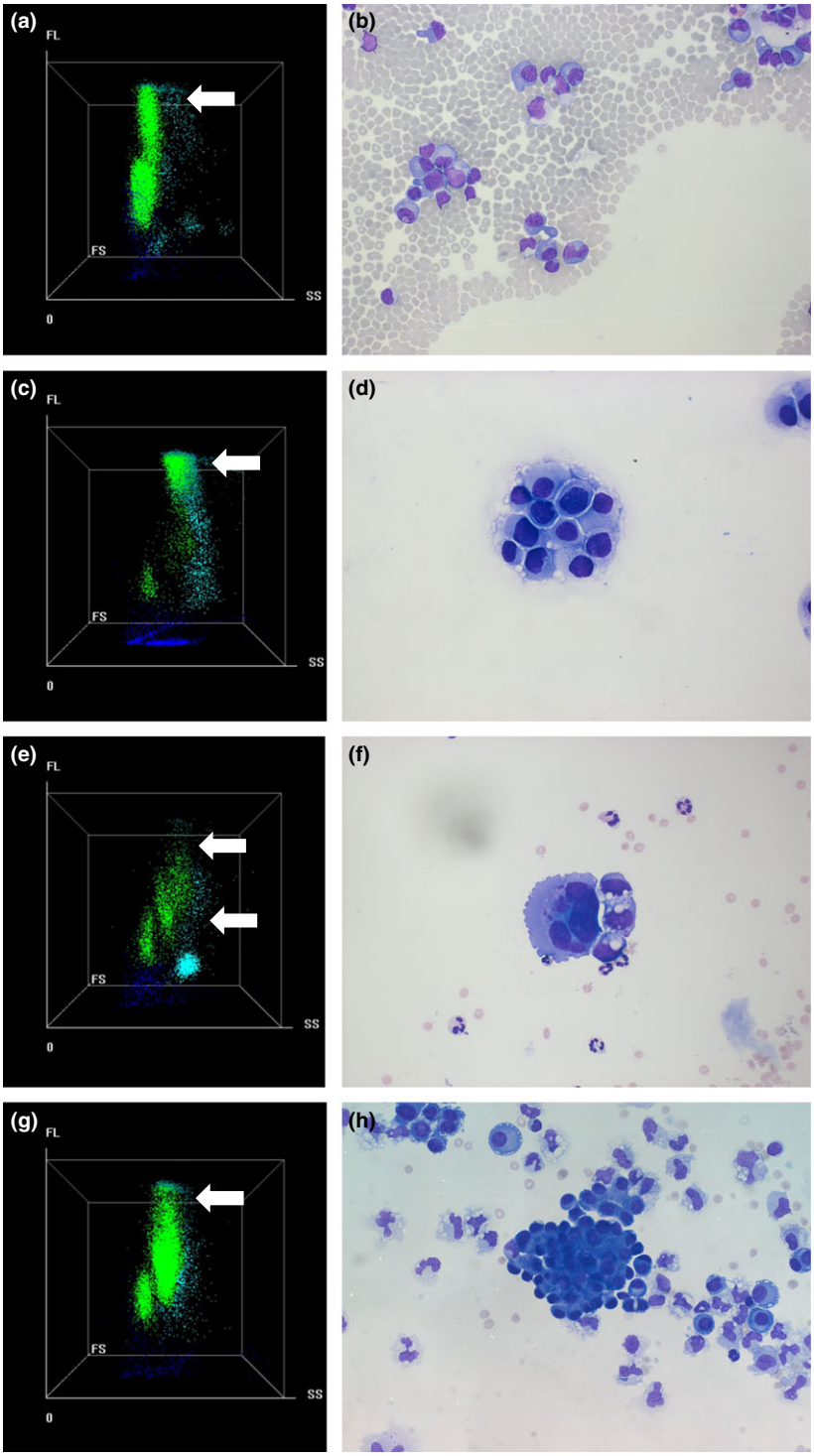
Sample E cytological examination showed the presence of small lymphocytes (B lymphocytes: CD19+, CD20+, CD5+, CD10−, CD23−, and with light chain restriction) (data not shown).

DISCUSSION

Although OM analysis is still considered the reference method for cellular analysis of body fluid samples, clinical laboratories are searching to replace manual techniques with automation, in order to provide clinicians with cost-effective, rapid, and accurate results.

This study evaluates the analytical performance of BC-6800-BF for ascitic and pleural samples analysis, but not for other BFs. For example for cerebrospinal fluid, synovial fluids, pericardial fluid, etc., in agreement with guidelines, dedicated studies are necessary [1, 13].

Data showed a negligible sample carryover, a clinically usable LoB (TC-BF and WBC-BF of 1.0×10^6 cells/L) and LoD (TC-BF and WBC-BF of 3.0×10^6 cells/L) as well as extended linearity covering cell counts in the majority of body fluid samples. LoQs were also excellent: particularly, a CV of 20% was attained at 4×10^6 cells/L for TC-BF and 3×10^6 cells/L for WBC-BF. PMN and MN showed under the same conditions LoQs of 12×10^6 cells/L and 22×10^6 cells/L, respectively. Among published studies, similar results of LoQ for the WBC count



were only reported using the UF1000i urine mode (CV = 16% at 3.5×10^6 cells/L) [10–12] or Sysmex XN (CV = 20% at 5×10^6 cells/L) [8]. This verification study confirmed the manufacturer's performances previously described as requested by ICSH guideline in 2014 [13].

