Automated Cerebrospinal Fluid Cell Counts Using the New Body Fluid Mode of Sysmex UF-1000i

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Abstract: *Background:* We evaluated the new body fluid module on Sysmex UF1000-i (UF1000i-BF) for analysis of white blood cell (WBC) and red blood cell (RBC) in cerebrospinal fluid (CSF). *Methods:* WBC and RBC counting were compared between UF1000i-BF and Fuchs–Rosenthal counting chamber in 67 CSF samples. This study also included the evaluation of between-day precision, limit of blank (LoB), limit of detection (LoD), functional sensitivity (limit of quantitation, LoQ), carryover and linearity. Diagnostic agreement for differentiation between normal and increased WBC counts ($\geq 5.0 \times 10^6/\text{L}$) was also assessed. *Results:* The agreement between UF1000i-BF and manual WBC counts was optimal in all CSF samples ($r = 0.99$; $y = 1.05x + 0.09$). A modest overestimation was noticed in samples with $\text{WBC} < 30 \times 10^6/\text{L}$ ($r = 0.95$; $y = 1.21x - 0.15$). A good agreement was observed for RBC counts ($r = 0.98$; $y = 1.15x + 0.55$), particularly in samples with RBC

$\geq 18 \times 10^6/\text{L}$ ($r = 0.98$; $y = 1.01x + 8.90$). Between-day precision was good, with coefficient of variations (CVs) lower than 7.2% for both WBC and RBC. The LoBs were 0.1×10^6 WBC/L and 1.2×10^6 RBC/L, the LoDs were 0.7×10^6 WBC/L and 5.5×10^6 RBC/L, the LoQs were 2.4×10^6 WBC/L and 18.0×10^6 RBC/L, respectively. Linearity was excellent ($r = 1.00$ for both WBC and RBC). Carryover was negligible. Excellent diagnostic agreement was obtained at 4.5×10^6 WBC/L cut-off (sensitivity, 100%; specificity, 97.4%). *Conclusion:* The UF1000i-BF provides rapid and accurate WBC and RBC counts in clinically relevant values of CSF cells. The use of UF1000i-BF may hence allow to replace routine optical counting, except for samples displaying abnormal WBC counts or abnormal scattergram distribution, for which differential cell counts may still be required. *J. Clin. Lab. Anal.* 30: 381–391, 2016. © 2015 Wiley Periodicals, Inc.

Key words: cerebrospinal fluid; automated cell counting; white blood cells; red blood cells; body fluids Sysmex UF-1000i-BF

INTRODUCTION

Cellular analysis in cerebrospinal fluid (CSF) is an essential part of diagnosis and follow-up of many central nervous system (CNS) disorders (1,2). An increased number of white blood cell (WBC) in CSF samples (i.e., $> 5 \times 10^6/\text{L}$ in adults, $> 7 \times 10^6/\text{L}$ in children, or $> 27 \times 10^6/\text{L}$ in neonates) is a cornerstone for diagnosing meningitis,

encephalitis, neurologic diseases, and leukemic CSF infiltrations (3). Normal CSF samples contain no red blood cells (RBC), whereas an increased RBC count reflects the presence of subarachnoid or intracerebral hemorrhage, and is also helpful to rule out a traumatic spinal tap or surgical procedure bleeding as the underlying cause of an elevated WBC count (3).

Although optical microscopy is still the reference method for cellular analysis of CSF, this technique has several drawbacks such as high interobserver variability and poor reproducibility, and is also labor-intensive and requires trained laboratory staff, especially in emergencies (3,4). A number of automated cell counters have hence been evaluated for applicability in CSF analysis (5–9). These studies revealed that automated cell counts in CSF

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may be of limited value due to large imprecision and poor accuracy, especially in samples with less than $50 \text{ WBC} \times 10^6/\text{L}$. To overcome these limitations, manufacturers have designed a new generation of analyzers with specific applications for body fluid analysis (10–20).

