

**ANALYTICAL AND CLINICAL EVALUATION OF SYSMEX UF1000I FOR AUTOMATED SCREENING OF CEREBROSPINAL FLUIDS**

## ANALITIČKA I KLINIČKA EVALUACIJA UREĐAJA SYSMEX UF1000I ZA AUTOMATSKI SKRINING CEREBROSPINALNIH TEČNOSTI

Sabrina Buoro<sup>1</sup>, Cosimo Ottomano<sup>2</sup>, Sara Appassiti Esposito<sup>1</sup>, Patrizia Gherardi<sup>1</sup>, Maria Grazia Alessio<sup>1</sup>, Alberto Crippa<sup>1</sup>, Claudio Farina<sup>3</sup>, Annibale Raglio<sup>3</sup>, Giuseppe Lippi<sup>2</sup>

<sup>1</sup>Laboratorio Analisi Chimico Cliniche, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy

<sup>2</sup>Unità Operativa di Diagnostica Ematochimica, Azienda Ospedaliero – Universitaria di Parma, Italy

<sup>3</sup>Laboratorio Microbiologia e Virologia, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy

**Summary**

**Background:** We evaluated the performance of Sysmex UF-1000i for cell counting and differential cell count, as well as for assessment of bacteria load in cerebrospinal fluid (CSF), as a potential approach for the rapid screening of meningitis or bacterial encephalitis.

**Methods:** We analyzed 77 consecutive CSF samples, 34 of which (44%) displayed leukocyte count >5 white blood cell (WBC)/ $\mu$ L with optical microscopy. Results on the UF-1000i were compared with those obtained by microscopic analysis. Imprecision was evaluated by testing three CSF samples with leukocyte values between 3.5 and 28.8 WBC/ $\mu$ L in 10 replicates. Carry-over was evaluated with the Broughton formula on three CSF pools with leukocyte counts between 93.5 and 132.5 WBC/ $\mu$ L. Linearity was assessed according to CLSI document EP6-A. In the presence of bacteria, identification and antibiogram were performed with Vitex (Biomerieux), except for *Neisserie meningitidis* (ApiNH, Biomerieux). Sensitivity tests were performed with Vitex and disc diffusion.

**Results:** Optimal correlation was found between UF-1000i and optical microscopy, displaying Pearson's correlation of 0.99 and mean bias of  $-3.5$  WBC/ $\mu$ L (95% CI, from  $-7.0$  to  $0.0$  WBC/ $\mu$ L). Imprecision varied between 12 and 16%. Linearity was excellent,  $4-278$  WBC/ $\mu$ L. Carry-over was negligible. ROC analysis yielded AUC of 0.99 for both WBC and bacterial counts. The agreement at threshold  $>4$  WBC/ $\mu$ L was 0.91, with sensitivity and specificity of 1.00 and 0.84. At

**Kratak sadržaj**

**Uvod:** Ispitali smo performanse uređaja Sysmex UF1000i za brojanje ćelija i diferencijalno brojanje kao i procenu bakteriološkog opterećenja u cerebrospinalnoj tečnosti (CST), u cilju njegove potencijalne upotrebe u brzom skriningu za meningitis ili bakterijski encefalitis.

**Metode:** Analizirali smo 77 konsektivnih uzoraka CST, od kojih je u 34 (44%) broj leukocita bio  $>5$  belih krvnih zrnaca (BKZ) po  $\mu$ L, što je utvrđeno optičkim mikroskopom. Upoređeni su rezultati dobijeni na UF1000i sa onima dobijenim pomoću mikroskopske analize. Nepreciznost je određena testiranjem tri uzorka CST sa vrednostima leukocita između 3,5 i 28,8 BKZ/ $\mu$ L u 10 ponavljanja. Prenos je izračunat pomoću Broughtonove formule na osnovu tri količine CST sa brojem leukocita između 93,5 i 132,5 BKZ/ $\mu$ L. Linearnost je procenjena na osnovu dokumenta EP6-A Instituta za kliničke i laboratorijske standarde (Clinical and Laboratory Standards Institute). U slučaju prisustva bakterija, obavljena je identifikacija i izrađen antibiogram pomoću »vitexa« (Biomerieux) osim za *Neisserie meningitidis* (ApiNH, Biomerieux). Testovi osetljivosti su izvršeni pomoću »vitexa« i difuzije diska.

