Original Article

Comparison between body fluid mode of Sysmex XN-9000 and optical microscopy for the counting of cells in ascitic fluid

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Background: This study was designed to evaluate basic analytical performance of the body fluid (BF) mode Sysmex XN-9000 (XN-BF) and to classificate and enumerate white blood cells (WBCs) in ascitic fluid (AF) comparing with optical microscopy (OM).

Methods: Sixty-six AF samples were analyzed by XN-BF and by conventional OM. The agreement between XN-9000 and OM have been evaluated with Pearson's correlation coefficient (r) and Passing-Bablok regression, while the bias between the two methods was assessed with Bland-Altman plot analysis. The study included assessment of carryover, imprecision and linearity.

Results: A good agreement was found between the results obtained by XN-BF and OM. The correlation coefficients for default parameter is comprised between 0.94 and 1.00, while for the different cells populations Pearson's correlation is comprised between 0.80 and 0.99. The Sysmex XN-9000 BF mode displayed excellent linearity and imprecision. The carryover was negligible.

Conclusions: The XN-BF shows excellent analytical performance, accurate count and cell differentiation comparable with conventional OM. Nevertheless, in all samples with abnormal WBC differential scattergram, OM continues to be the "gold standard" for a correct clinical outcome.

Keywords: Ascitic fluid (AF); optical microscopy (OM), Sysmex XN-9000 (XN-BF); leukocytes; cells with high fluorescence (HF).

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Introduction

The term "ascites" is derived from the Greek word "Askitos" meaning bladder or bag. The main conditions in which ascites may significantly increase include the cirrhosis, hepatic venous outflow obstruction, hepatic cancer, nephrotic syndrome, congestive cardiac failure and constrictive pericarditis, infection as tuberculous peritonitis, bacterial peritonitis, malignancy (primary peritoneal cancer, pancreatic cancer, hepatobiliary cancer, etc.) (1). For the identification and classification of ascites is crucial the chemical and morphological analysis of the peritoneal fluid. Patients with a total cell counts (TC) ≥500/μL and absolute neutrophil count ≥250/μL are the standards for establishing a diagnosis of spontaneous bacterial peritonitis (2). In tuberculous peritonitis, cell counts are typically greater than >1,000 and lymphocytes predominate (i.e., 50%) (2-4).

For identification and enumeration of white blood cell

(WBC) in ascitic fluid (AF), optical microscopy (OM) is traditionally used. This technique is still regarded as the "gold standard", though it presents lengthy turnaround time (TAT), needs to educate and train specialized personnel for this type of manual analysis and has a high inter and intra-assay imprecision (2,5,6).

From 2006, the Sysmex (Kobe, Japan) has integrated a specific body fluid (BF) mode in its automatic hematologic analyzers, which is hence mainly aimed to be used for analysis of pericardial, ascites, synovial, pleural and cerebrospinal fluids. Automated counting has several advantages: rapid TAT, it doesn't need for highly qualified personnel and management of specimens more cost-effective than using OM. Besides, the use of a larger sample volume as compared to counting chamber leads to more cells being counted enhancing precision and accuracy (7-9).

The Sysmex XN-9000, in addition to the default parameters—total nucleated cells (TC), WBC counts, differential cell count for mononuclear cells (MN) and polymorphonuclear cells (PMN)—is equipped with a series of research parameters including neutrophils (NE), lymphocytes (LY), monocytes (MO), eosinophils (EO) and cells with high fluorescence (HF).

The performance of XN-BF for analysis of AF default parameters has been evaluated in other studies (6,7,9). However, the analytical performance of research parameters has not been evaluated. The aim of this study was, in the first instance, to verify the basic performance of the fully automated XN-BF and then to compare data obtained using manual microscopy with those ones obtained using XN-BF not only for default parameters but also for new research parameters, in the analysis of AF samples.

Methods

Samples

This comparison study was carried using 66 AFs samples received to the local laboratory from various clinical departments for routine analysis. All samples were collected in 2.0 mL, K_2EDTA anticoagulated tubes and tested within 2 h from arrival in the laboratory (2). The results of total WBC count were directly compared with those obtained on the same AF sample by OM on Nageotte chamber, after diluted (1:20 or 1:200) with Turk's solution. The slides for differential WBC count were prepared with cytospin (Cytospin 3 Thermo Shandon, France), and subsequently stained whit May-Grunwald-Giemsa (Carlo Erba, Italy). Microscopic analysis was performed with light microscopy under oil immersion, at 50× magnification. The differential WBC count included the following classes: NE, LY, monocytes/macrophages (MN/MACRO), EO, and other cells (mesothelial cell and tumor cells).