The Sysmex UF1000i (Sysmex Co., Kobe Japan) is an automated analyzer encompassing fluorescent flow cytometry and hydrodynamic focusing to identify and enumerate cells and formed particles in urine. Although previous studies showed that UF1000i exhibits good performance for counting nucleated cells in CSF and peritoneal fluid (21–23), a specific software module for RBC, WBC, and TNC (total nucleated cells) counting in body fluid samples (UF1000i-BF) has recently been developed (24), but no information for CSF analysis exists to the best of our knowledge.

This study was hence aimed to validate the analytical and diagnostic performances of UF1000i-BF for cellular analysis in CSF samples, in accord with the reference documents published by the Clinical Laboratories Standard Institute (CLSI) (3) and, recently, by the International Council for Standardization in Hematology (ICSH) (25).

MATERIALS AND METHODS

CSF Samples

The study included 67 consecutive CSF samples collected from adult patients (older than 16 years), submitted to the local laboratory for diagnostic investigation over 1 month. All samples (37 specimens obtained from lumbar puncture, 24 from external ventricular drainage, 6 from reservoir) were collected in sterile tubes without additives, and analysis was performed within 2 hr from collection. The investigation was based on pre-existing samples, so that ethical permission and informed consent were unnecessary. The study was carried out in accordance with the declaration of Helsinki and with the terms of local legislation.

Manual Microscopy

Manual microscopic cell counting was performed in a Fuchs–Rosenthal counting chamber. RBCs were counted unstained on undiluted samples. To count WBCs, CSF samples were diluted (9:10) with Turk's solution (Carlo Erba, Italy). For each sample, cells in the entire chamber ($3.2 \mu\text{L}$) were counted at $\times 400$ magnification. WBC differential was performed by an expert technician by light microscopy at $\times 400$ magnification. The slides were prepared by cytocentrifugation of CSF samples at $100 \times g$ for 3 min (Cytospin2 Thermo Scientific, MA, USA), followed by May–Grunwald–Giemsa staining.

Sysmex UF1000i-BF Mode

As for analysis of urine samples, the UF1000i-BF employs fluorescent flow cytometry with hydrodynamic focusing to provide quantification of corpuscular elements in body fluid samples. Cells are categorized on the basis of their forward scatter light (volume), side scatter light (internal complexity), and fluorescence intensity (DNA/RNA content). The RBCs are counted in sediment channel. The WBCs and TNCs are stained with fluorescent dye and counted in bacteria channel after lysis of RBCs, by tuning the sensitivity of the channel for WBCs and other nucleated cells analysis. The data are then displayed as histograms, scattergrams, and cells number. Cells other than WBCs (i.e., mesothelial cells, macrophages, malignant nonhematopoietic cells) are reported as LC (large cells, a parameter for research purposes only) and included in the TNC count.

The UF1000i-BF uses $398 \mu\text{L}$ of body fluid sample, which is less than half of the volume required for urinalysis. The analyzer does not require any manual sample treatment and the throughput is 25 body fluid samples per hour. When switched from urine mode, the UF1000i-BF automatically performs a rinse cycle, followed by a background check, to avoid cross-contamination from urine samples. To avoid sample carryover in the BF mode, an automatic rinsing is performed before sample analysis.

Sample Carryover

Carryover was assessed on three CSF samples with a high cell count (WBC from 104 to $153 \times 10^6/\text{L}$; RBC from 770 to $822 \times 10^6/\text{L}$). Each sample was measured three times (H1, H2, H3) followed by three measurements of saline (B1, B2, B3). Percentage of carryover was calculated using the formula “carryover = $((B1-B3)/(H3-B3)) \times 100$ ” (26).

Between-Day Precision

Between-day precision was assessed by daily analysis throughout 32 days of two levels (low and high) of Sysmex UF-II control material. Mean values, standard deviation (SD), and coefficient of variation (CV) were calculated for both WBC and RBC.