**Rezultati:** Utvrđena je optimalna korelacija između UF1000i i optičke mikroskopije, sa Pearsonovom korelacijom od 0,99 i srednjim odstupanjem od  $-3,5$  BKZ/ $\mu$ L (95% CI, od  $-7,0$  do  $0,0$  BKZ/ $\mu$ L). Nepreciznost je varirala 12–16%. Linearnost je bila odlična,  $4-278$  BKZ/ $\mu$ L. Prenos je bio zamearljiv. ROC analiza dala je krivu od 0,99 za BKZ i brojeve

Address for correspondence:

Prof. Giuseppe Lippi  
U.O. Diagnostica Ematochimica  
Azienda Ospedaliero-Universitaria di Parma, Via Gramsci, 14  
43126 – Parma, Italy  
Tel. 0039-0521-703050  
Fax. 0039-0521-703791  
e-mail: glippi@ao.pr.it, ulippi@tin.it

≤19 bacteria/μL cut-off, accuracy was 0.98, sensitivity 1.00 and specificity 0.97.

**Conclusions:** According to these results, CSF screening with UF-1000i seems a reliable approach in terms of instrument performance, turnaround time and overall laboratory efficiency.

**Keywords:** cerebrospinal fluid, meningitis, automation, cytofluorimeter

## Introduction

The laboratory assessment of cerebrospinal fluid (CSF) is a mainstay for diagnosis and follow-up of a large number of disorders of the central nervous system (CNS), including meningitis, infectious and noninfectious encephalitis, cancer, metastasization, carcinomatosis or meningeal lymphomatosis, subarachnoidal hemorrhage and other neurodegenerative disorders (1, 2). In urgent settings, the more useful tests for supporting the clinical decision making are indeed represented by cytometric analysis, macroscopic evaluation, measurement of proteins and the ratio between the concentration of glucose in serum and CSF (1–3).

The count and differential of cells eventually present in CSF are crucial for troubleshooting CNS disorders. In healthy conditions, the overall number of white blood cells (WBCs) in CSF is typically comprised between 0 and 27 cell/μL in neonates aged less than 1 month, between 0 and 7 cell/μL in children, and between 0 and 5 cell/μL in adults, respectively (2, 3), with a prevalence of mononuclear cells and polymorphonuclear leukocytes lower than 2%. Despite the inherent limitations (e.g., long turnaround time, low throughput, high inter-observer imprecision and need for specifically trained personnel), the gold standard for enumeration and classification of cellular elements in CSF is still represented by optical microscopy (2, 4, 5). It is now undeniable that the possibility to automate this crucial step of diagnostic workout by using hemocytometers or cytofluorimeters normally designed for blood or urine analysis would generate a large number of analytical, organizational and clinical benefits, ultimately providing faster and more analytically robust results. Recent evidence suggests, however, that analytical sensitivity and imprecision of most laboratory hemocytometers at the diagnostic thresholds of <7 WBC/μL in children and >5 WBC/μL in adults are suboptimal, or even unacceptable (6–16). As regards urine cytofluorimeters, despite the globally acceptable analytical performance in terms of linearity, carry-over and bias with optical microscopy, these instruments also revealed notable problems of analytical sensitivity and low imprecision in the presence of leukocyte aggregates (14, 15).

The UF-1000i (Sysmex Co., Kobe, Japan; commercialized in our country by Dasit SpA, Cornaredo,

bakterija. Slaganje na pragu >4 BKZ/μL bilo je 0,91, uz osetljivost i specifičnost od 1,00 i 0,84. Uz cutoff vrednost od ≤19 bakterija/μL, tačnost je bila 0,98, osetljivost 1,00 a specifičnost 0,97.