Analytical performance assessment

The linearity was assessed using 2 AF samples with low cell counts (sample 1: WBC, 65×10^6 cells/L) and a high cell counts (sample 2: WBC, $4{,}750\times10^6$ cells/L). Each sample was serially diluted with Cellpack to get scalar values, which have been then measured three consecutive times each. Results were plotted against with expected values, as according of CLSI document EP06-A (10).

The imprecision of the XN-BF was evaluated by analyzing in 20 measurements of 2 AF samples with low (sample 1: mean value, 26×10^6 cells/L) and high (sample 2: mean value, $2{,}340\times10^6$ cells/L) WBC counts, expressed in percentage by the coefficient of variation (CV), as according to the Clinical and Laboratory Standards Institute (CLSI) document EP5-A29 (11).

Carryover was performed according to the ICSH guideline: on one AF samples with high cell count in triplicate (AF1, AF2, AF3), followed by three measurements of a blank (Cellpack; B1, B2, B3) (12). Percentage of carryover was calculated as follows: [(B1 – B3)/(A3 – B3)] ×100.

Ethical statement

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

Statistical analysis

The bias between XN-BF and OM was estimated with Bland-Altman plot analysis, while the agreement was assessed with Pearson's correlation coefficient (r) and Passing-Bablok regression. Statistical analysis was performed using Analyse-it software version 3.90.1.

Results

For our study were used 66 samples, the mean cellularity value was 461.8×10^6 cells/L (95% CI: 244.8–678.8) by OM and 472.2×10^6 cells/L (95% CI: 253.2–691.2) with Sysmex

Table 1 Comparison of cell counts between XN-BF and OM for the main results using 66 ascitic fluid

Ascitic fluid parameters (10^6 cells/L)	Optical microscopy	Sysmex XN-9000	P value
TC	461.8±899.3	472.2±908.3	0.947
WBC	423.2±902.0	453.5±912.6	0.900
PMN	273.5±849.0	272.4±855.8	0.994
MN	191.4±198.6	181.1±191.4	0.762
NE	45.25±75.15	45.02±77.27	0.986
LY	100.2±140.4	95.95±138.6	0.859
MO/MACRO	77.95±77.72	85.32±83.77	0.604
EO	1.307±3.703	0.907±1.568	0.424
HF	40.49±67.845	35.12±37.852	0.578

Data were presented as mean and standard deviation and compared with paired *t*-test. TC, total cell count; WBC, white blood cells; PMN, polymorphonuclear cells; MN, mononuclear cells; NE, neutrophil; LY, lymphocyte; MO, monocyte; MACRO, macrophage; EO, eosinophils; HF, cells with high fluorescence (including mesothelial cell and tumor cells).

Table 2 Comparison of cell counts between XN-BF and OM by Bland-Altman bias, Passing-Bablok regression and Pearson's correlation

Ascitic fluid parameters (10^6 cells/L)	Bland-Altman bias (95% CI)	Passing-Bablok regression			Pearson's correlation	
		Regression model	Slope (95% CI)	Intercept (95% CI)	Coefficient (95% CI)	P
TC	−10.5 (−17.5 to −3.30)	Y =−0.185+1.00x	1.00 to 1.01	−0.66 to −0.00	1.00	<0.0001
WBC	−21.4 (−51.9 to 9.27)	Y =−0.401+1.01x	1.00 to 1.02	−1.28 to −0.0242	0.99	<0.0001
PMN	3.30 (−7.3 to 13.9)	Y =0+1x	1.00 to 1.01	−0.11 to 0.00	1.00	<0.0001
MN	10.3 (−6.55 to 27.3)	Y =0+1x	1.00 to 1.01	−069 to 0.00	0.94	<0.0001
NE	0.29 (−2.90 to 3.36)	Y =0+1x	1.00 to 1.0o	0.00 to 0.00	0.99	<0.0001
LY	−0.22 (−0.73 to 0.27)	Y =0+1x	1.00 to 1.01	−0.20 to 0.00	0.91	<0.0001
MO/MACRO	−7.36 (−16.1 to 1.40)	Y =−0.79+1.03x	1.00 to 1.15	6.32 to 0.00	0.90	<0.0001
EO	0.40 (−0.33 to 1.13)	Y =0+1x	0.80 to 1.00	0.00 to 0.00	0.80	<0.0001
HF	5.36 (−8.40 to 19.1)	Y =0+1x	1.00 to 1.00	0.00 to 0.00	0.67	<0.0001

TC, total cell count; WBC, white blood cells; PMN, polymorphonuclear cells; MN, mononuclear cells; NE, neutrophil; LY, lymphocyte; MO, monocyte; MACRO, macrophage; EO, eosinophils; HF, cells with high fluorescence (including mesothelial cell and tumor cells); CI, confidence interval. Y, the dependent variable; x, the independent variable.