LoB and LoD

The LoB and LoD were assessed according to the CLSI reference document EP17-A2 (27). LoB was determined by using nonparametric analysis as the 95th percentile value from 60 replicates of sample diluent of the UF1000i-BF (UF Pack Sed Sysmex Co., Kobe, Japan). The LoD was then assessed on 12 CSF samples diluted

with saline, to obtain very low concentrations of both WBCs and RBCs. Five replicates of each sample were tested, for a total of 60 measurements. The mean values of samples ranged from 0.7 to 5.0×10^6 WBC/L and from 2.4 to 6.7×10^6 RBC/L, respectively. The LoD was determined as the lowest WBC and RBC concentrations that could be detected above their respective LoB with probability of 95% (27).

Functional Sensitivity (Limit of Quantitation)

The functional sensitivity was assessed on ten replicates of eight samples displaying different cell values (WBC from 1.3 to 84.7×10^6 /L; RBC from 6.8 to 321.2×10^6 /L). Samples were prepared by adding WBCs and RBCs isolated from peripheral blood after treatment with HetaSep (Sigma–Aldrich, Italy) to a pool of cell-free CSF. The mean WBC count and RBC count of each sample was plotted against the CV. Functional sensitivity was mathematically determined from the power regression equation at the concentration in which the CV equals 20%. This value was defined as limit of quantitation (LoQ) (4, 27).

Linearity

For linearity testing, WBCs and RBCs obtained from peripheral blood after treatment with HetaSep were added to a pool of cell-free CSF. The concentrated sample was then serially diluted with phosphate buffered saline (PBS) to produce five aliquots in the low range of values (WBC from 0.8 to 405.0×10^6 /L, RBC from 1.9 to 970.0×10^6 /L). Each sample was measured five times consecutively. Results were plotted against the expected cell counts and linearity was evaluated according to the CLSI reference document EP06-A (28).

Comparison of Patient Samples

Comparison between UF1000i-BF and the Fuchs–Rosenthal chamber counts was evaluated using Passing–Bablok regression analysis, Pearson's correlation coefficient (r), and Bland–Altman bias plot in 67 CSF samples. For RBC counts, 11 samples were not included in the statistical analysis for the following reasons: lysis and degeneration of RBCs observed at manual microscopy (two samples), data of manual counting unavailable (five samples), RBC counts above measuring range (up to $99,999 \times 10^6$ RBC/L of UF1000i-BF [four samples]). The slope and intercept of Passing–Bablok regression were calculated with their 95% confidence interval (95% CI) to check whether statistical significance is proportional or systematically different between methods. In Bland–Altman plot, absolute differences were plotted against results of counting chamber. A

significant bias is present when the 95% CI of mean of differences does not contain the value 0.

Diagnostic Accuracy

The diagnostic accuracy of UF1000i-BF to distinguish normal from abnormal CSF samples was assessed by the area under the curve (AUC) in receiver operating characteristic (ROC) curve. Using the counting chamber as reference method, sensitivity, specificity, and agreement (proportion of true-negative and true-positive samples correctly identified by the UF1000i-BF) were calculated. The Youden index (i.e., maximum value of sensitivity + specificity) was used to assess the optimal cut-off value for discriminating samples in negative and positive groups.

Manual WBC counts were grouped into five clinically relevant categories ranging from normal (0 – 5×10^6 WBC/L) to markedly high ($>200 \times 10^6$ WBC/L; (3, 9, 11, 13). WBC counts in UF1000i-BF were then matched to these categories. Intergroups agreement between the two methods was quantified by the linear weighted kappa (κ) statistic along with the 95% CI (27, 29).

Statistical Analysis

Statistical analysis was performed using Analyse-it software version 3.80 (Analyse-it Software, Ltd., Leeds, UK), Microsoft Excel 2010, and CLSI Statis-Pro software version 3.0.