**Zaključak:** Prema ovim rezultatima, skringing CST pomoću uređaja UF1000i deluje kao pouzdan metod u smislu performansi instrumenta, srednjeg vremena trajanja procesa i ukupne laboratorijske efikasnosti.

**Ključne reči:** cerebrospinalna tečnost, meningitis, automatizacija, citofluorimetar

Italy) is an automated urine cytofluorimeter that combines fluorescence flow cytometry with semi-conductor laser and hydrodynamic focusing conductometry for differentiating different cell populations. The instrument allows simultaneous enumeration and classification of urine cells (thus including WBC, erythrocytes, epithelial and small round cells), bacteria and mycetes, as well as the identification of cylinders. In a preliminary evaluation of UF-1000i in CSFs, Nanos and Delanghe showed good performance for differential diagnosis of viral, bacterial or fungal meningitis. The aim of this study was hence to evaluate the analytical and clinical performance of this cytofluorimeter for cell count and differential, as well as for assessing bacterial load in order to establish whether it may be a reliable perspective for initial screening of CSF in patients with meningitis or bacterial encephalitis.

## Materials and Methods

The study was based on all consecutive CSFs of adult patients with suspected meningitis or encephalitis, that had been referred for routine diagnostics to the clinical chemistry laboratory of the general hospital of Bergamo, over one month. The samples, which were collected in sterile tubes containing no additives, included 23 specimens obtained from external ventricular drainage, 9 from reservoir and 45 from lumbar puncture. Results obtained on the UF-1000i according to manufacturer's specifications were compared with those of microscopic analysis by Person's correlation, Passing and Bablok regression and Bland-Altman plots. Optical microscopy was performed after cytocentrifugation of fresh samples in Nageotte chamber and staining with May-Grunwald-Giemsa reagent, in accord with current recommendations (3). Imprecision was assessed on ten replicates of three CSF samples enriched in leukocytes obtained from buffy coats of whole blood with WBC values comprised between 3.5 and 28.8 cell/μL. Carry-over was assessed on three CSF pools displaying leukocyte values between 93.5 and 132.5 WBC/μL, according to the protocol suggested by Broughton (17). Linearity was assessed according to CLSI document EP6-A (18). In the presence of bacteria, identification and antibiogram were performed with Vitex (Biomerieux, Hazelwood, MO, USA) except for *Neisserie meningitidis* (ApiNH, Biomerieux). Sensitivity tests were per-