XN-9000 BF mode. The main results of this study are shown in *Table 1*.

Bland-Altman bias was -10.5×10^6 cells/L (95% CI: −17.5 to −3.30), -21.4×10^6 cells/L (95% CI: −51.9 to 9.27), 3.30×10^6 cells/L (95% CI: −7.3 to 13.9) and 10.3×10^6 cells/L (95% CI: −6.55 to 27.3) respectively, for TC-BF, WBC-BF, PMN-BF and MN-BF in all 66 samples (*Table 2*). The bias for WBCs subpopulations and cells with HF is shown in *Table 2*. The overall bias between the XN-BF and OM was always clinically meaningless.

A good correlation between Sysmex XN-9000 BF mode and OM counts has been found for all considered parameters except for HF cells (r=0.67) (*Table 2*).

The XN-9000 BF mode showed excellent linearity, with all correlation coefficients for TC, WBC, PMN and MN equal to 1.00 (P<0.05), in a wide range of values comprised between 65×10^6 and $4{,}750\times10^6$ cells/L. The imprecision was excellent, with CV <5% for AF samples with mean values of 26×10^6 cells/L and <4% for AF samples with mean values of $2{,}340\times10^6$ cells/L. The carryover was negligible for all

parameters (<0.01).

Discussion

The accurate classification and counting of cells in AF are fundamental needs for faster diagnosis and appropriate therapeutic treatment of patients with ascites. OM remains even today "gold standard" for total WBC counting and for differentiating WBCs subpopulations in this BF (13-15). However, the OM presents a high intra-operator inaccuracy, requires qualified technical personnel and requires longer analytical times (16-18). For these reasons, the use of automatic analyzers is increasing more and more in routine clinical laboratories.

In the last years the introduction of a new generation of automated hematological analyzers has allowed to overcome the main methodological problems for their use in the analysis of cavitary liquids, represented by the presence of macrophages or neoplastic cells. Furthermore, the count in automation can increase the level of analytical standardization even in the case of personnel who are not highly qualified for reading in OM (7,19,20).

For certain types of cavitary liquids, automated cell count has hence allowed to achieve a high degree of accuracy and precision, concomitantly reducing both inter-observer variability and TAT (21,22). In a previous investigation, Paris *et al.* (23) found an optimal agreement for PMN (r=0.99) and MONO (r=0.98) counts between the manual method and XE-5000 automated count on 81 AF samples. In another study (6), also showed a good correlation for TC (r=0.99), WBC (r=0.98), PMN (r=0.93) and MN (r=0.96) between XN-BF mode and OM. Furthermore, a satisfactory agreement was found between XN-BF and OM for the different WBC subpopulations, with correlation coefficients comprised between 0.84 and 0.93.

Our study was mainly aimed to assess the analytical performance of the new BF mode on the Sysmex XN-9000 using AF samples and comparing data with those obtained with the reference technique (i.e., OM) including neutrophils, lymphocytes, monocytes, eosinophils and cells with HF. The results of our investigation attest that the novel XN-9000 hemocytometer exhibits excellent analytical performance in terms of carryover, imprecision, linearity and throughout a broad range of cellularity in AF samples. In agreement with others studies, BF mode on the Sysmex XN-9000 confirmed a good agreement with default parameters as well as with new research parameters, as shown in *Table 2*.

At last, comparison between XN-BF and OM about HF cells parameter showed that the different cells instrument counts aren't overlapped with WBC (i.e., mesothelial cell and tumor cells). The Sysmex XN-9000 BF mode not only exhibits acceptable analytical performance, but it may be used as an alternative to OM, as a first-line screening technique for rapid analysis of AF samples either referred for routine or, especially, for urgent testing. Instead, in cases where an abnormal scattergram or difference between WBC and total cells count with consequent increase of cells with HF are present, the OM revision is fundamental (6,20).

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jlpm.2018.10.01). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was carried out in accordance with the Declaration of Helsinki (as revised in 2013), under the terms of all relevant local legislation. All samples were anonymized before testing, and test results did not impact the clinical management of patients, patient's permission to use the samples for this study were cleared by the local institutional review board.

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