RESULTS

Sample Carryover

The carryover was negligible, being 0.00% for WBC count and never exceeding 0.13% for RBC count, respectively.

Between-Day Precision

The results of between-day imprecision are shown in Table 1. The CV values for WBC counts and RBC counts were lower than 7.2% in both levels of UF-II control material.

TABLE 1. Between-Day Precision

	Sample	Mean (10^6 /L)	SD	CV (%)
WBC	UF II control low	40.5	2.89	7.1
RBC	UF II control low	39.2	2.29	5.9
WBC	UF II control high	761.7	16.97	2.2
RBC	UF II control high	193.0	5.60	2.9

Results of between-day precision for WBC count and RBC count of Sysmex UF-1000i on two levels of Sysmex UF-II control material (low and high) analyzed daily for 32 days.

TABLE 2. Comparison Between the UF1000i-BF and Fuchs–Rosenthal Counting Chamber for WBC Counts

	<i>r</i> Value	Passing–Bablok regression		Bland–Altman difference plot	
		Slope (95% CI)	Intercept (95% CI)	Mean bias (95% CI)	95% Limits of agreement (mean bias $\pm$ 1.96 SD)
All samples (<i>n</i> = 67)	0.99	1.05(0.97–1.12)	0.09(–0.21 to 0.38)	–1.67 (–5.43 to 2.09)	–31.88 to 28.54
WBC <30 $\times 10^6$ /L (<i>n</i> = 55)	0.95	1.21(1.09–1.43)	–0.15(–0.41 to 0.10)	0.90 (0.23 to 1.58)	–3.97 to 5.78
WBC $\geq 30 \times 10^6$ /L (<i>n</i> = 12)	0.98	0.99(0.73–1.09)	–5.32(–22.13 to 18.73)	–13.46 (–35.57 to 8.65)	–81.66 to 54.74

r Value, Pearson's coefficient correlation.

LoB, LoD, and LoQ

The LoB was 0.1×10^6 /L for WBC count and 1.2×10^6 /L for RBC count, respectively. The LoD, calculated using the formula $\text{LoD} = \text{LoB} + 1.645 \times \text{SDs}$ (where SDs is the pooled standard deviations of results on low-level samples), was 0.7×10^6 /L for WBCs and 5.5×10^6 /L for RBCs, respectively. The LoQ was 2.4×10^6 /L for WBC count and 18.0×10^6 /L for RBC count, respectively.

Linearity

The best fitting model was linear regression for both WBC count ($y = 1.00x + 1.09$; $r = 1.00$) and RBC count ($y = 1.00x - 3.51$; $r = 1.00$). The bias between mean values of WBCs or RBCs and their predicted values were within $\pm 10\%$ in the ranges of $8\text{--}405 \times 10^6$ WBC/L and $19\text{--}970 \times 10^6$ RBC/L. Notably, the bias was comprised within ± 0.5 cells $\times 10^6$ /L at the lowest expected cell value (0.8×10^6 WBC/L and 1.9 RBC $\times 10^6$ /L, respectively).

Comparison on Patient Samples

The comparison of WBC and RBC counts obtained with UF1000i-BF and the reference counting chamber on patient samples are summarized in Tables 2 and 3, respectively. In counting chamber, WBC counts ranged from 0.0 to 548.6×10^6 /L and RBC counts from 0.0 to 3656.3×10^6 /L. A good agreement was observed between manual and automated WBC counts in all CSF samples ($r = 0.99$; $y = 1.05x + 0.09$; $n = 67$; Fig. 1A; Table 2). The

