Clinica Chimica Acta 473 (2017) 133–138

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Preliminary evaluation of UF-5000 Body Fluid Mode for automated cerebrospinal fluid cell counting

Michela Seghezzi, Barbara Manenti, Giulia Previtali, Maria Grazia Alessio, Paola Dominoni, Sabrina Buoro*

Clinical Chemistry Laboratory, Hospital Papa Giovanni XXIII, Bergamo, Italy

ARTICLE INFO

Keywords:
Sysmex UF-5000
Automated cell counting
Cerebrospinal fluid
Body fluids

ABSTRACT

Background: Cellular analysis of cerebrospinal fluid (CSF) provides important diagnostic information in various medical conditions. The aim of this study was to evaluate the application of Sysmex UF-5000 body fluid mode in cytometric analysis of CSF compared to Light Microscopic (LM).

Methods: Eighty-one consecutive CSF samples were analyzed by UF-5000 body fluid mode and by LM. The study also included the evaluation of: limit of Blank (LoB), limit of Detection (LoD), limit of Quantitation (LoQ), carryover and linearity.

Results: For total nucleated cells (TNC-UF) and white blood cells (WBC-UF) LoB, LoD and LoQ were 1×10^6 cells/L, 1.8×10^6 cells/L and 1.9×10^6 cells/L respectively. For red blood cells (RBC) LoB was 2×10^6 cells/L, LoD was 3.5×10^6 cells/L and LoQ was 14×10^6 cells/L respectively. Linearity was excellent, carryover was negligible. The agreement between UF-5000 body fluid mode parameters and manual cell counts was good in all CSF samples with bias ranged between -0.5 and 25.1×10^6 cells/L. The ROC curve analysis showed an area under curve of 0.99 for both TNC-UF and WBC-UF parameters.

Conclusions: The UF-5000 body fluid mode offers rapid and accurate counts in clinically relevant concentration ranges, replacing the LM for most samples. However, in samples with abnormal cell counts or with abnormal scattergram the need for microscopic review remains.

1. Introduction

The cell counting in cerebrospinal fluid (CSF) provides important diagnostic information in various medical conditions [1–7]. High cell count in CSF samples ($> 5 \times 10^6$ cells/L in adults, $> 7 \times 10^6$ cells/L in children or $> 27 \times 10^6$ cells/L in neonates) are observed in meningitis and encephalitis, as well as in other neurological disorders and neoplastic or leukaemic central nervous system infiltrations [7]. In these cases, additional diagnostic information can be provided by the differential cell count and cellular analysis [1,3,5]. Although the presence of red blood cells (RBC) in CSF is difficult to interpret and may result from subarachnoid haemorrhage, contamination due to traumatic puncture or surgical procedures, the knowledge of the RBC count may help to interpret certain constellations in CSF diagnostics [4–7]. The reference method for cell counts in CSF is the manual method by light microscopy in a counting chamber [5] This procedure is time-consuming, labour intensive and requires experienced laboratory workers, furthermore, this method has high intra and inter-operator variability and shows insufficient precision [8–10]. In the last decade, the use of automatic haematological analysers improved the accuracy and workflow of CFS and other body fluids examination [1,7–18]. More recently CSF analysis was performed even on automated urine microscopy analysers (Iris Diagnostics iQ200) and urine flow cytometers (Sysmex UF-1000i, UF-100) [18–22]. The inadequate sensitivity of some automated analysers at low total nucleated cell (TNC) counts and the inability to differentiate between pathological and non-pathological samples could be the limiting factor for a wider use of automated cell counting analysers in CSF diagnostics as well as the lack of differential cell counts [15–22].

The UF-5000 (Sysmex Co., Kobe Japan) is the next generation of automated urine analysers by Sysmex, using fluorescent flow cytometry technology and hydrodynamic focusing to identify and enumerate cells

Abbreviations: AUC, Area Under Curve; BACT, Bacteria; BF, Body Fluid; CI, Confidence Interval; CSF, Cerebrospinal Fluid; CV, Coefficient of Variation; EC, Epithelial Cells; FCM, Flow Cytometry Method; In, Intercept; LM, Light Microscopy; LoB, Limit of Blank; LoD, Limit of Detection; LoQ, Limit of Quantitation; MN, Mononuclear Cells; PMN, Polymorphonuclear Cells; RBC, Red Blood Cells; RBC-UF, Red blood cells count by Body Fluid module of Sysmex UF-5000; Sl, Slope; SD, Standard Deviation; TNC, Total Cells; TNC-UF, Total cells count by Body Fluid module of Sysmex UF-5000; WBC, White Blood Cells; WBC-UF, White blood cells count by Body Fluid module of Sysmex UF-5000

* Corresponding author at: Clinical Chemistry Laboratory, Hospital Papa Giovanni XXIII, Piazza OMS 1, 24128 Bergamo, Italy.
E-mail address: sbuoro@hpg23.it (S. Buoro).

http://dx.doi.org/10.1016/j.cca.2017.08.026
Received 6 June 2017; Received in revised form 22 August 2017; Accepted 22 August 2017
Available online 24 August 2017
0009-8981/

and formed particles in urine. UF-5000 is also able to perform body fluid analysis in a dedicated body fluid module including the differential cell counts. Previous studies showed that UF-1000i exhibits good performance for counting nucleated cells in CSF and peritoneal fluid [19,20,22,23], but there are yet no studies available regarding the performance of UF-5000 in the analysis of CSF.