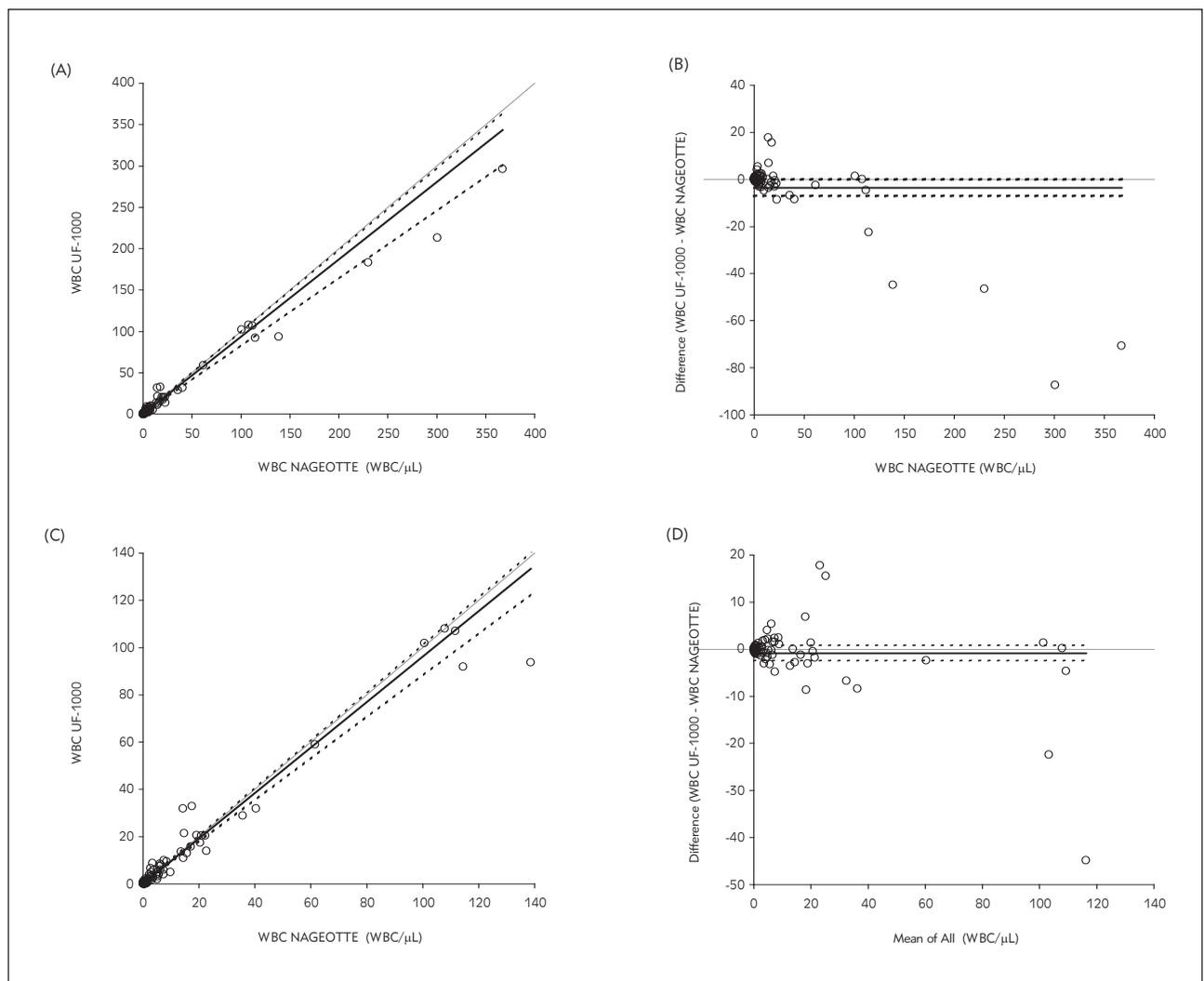
formed with Vitex and disc diffusion. The diagnostic performance as compared with the gold standards (i.e., optical microscopy and Vitex) was assessed with receiver operating characteristics (ROC) curve analysis. The statistics was carried out with Analyse-it for Microsoft Excel (Analyse-it Software Ltd, Leeds, UK). The study was performed in accordance with the Declaration of Helsinki, under the terms of all relevant local legislation.

## Results

Thirty-four of the 77 samples (44%) displayed an optical microscopy count of  $>5$  WBC/ $\mu$ L and were hence considered as positive for CNS infection according to CLSI guideline H56-A (3). An optimal correlation was found between the UF-1000i and optical microscopy, after excluding one sample due to

an excessive number of cells (i.e., 9936 WBC/mL and 10011 WBC/mL with optical microscopy and flow cytometry, respectively), since this would have misleadingly biased the comparison analysis. In the whole set of samples, the Pearson's correlation was 0.99 ( $p < 0.001$ ), the Passing and Bablok regression was  $y = 0.93 + 0.07x$ , and the mean bias calculated with Bland-Altman plots was  $-3.5$  WBC/ $\mu$ L (95% CI, from  $-7.0$  to  $0.0$  WBC/ $\mu$ L) (Figure 1A and 1B). In the 73 samples with a WBC count  $< 150$  WBC/ $\mu$ L, the Pearson's correlation was 0.98 ( $p < 0.001$ ), the Passing and Bablok regression was  $y = 0.96 + 0.00x$ , and the mean bias calculated with Bland-Altman plots was  $-0.8$  WBC/ $\mu$ L (95% CI, from  $-2.5$  to  $0.8$  WBC/ $\mu$ L) (Figure 1C and 1D).