mean bias was -1.67×10^6 /L (Fig. 1C; Table 2). Fifty-five of the 67 CSF samples (82.1%) had a manual WBC count $<30 \times 10^6$ /L, but the agreement between the methods remained excellent in either of this subset of samples ($r = 0.95$; $y = 1.21x - 0.15$) (Fig. 1B; Table 2). However, the UF1000i-BF demonstrated a significant proportional overestimation with Passing–Bablok regression (95% CI slope: 1.09–1.43). This trend was confirmed by the Bland–Altman plot that revealed a statistically significant bias, even if rather limited, of 0.90×10^6 /L (95% CI: $0.23\text{--}1.58 \times 10^6$ /L) (Fig. 1D; Table 2). An excellent agreement was observed in the 12 samples with a manual WBC count $\geq 30 \times 10^6$ /L ($r = 0.98$; $y = 1.00x - 5.32$; mean bias -13.46×10^6 /L; Table 2).

The differential count performed on cytospin preparations showed the presence of macrophages in four samples. The agreement between UF1000i-BF and manual TNC counts in all CSF samples showed results similar to those obtained for WBCs ($r = 0.99$, $y = 1.04x + 0.17$, mean bias -1.79×10^6 /L; $n = 67$, data not shown).

For RBC counts, a good agreement between UF1000i-BF and the counting chamber was observed in all CSF samples ($r = 0.98$; $y = 1.15x + 0.55$) (Fig. 2A; Table 3). Despite the RBC counts with UF1000i-BF tended to be slightly higher compared to the counting chamber, the slope and intercept of Passing–Bablok regression did not significantly deviate from 1 and 0, respectively. The mean bias was 21.02×10^6 /L (95% CI: -14.69 to 56.72 ; Fig. 2B; Table 3). A separate analysis was performed in samples with RBC count below ($n = 28$) and above ($n = 28$) the

TABLE 3. Comparison Between the UF1000i-BF and Fuchs–Rosenthal Counting Chamber for RBC Counts

	<i>r</i> Value	Passing–Bablok regression		Bland–Altman difference plot	
		Slope (95% CI)	Intercept (95% CI)	Mean bias (95% CI)	95% Limits of agreement (mean bias $\pm$ 1.96 SD)
All samples (<i>n</i> = 56)	0.98	1.15 (0.99–1.36)	0.55(–0.32 to 2.38)	21.02 (–14.69 to 56.72)	–240.29 to 282.32
RBC <18 $\times 10^6$ /L (<i>n</i> = 28)	0.75	1.24 (0.95–3.33)	–0.13(–0.41 to 1.84)	1.12 (–0.03 to 2.27)	–4.68 to 6.93
RBC $\geq 18 \times 10^6$ /L (<i>n</i> = 28)	0.98	1.01 (0.84–1.33)	8.90(–3.05 to 26.05)	40.91 (–32.02 to 113.85)	–327.73 to 409.56

r Value, Pearson's coefficient correlation.

Fig. 1. Comparison for WBC count between the UF1000i-BF and the reference Fuchs–Rosenthal counting chamber (manual count). Left panel: Passing–Bablok regression analysis in all CSF samples ($n = 67$) (A) and in samples with WBC count $< 30 \times 10^6/L$ ($n = 55$) (B). Solid and dashed red lines indicate the regression line and 95% CI of the slope, respectively. The gray line represents the identity line ($y = x$). Right panel: Bland–Altman plot in all CSF samples ($n = 67$) (C) and in samples with WBC count $< 30 \times 10^6/L$ ($n = 55$) (D). The absolute differences between the results of WBC counts by the two methods are plotted against the WBC count of the manual reference method. Solid and dashed blue lines indicate the mean of differences (mean bias) and the 95% limit of agreement (defined as the mean bias ± 1.96 SD), respectively. The gray line represents the identity line (bias = 0). See Table 2 for the data of Passing–Bablok regression analysis and Bland–Altman plots.