This preliminary study was hence aimed to validate the analytical and diagnostic performance of UF-5000 body fluid mode for cellular analysis in CSF samples, according to the reference documents published by the Clinical Laboratories Standard Institute (CLSI) document H56-A [5] as well as to the International Council for Standardization in Haematology (ICSH) guidelines for the verification and performance of automated cell counters for body fluids, [24].

2. Materials and methods

2.1. CSF samples

The preliminary study was performed on 81 consecutive CSF samples of hospitalized adult patients (30 from subjects in follow-up for acute lymphoblastic leukaemia, 20 from patients admitted to Neurology Unit, 5 from patients admitted to Neurosurgery Unit, 10 from patients admitted to Infectious Diseases Unit and 16 patients from the emergency department). Each sample was collected in sterile tubes (Becton Dickinson, Franklin Lakes, NJ) without additives and was processed within 1 h. The study, that lasted six months, was approved by ethical committee and was carried out in accordance with the local legislation and the declaration of Helsinki.

2.2. Manual light microscopy (LM)

Manual microscopic cell counting was performed in Fuchs–Rosenthal and Nageotte counting chambers. The samples were analyzed according to CLSI document H56-A [5] and ICSH guideline [24]. In order to ensure the standardization of the procedure for manual counting appropriate materials were used such as certified pipettes and cleaned chambers.

RBCs of each sample were counted unstained in Fuchs–Rosenthal chamber. The remaining cell-types were counted throughout the entire chamber corresponding to 3.2 μL of CSF. TNCs were counted in Nageotte chamber. CSF samples were diluted (1:1) with Turk's solution (Carlo Erba, Italy). The cells were counted in 12 squares, corresponding to 7.5 μL of CSF according to Buoro et al. [17]. In addition, Turk's staining has been used to enhance the cell recognition. Finally, the cell counting was performed by two skilled and independent operators with a light microscope at × 400 magnification (blinded method) An additional count was performed by a third operator the case of > 5% disagreement between the results of the two operators.

2.3. UF-5000 body fluid mode

Flow cytometry method (FCM) shines laser light on particles and cells, and measures the resulting scattered light and fluorescence to determine the characteristics of the particles. Fluorescent dyes are applied to highlight specific parts of the cells. The cells in suspension are sent through a nozzle one by one, directed by a constant flow of sheath fluid and a tightly focused laser beam is then directed onto the cells, which results in scattered light and fluorescence. Translating these light signals into parameters, a one-dimensional histogram based on light intensity and a two-dimensional scattergram based on fluorescent intensity and scattered light intensity are generated to enable detailed analysis of the cells.

The incident laser light that is scattered forward and to the side is called scattered light and the intensity of this scattered light indicates the size and the internal complexity of the cells. The fluorescence signal reflects quantitatively surface and intracellular cell characteristics, such as the amount of RNA and DNA. Based on the principle of flow cytometry, the UF-5000 analyser identifies cells in body fluids into RBC Red blood cells (RBC-UF), White blood cells (WBC-UF), Mononuclear cells in absolute counts (MN#) and in ratio (MN%), Polymorphonuclear cells in absolute counts (PMN#) and in ratio (PMN%), Epithelial cells (EC), Total nucleated cells (TNC-UF) and Bacteria (BACT) and expresses these quantitatively.

The UF-5000 body fluid mode requires 600 μL of body fluid sample and aspirates 450 μL. The analyser does not require any manual sample pre-treatment and the throughput is 20 body fluid samples per hour. When switched from urine mode to body fluid mode, the UF-5000 automatically performs a rinse cycle, followed by a background check to avoid carryover from urine samples. To avoid sample carryover in the body fluid mode, an automatic rinsing is performed before sample analysis. All UF-5000 body fluid measurements were performed in accordance with manufacturer's instructions and following daily two level-quality control (UF-Control, Sysmex Europe GmbH).

2.4. Carryover

Carryover was assessed on three CSF samples with a high cell count (WBC of 9568×10^6 cells/L and RBC of $59{,}623 \times 10^6$ 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 [5,24].

2.5. Linearity evaluation

The linear reportable range for cell count on UF-5000 body fluid mode was assessed by checking the counting performance of UF-5000 body fluid mode 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, standard 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 TNC of 2958×10^6 cells/L and RBC of $97{,}359 \times 10^6$ cells/L, was serially diluted with Phosphate Buffered Saline (PBS) to produce, 6 values in the low range, respectively (i.e. TNC from 3 to 2958×10^6 cells/L; RBC from 928 to 97359×10^6 cells/L). Each dilution was measured for 5 consecutive times. Results were plotted against the expected cell counts, and linearity was then evaluated according to the CLSI document EP06-A [25].

2.6. Imprecision

The within-run imprecision of UF-5000 body fluid mode was evaluated by measuring 10 replicates of 7 fresh CSFs routine samples, and results analyzed according to the CLSI document EP05-A3 [26]. The mean sample values ranged from 1 to 1620×10^6 cells/L.

2.7. Limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ)

The LoB, LoD and LoQ were performed according to the CLSI document EP17-A2 [27], by considering 60 measurements for each specification.

2.8. Method comparison, bias estimation and diagnostic agreement between UF-5000 BF mode and LM

UF-5000-BF parameters were compared versus cell counts obtained by LM Nageotte and Fuchs-Rosenthal counting chambers and LM differential counts on Cytospin slides using samples with cell counts up to 20 $x10^6$cells/L. In LM cell-differentiation was carried out according to