The Imprecision of UF-1000i varied between 12% in the sample with 28.8 WBC/ $\mu$ L to 16% in that with a very limited number of WBC (i.e., 3.5



**Figure 1** Comparative analysis of white blood cell (WBC) count between optical microscopy and Sysmex UF-1000i. (A) Passing and Bablok regression and (B) Bland-Altman plot for all samples. (C) Passing and Bablok regression and (B) Bland-Altman plot for samples with  $< 150$  WBC/mL.

WBC/ $\mu\text{L}$ ) (Table I). Within-run imprecision was always lower than 20%, i.e., the threshold usually selected for defining the optimal analytical sensitivity of a laboratory measurement (9,19). The carry-over using pools with a high number of cells (i.e., between 93.5 and 132.5 WBC/ $\mu\text{L}$ ) was negligible (i.e., <0.3%). Linearity performed according to CLSI document EP6-A (18) was excellent (i.e.,  $r=1.00$ ;  $p<0.001$ ) in the range between 4 and 278 WBC/ $\mu\text{L}$ .

The ROC curve analysis for WBC count yielded a remarkable area under the curve (AUC 0.99;  $p<0.001$ ) (Figure 2). The diagnostic agreement of UF-1000i with optical microscopy was 0.98 ( $p<0.001$ ) at the diagnostic threshold of  $>5$  WBC/ $\mu\text{L}$ . The

**Table I** Imprecision (expressed as coefficient of variation, CV) of Sysmex UF-1000i for white blood cell (WBC) count in cerebrospinal fluid.

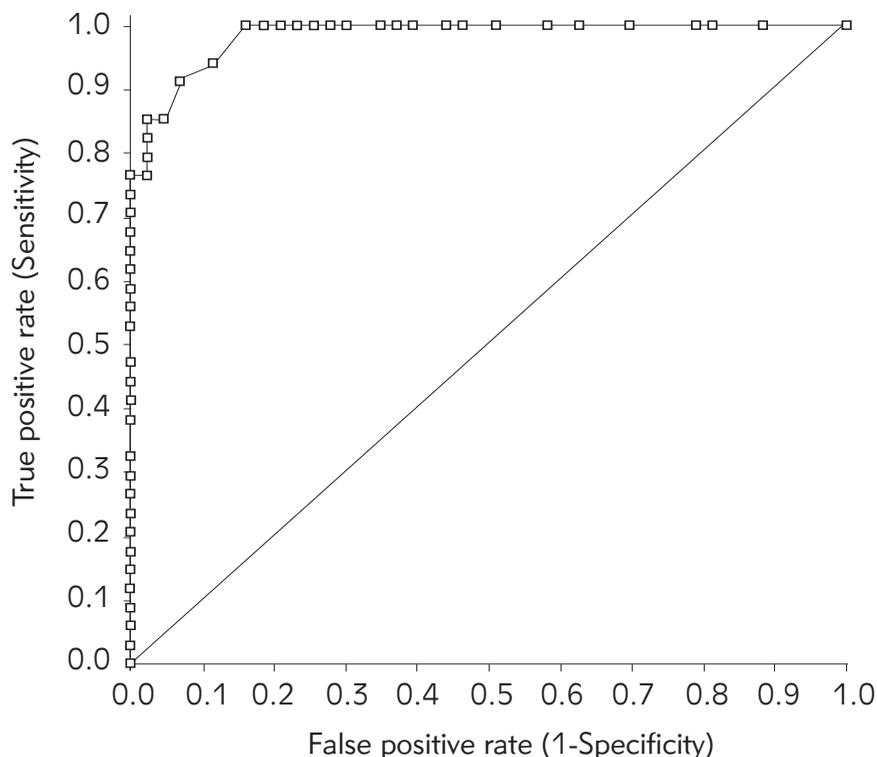
	n	WBC/ $\mu\text{L}$ (mean $\pm$ SD)	CV
Sample A	10	3.5 $\pm$ 0.6	16%
Sample B	10	18.1 $\pm$ 2.8)	15%
Sample C	10	28.8 $\pm$ 3.4	12%

agreement at the diagnostic cut-off of  $>4$  WBC/ $\mu\text{L}$  on UF-1000i was 0.91, with sensitivity and specificity of 1.00 and 0.84.