Fig. 1. Continued

LoQ of UF1000i-BF ($18.0 \times 10^6/\text{L}$). No statistically significant difference was observed in samples with RBC count $\geq 18.0 \times 10^6/\text{L}$ ($r = 0.98$; $y = 1.01x + 8.90$; mean bias $40.9 \times 10^6/\text{L}$; Table 3). In samples with RBC count $< 18.0 \times 10^6/\text{L}$, a slight positive bias ($1.12 \times 10^6/\text{L}$; 95% CI: -0.03 to 2.27 ; Fig. 2D) along with a major scatter around the regression line was observed ($r = 0.75$; $y = 1.24x + 0.13$; 95% CI slope: 0.95 – 3.33 ; Fig. 2C; Table 3).

Diagnostic Accuracy

Twenty-nine of the 67 CSF samples (43.3%) had a manual WBC count $> 5.0 \times 10^6/\text{L}$ and were hence considered positive. The ROC curve analysis for WBC count yielded

an excellent AUC of 1.00 (95% CI: 0.99 – 1.00 ; $P < 0.001$; Fig. 3). At a cut-off value of $5.0 \text{ WBC} \times 10^6/\text{L}$, the agreement between UF1000i-BF and counting chamber was 97.1%. Only 1 of the 29 positive samples was classified as negative (manual count, $6.9 \times 10^6/\text{L}$ vs. UF1000i-BF, $4.6 \times 10^6/\text{L}$). Only 1 of the 38 negative samples was classified as positive (manual count, $3.1 \times 10^6/\text{L}$ vs. UF1000i-BF, $7.2 \times 10^6/\text{L}$). Therefore, sensitivity and specificity were 96.6% and 97.4%, respectively. At the optimal cut-off value of $4.5 \text{ WBC} \times 10^6/\text{L}$, the agreement between UF1000i-BF and counting chamber increased to 98.5%, with 29/29 of positive samples and 37/38 of negative samples being correctly identified. Sensitivity and specificity were 100% and 97.4%, respectively.

Fig. 2. Comparison for RBC count between the UF1000i-BF and the reference Fuchs–Rosenthal counting chamber (manual count). Left panel: Passing–Bablok regression analysis in all CSF samples ($n = 56$) (A) and in samples with RBC counts $< 18 \times 10^6/\text{L}$ ($n = 28$) (B). Solid and dashed red lines indicate the regression line and 95% CI of the slope, respectively. The gray line represents the identity line ($y = x$). Right Panel: Bland–Altman plot in all CSF samples ($n = 56$) (C) and in samples with RBC counts $< 18 \times 10^6/\text{L}$ ($n = 28$) (D). The absolute differences between the results of RBC counts by the two methods are plotted against the results of the manual reference method. Solid and dashed blue lines indicate the mean of differences (mean bias) and the 95% limit of agreement (defined as the mean bias ± 1.96 SD), respectively. The gray line represents the identity line (bias = 0). Refer to Table 3 for the data of Passing–Bablok regression analysis and Bland–Altman plots.

The manual WBC counts were grouped into five categories classified as normal ($0\text{--}5.0 \times 10^6/\text{L}$, $n = 38$), suspicious for disease ($>5.0\text{--}10.0 \times 10^6/\text{L}$, $n = 8$), mildly high ($>10.0\text{--}30.0 \times 10^6/\text{L}$, $n = 11$), moderately high ($>30.0\text{--}200.0 \times 10^6/\text{L}$, $n = 5$), and consistently high ($>200 \times 10^6/\text{L}$, $n = 5$). The corresponding WBC counts of UF1000i-BF were matched to these categories (Table 4). Intergroup agreement between manual and automated

Fig. 2. Continued

WBC counts was excellent, resulting in a weighted κ value of 0.97 (95% CI: 0.93–1.00). Percentages of agreement were 97.4% (37/38) in the category of normal WBC count; 77.8% (7/9) in the category of suspicious for disease; and 100% in the categories of mildly high (10/10), moderately high (5/5), and consistently high (5/5) WBC count, respectively.