The comparison between BC-6800-BF and OM showed a good agreement for total nucleated cell and

Figure 2. Ascitic and pleural fluid samples with abnormal DIFF scattergrams showed in three-dimensional scattergram (3D). (a) Sample A in the DIFF scattergram shows the MN area very close to the HF-BF area, with inefficient discrimination between MN and HF-BF (highlighted with an arrow) (TC-BF: 5963 × 10^6 cells/L; PMN: 4.6%; MN: 95.4%; HF-BF: 226 × 10^6 cells/L by BC-6800-BF). (b) Morphological cells feature abnormal scattergrams of sample A by optical microscopy (OM) (400× magnification) on cytospin-stained May–Grunwald–Giemsa (MGG). Differential count shows neutrophils 3%; lymphocytes 55%; macrophages 9%; mesothelial cells 10%; other neoplastic/atypical cells 23%; and OM-TC 5867 × 10^6 cells/L. Cytologic examination was positive for a lymphoproliferative disease. (c) Sample B in the DIFF scattergram shows the MN area very close to the HF-BF area (highlighted with an arrow), with a prevalence of HF-BF (TC-BF: 5445 × 10^6 cells/L; PMN: 26.2%; MN: 73.8%; HF-BF: 4067 × 10^6 cells/L). (d) Morphological cells feature abnormal scattergrams of sample B by OM (400× magnification) on cytospin-stained MGG. Differential count shows neutrophils 2%; lymphocytes 8%; other neoplastic/atypical cells 90%; and OM-TC 5120 × 10^6 cells/L. Cytologic examination was positive for malignant tumor cells. (e) Sample C: in the DIFF scattergram, the MN area is very close to the PMN area and the HF-BF area, with inefficient discrimination between them (highlighted with an arrow) (TC-BF: 1634 × 10^6 cells/L; PMN: 43.3%; MN: 56.7%; HF-BF:76 × 10^6 cells/L). (f) Morphological cells feature abnormal scattergrams of sample C by OM (400× magnification) on cytospin-stained MGG. Differential count shows neutrophils 59%; lymphocytes 7%; monocytes 3%; macrophages 18%; other neoplastic/atypical cells 12%; and OM-TC 1056 × 10^6 cells/L. Cytologic examination was positive for metastatic cells from an undifferentiated ovarian carcinoma. (g) Sample D in the DIFF scattergram shows the MN area very close to the HF-BF is with inefficient discrimination between MN and HF-BF (highlighted with an arrow) (TC-BF: 7295 × 10^6cells/L; PMN: 3.4%; MN: 96.6%; HF-BF: 350 × 10^6cells/L). (h) Morphological cells feature abnormal scattergrams of sample D by OM (400× magnification) on cytospin-stained MGG. Differential shows lymphocytes 15%; monocytes 62%; mesothelial cells 3%; other neoplastic cells 20%; and OM-TC 6973 × 10^6 cells/L. Cytologic examination was positive for atypia in mesothelial proliferation.

WBC count as well as for the differential count (Table 1). The slight positive bias observed, with the exception of TC-BF and PMN absolute and percent count, did not seemingly compromise the ability of BC-6800-BF to correctly categorize abnormal WBC counts and their differential counts in proper clinical category as indicated in CLSI document H56-A [1]. Indeed, the diagnostic performance of WBC-BF and MN showed an AUC of 0.99 ($P < 0.0001$) and AUC of 0.79 ($P < 0.0001$), respectively, and standard cell cut-off [1] showed a sensitivity 1.00 for both (Table 3).

The overall performance of the MN parameters, both as a percentage and in absolute value, is seemingly lower, showing overestimation (Table 2). This is due to MN-BF that includes a variety of cells, namely lymphocytes, monocytes, and macrophages.

In pleural fluids, the MN% parameter is characterized by a modest diagnostic performance, with an AUC that is much lower than those of PMN% (i.e., 0.79 *vs.* 0.93). Introducing an instrument-specific cut-off (i.e., ≥70%), the diagnostic agreement for MN% in pleural fluids could be improved. Conversely, in ascitic fluids, PMN# at conventional threshold (250 × 10^6cells/L) [1] showed a better diagnostic performance which, in the same way as above, could be further improved by introducing an instrument-specific cutoff (i.e., ≥170 × 10^6cells/L) (Table 3) as also suggested by CLSI document H56-A [1]. Four misclassified samples as false negative obtained after the application of conventional or instrument-specific threshold showed abnormalities in DIFF scattergrams. In these samples, the microscopic review showed cellular degeneration and other nonpathological morphological abnormalities (such as cluster cells, erythrophage, lipophage, siderophage, macrophage with cytoplasmic inclusion, etc.). This confirms the need to perform a microscopic review of all samples with abnormal scatterplot distributions.