Thirty-seven of the 42 CSFs (88%) for which cultural exams was performed were negative, whereas in the remaining 5 samples a positivity was found for coagulase-negative *Staphylococcus* ( $n=2$ ), *Klebsiella pneumoniae* ( $n=1$ ), *Serratia marcescens* ( $n=1$ ) and *Enterococcus faecium* ( $n=1$ ). In two out of these five samples, however, the bacterial load was very low (i.e.,  $<19$  bacteria/ $\mu\text{L}$ ), the Gram staining was negative, so that they were considered as contaminated. Therefore, the samples were finally reclassified as 3 positive and 39 negative. At a cut-off of 19 bacteria/ $\mu\text{L}$ , the diagnostic accuracy of UF-1000i was 0.98, displaying an area under the ROC curve of 0.99 (95% CI, 0.97–1.00;  $p<0.001$ ), a sensitivity of 1.00 and a specificity of 0.97.

## Discussion

The automation of several steps in laboratory diagnostics carries a kaleidoscope of technical, organizational and clinical advantages (21). The gold standards for WBC enumeration and identification of



**Figure 2** Receiver Operating Characteristic (ROC) curve analysis of white blood cell (WBC) count between optical microscopy and Sysmex UF-1000i.

both bacterial meningitis and encephalitis are currently represented by optical microscopy and cultural examination of CSF (1–3). These techniques are inherently cumbersome, require trained personnel and are plagued by suboptimal analytical performance, especially by the large inter-observed agreement that typically characterizes cell count and classification in biological fluids. An appealing alternative for increasing the efficiency and accuracy of CSF analysis is hence represented by the use of conventional hemocytometers and cytofluorimeters, which have been originally developed and commercialized for blood and urine analyses. Previous studies have shown that this approach has some drawbacks, since the analytical sensitivity of most hematological analyzers seems unsuitable for enumeration of a modest number of cells in biological fluids (6–16), whereas urine cytofluorimeters showed unacceptable error rates at counts less than 50/ $\mu\text{L}$  (i.e., the vast majority of specimens) (15), thus raising serious doubts as to whether this type of instrumentation would be really effective to improve laboratory efficiency.

It is hence noteworthy that the results of the present study confirm those previously reported in another article using the same instrumentation (16), and show that the UF-1000i is indeed an appealing perspective for rapid screening of CSFs in patients with suspected CNS infections. The correlation stud-

ies with optical microscopy revealed a substantial agreement of measures, much greater than that observed by Nanos and Delanghe (i.e.,  $r=0.99$  versus  $r=0.73$ ), with a rather limited or even negligible bias, especially in samples with  $\text{WBC} < 150/\mu\text{L}$ . The diagnostic performance was also optimal, showing an AUC of 0.99, a sensitivity of 1.00 and a specificity of 0.84 at a diagnostic cut-off of 4  $\text{WBC}/\mu\text{L}$ . At variance with the study of Nanos and Delanghe, we also showed that the WBC count with the UF-1000i in CSF is characterized by acceptable within-run imprecision (always lower than 20%), extended linearity (up to 278  $\text{WBC}/\mu\text{L}$ ) and virtually absent carry-over (less than 0.3%). The good performance for bacterial load assessment is another important and innovative finding of this study, wherein the UF-1000i displayed an AUC of 0.99, a sensitivity of 1.00 and a specificity of 0.97 at a threshold of 19 bacteria/ $\mu\text{L}$ . It is thus likely that the UF-1000i may be reliably used for the routine screening of bacterial load in CSFs, as already suggested for the screening of urinary tract infections (22, 23).

### Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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