DISCUSSION

Although optical microscopy analysis remains the reference method for cellular analysis of CSF samples, clinical laboratories are under pressure to replace manual techniques with automation, in order to provide cost-effective, rapid, and accurate results to clinicians. The unsatisfactory precision of automated analyzers at very low cell

Fig. 3. ROC curve for UF1000i-BF in distinguish normal from abnormal WBC counts ($>5.0 \times 10^6$ WBC/L; $n = 29$, 43.3%) in 67 CSF samples using the Fuchs–Rosenthal counting chamber as the reference method. Results of ROC curve analysis were AUC 1.00 (95% CI: 0.99–1.00; $P < 0.0001$); optimal cut-off value UF1000-i BF 4.5×10^6 /L; sensitivity 100%, specificity 97.4%. Of the 67 CSF samples, only one false-positive result was found.

TABLE 4. Reliability of UF1000i-BF in Classification of CSF Samples According to the Reference WBC Counts (Fuchs–Rosenthal Counting Chamber)

Group of CSF samples	WBC counting chamber ($\times 10^6$ /L)	WBC UF1000i-BF ($\times 10^6$ /L)				
		0–5	>5–10	>10–50	>50–200	>200
Normal ($n = 38$)	0–5	37	1	0	0	0
Suspicious for disease ($n = 9$)	>5–10	1	7	1	0	0
Mildly elevated ($n = 10$)	>10–50	0	0	10	0	0
Moderately elevated ($n = 5$)	>50–200	0	0	0	5	0
Highly elevated ($n = 5$)	>200	0	0	0	0	5

Data are given as the number of cases in each clinically relevant group. Numbers underlined in boldface indicate the number of cases in complete agreement by both methods in each clinically relevant group. Reliability of UF1000i-BF: weighted κ , 0.97; 95% CI: 0.93–1.00.

counts has considerably challenged their widespread use in CSF diagnostics (10–13).

In this study, we evaluated the analytical performance of Sysmex UF1000i-BF for CSF analysis, showing a neg-

ligible sample carryover, clinically usable LoB (0.1×10^6 WBC/L, 1.2×10^6 RBC/L) and LoD (0.7×10^6 WBC/L, 5.5×10^6 RBC/L), optimal between-day imprecision (with CV values lower than 7.2% for both WBC and RBC), as well as extended linearity, which covers cell counts in the vast majority of clinically meaningful CSF values. The LoQs were also excellent. More specifically, a CV of 20% was obtained at 2.4×10^6 WBC/L and 18.0×10^6 RBC/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 WBC/L) (21) or Sysmex XN1000 (CV = 20% at 5×10^6 WBC/L) (16). It is also noteworthy that our results are in accord with manufacturer's specification of UF1000i-BF for CSF measurement (LoQ = 2.0×10^6 WBC/L; 15.0×10^6 RBC/L), and even better than those reported by Fleming et al. for analysis of serous fluids (LoQ: 9.0×10^6 WBC/L and 25.0×10^6 RBC/L) (24).

The comparison between UF1000i-BF and manual microscopy showed a good agreement for WBC counting. The slight positive bias observed in samples with WBC count $<30 \times 10^6$ /L did not seemingly compromise the ability of UF1000i-BF to correctly categorize abnormal WBC counts in proper clinical category, as observed by

the excellent results of intergroup agreement between manual and automated WBC counts (Table 4). Diagnostic accuracy was also excellent. At the threshold value of $4.5 \times 10^6/\text{L}$, the UF1000i-BF proved to be highly sensitive (100%) and specific (97.4%) for differentiating normal and pathologic WBC counts ($>5.0 \times 10^6/\text{L}$). To our knowledge, similar results of diagnostic accuracy on CSF samples were reported in published studies only using Sysmex XN1000 (sensitivity = 100%, specificity 97.4%; $n = 67$) (16) and UF1000i urine mode (sensitivity = 100%, specificity = 84%; $n = 77$) (21).