The results suggest that the use of BC-6800-BF may be a reliable strategy to perform cell and differential counts in ascitic and pleural fluids. In particular, BC-6800-BF may be useful for initial screening on these BFs in urgent settings or outside the routine activity, when the availability of skilled and trained personnel cannot be assured. Moreover, analysis is rapid (i.e., <3 min) and does not require pretreatment; this may be effective to save time and manual labor in clinical laboratories.

In ascitic and pleural fluids, BC-6800-BF showed sensitivity and specificity equal and/or sometimes superior to the other analyzers with BF platforms [2, 6, 8, 10, 12, 21, 22].

In conclusion, despite the excellent analytical performance observed in our study, BC-6800-BF cannot completely replace manual microscopy so far. In routine work, the more accurate and systematic evaluation of DIFF scattergram and of the HF-BF parameter count can help to do a reflex test for microscopic review. The use of BC-6800-BF may allow to replace routine optical counting, except for samples displaying abnormal cell counts (i.e., HF-BF >50 cells $\times$ 10^6/L) and/or abnormal DIFF scattergram.

CONFLICT OF INTEREST

The authors declared no potential conflict of interests with respect to the research, authorship, and/or publication of this article.

FUNDING

The authors received no financial support for the research, authorship, and/or publication of this article.

REFERENCES

1. CSLI. Body Fluid Analysis for Cellular Composition; Approved Guidelines. CLSI document H56-A. Wayne, PA: Clinical and Laboratory Standard Institute; 2006.
2. De Jonge R, Brouwer R, de Graaf MT, Luitwieler RL, Fleming C, de Frankrijker- Merkestijn M, Sillevis Smitt PA, Boonstra JG, Lindemans J. 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.
3. Runyon BA, AASLD Practice Guidelines Committee. Management of adult patients with ascites due to cirrhosis: an update. Hepatology 2009;49:2087–107.
4. Light RW, Macgregor MI, Luchsinger PC, Ball WC. Pleural effusion: the diagnostic separation of transudates and exudates. Ann Intern Med 1972;77:507–13.
5. Aune MW, Becker JL, Brugnara C, Canfield W, Dorfman DM, Fiehn W, Fischer G, Fitzpatrick P, Flaming TH, Henriksen HK, Kunicka JE, Lackner KJ, Minchello E, Mullenix PA, Myers M, Petersen A, Ternstrom W, Wilson SJ. Automated flow cytometric analysis of blood cells in cerebrospinal fluid. Am J Clin Pathol 2004;121:690–700.
6. De Jonge R, Brouwer R, van Ryn M, Van Acker BA, Otten HJ, Lindemans J. Automated analysis of pleural fluid total and differential leukocyte counts with the Sysmex XE-2100. Clin Chem Lab Med 2006;44:1367–71.
7. Zimmermann M, Ruprecht K, Kainzinger F, Heppner FL, 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.
8. Fleming C, Brouwer R, Lindemans J, de Jonge 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.
9. Walkert J, Nelson LD, Dunphy BW, Anderson DM, Kicklert S. Comparative evaluation of the Iris iQ200 body fluid module with manual hemocytometer count. Am J Clin Pathol 2009;131:333–8.
10. Buoro S, Gustinetti R, Dominoni P, Alessio MG, Filisetti P, Lucà MG, Fagiuoli S, Ottomano C, Lippi G. Analytical evaluation of Sysmex UF-1000i for flow cytometric analysis of peritoneal fluid. Clin Biochem 2012;45:1263–5.
11. Buoro S, Ottomano C, Apassiti Esposito S, Gherardi P, Alessio MG, Crippa A, Farina C, Raglio A, Lippi G. Analytical and Clinical evaluation of Sysmex UF1000i for automated screening of cerebrospinal fluids. J Med Biochem 2013;32:1–6.
12. Fleming C, Brower R, van Alphen A, Lindemans J, de Jonge R. UF-1000i: validation of the body fluid mode for counting cells in body fluids. Clin Chem Lab Med 2014;52:1781–90.
13. Bourner G, De La Salle B, George T, Tabe Y, Baum H, Culp N, Keng TB. ICSH Guidelines for the verification and performance of automated cell counters for body fluids. Int J Lab Hematol 2014;36:598–612.
14. CLSI. Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods; Approved Standard, 2nd edn. CLSI document H20-A2.Wayne, PA: Clinical and Laboratory Standards Institute; 2007.
15. 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 J Lab Hematol 2014;36:613–27.
16. CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline, 3rd edn. CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
17. Broughton P. Carry-over in automatic analyzers. J Autom Chem 1984;6:94–5.
18. CSLI. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline, 2nd edn. CLSI document EP17-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
19. CSLI. 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.
20. Koss LG, Melamed MR. Diagnostic Cytology and its Histopathologic Bases, Vol 2, 5th edn. Philadelphia, PA: Lippincott Williams & Wilkins (eds); 2006.
21. 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.
22. Lippi G, Cattabiani C, Benegiamo A, Gennari D, Pavesi F, Caleffi A, Pipitone S. Evaluation of white blood cell count in peritoneal fluid with five different hemocytometers. Clin Biochem 2013;46:173–6.