The comparison between UF1000i-BF and manual method also showed a good correlation for RBC counting, despite the presence of slight positive bias along with major scatter around the regression line in samples with RBC count $<18 \times 10^6/\text{L}$. The RBC count in CSF is useful for diagnosing intracranial hemorrhage and for establishing whether an elevated WBC count may be secondary to a traumatic tap. Most hematological analyzers can reliably measure RBCs when the number is $\geq 1000 \times 10^6/\text{L}$ (4–9, 11–16), thus limiting their use in acute intracranial hemorrhage and in visually blood-contaminated samples. However, pediatric oncology protocols indicate that the presence of RBC $>10 \times 10^6/\text{L}$ may be used as an index of blood contamination (4, 28, 29, 32). Compared to this cut-off, the diagnostic accuracy of UF1000i-BF remained excellent. In particular, the AUC was 0.98 (95% CI: 0.95–1.00), with optimal cut-off value of 13×10^6 RBC/L associated with 96.3% sensitivity and 86.7% specificity, respectively.

Macrophages were only present in four samples in our study. In one case with 100% of macrophages/100 WBC, the results of UF1000i-BF (TNC = $21.0 \times 10^6/\text{L}$, RBC = $20.4 \times 10^6/\text{L}$) compared with manual chamber (TNC = $18.0 \times 10^6/\text{L}$, WBC = $9 \times 10^6/\text{L}$) demonstrated that these cells were not classified as LC by UF1000i-BF (LC = $0.6 \times 10^6/\text{L}$), thus leading to a significant overestimation of WBCs. A similar case was reported by Fleming et al. in pleural and ascites samples (24).

The UF1000i-BF does not report yeast cells and bacteria counts, and no flags are generated in the presence of these elements. Published studies on UF1000-i showed good performance of bacteria counting and yeast cells in CSF samples (21–23). Beside the clinical interest of their rapid counting in patients with suspected CNS infections, it has been reported that microorganisms can interfere with automated cell counts performed in whole blood and body fluids (33–35). In two samples included in our study, the cytospin analysis revealed the presence of bacteria. A single microorganism (*Escherichia coli* and *Staphylococcus coagulase negative*) was identified in each sample after microbiological investigations. In both cases, no flags were generated by UF1000i-BF, although WBC and RBC counts were not significantly overestimated. Interference

from bacteria should be unlikely since their morphology (small in size and with less DNA/RNA content) is highly different from blood cells. However, as observed by Fleming et al. (24), aggregation of bacteria or yeast may generate signals that are interpreted as RBCs and WBCs, thus leading to overestimation. The sample also showed abnormalities in histograms and scattergram, confirming the need to perform manual microscopic review of samples with abnormal scatterplot distributions.

In conclusion, the results of this study suggest that the use of UF1000i-BF may be a reliable strategy for performing cell counts in CSF samples. In particular, UF1000i-BF may be useful for initial screening of CSF in urgent settings or outside the routine activity, when availability of expert and trained personnel cannot be ensured. Moreover, the analysis is rapid (i.e., <3 min), does not require pretreatment of samples or separate steps for RBC counting, so that UF1000i-BF can save time and manual labor in clinical laboratories. To further improve the diagnostic usefulness for CSF analysis, the UF1000i-BF should be implemented with differential WBC counts, at least for mononuclear cells and polymorphonuclear cells, and with count (or at least flagging) of bacteria and yeasts. These parameters, when available, could offer an extraordinary and cost-effective support for differential diagnosis of patients with suspected CNS infections.

Despite the excellent analytical performance observed in our study, the UF1000i-BF cannot completely replace manual microscopy so far, and this is mainly due to the fact that the analyzer does not provide a WBC differential count. Therefore, the need to manually perform cell count differential in samples with abnormal WBC counts or with abnormal scattergram distributions remains. Malignant fluids should also be excluded from automated analysis of WBCs, since cancer cells may be present in small numbers in CSF, and even in samples with normal WBC counts.

CONFLICT OF INTEREST

None declared.

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