Jornal Brasileiro de Patologia e Medicina Laboratorial
ISSN: 1676-2444
jbpml@sbpc.org.br
Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial
Brasil

Cognialli, Regielly Caroline R.; Comar, Samuel Ricardo; de Souza, Angela Maria; Singer, Gisele Maria B.

Evaluation of pleural and ascitic fluid analysis on the Sysmex XE-5000 hematology analyzer

Jornal Brasileiro de Patologia e Medicina Laboratorial, vol. 53, núm. 3, junio, 2017, pp. 150-158
Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial
Rio de Janeiro, Brasil

J Bras Patol Med Lab, v. 53, n. 3, p. 150-158, June 2017

10.5935/1676-2444.20170023

Evaluation of pleural and ascitic fluid analysis on the Sysmex XE-5000 hematology analyzer

Avaliação da análise dos líquidos pleural e ascítico no analisador hematológico Sysmex XE-5000

Regielly Caroline R. Cognialli; Samuel Ricardo Comar; Angela Maria de Souza; Gisele Maria B. Singer

Universidade Federal do Paraná (UFPR), Paraná, Brazil.

ABSTRACT

Introduction: Body fluid (BF) analysis is critical to the diagnosis and monitoring of several pathological conditions. The limitations of manual cell counts have led to greater interest in the development of automated BF analysis. **Objective:** To evaluate the analytical performance of the Sysmex XE-5000 hematology analyzer in the analysis of pleural and ascitic fluids in the laboratory routine of a large university hospital. **Methods:** A total of 56 samples (35 ascitic and 21 pleural fluids) were analyzed by manual optical microscopy (OM) and XE-5000. Analytical performance includes linearity, carryover, functional sensitivity and comparison of patient samples. **Results:** Performance studies showed linearity up to 25,825 WBC-BF/µl ($r^2 = 0.999$), WBC-BF showed carryover of 0.18%, and the lower limit of quantitation was set at 22 WBC-BF/µl. Good correlations between the methods were observed just for total cell (TC-BF) and white blood cell (WBC-BF) counts in pleural and ascitic fluids. The high-fluorescence cell count (HF-BF) showed poor correlation but high positive predictive value (PPV) for both fluids (94.74% for pleural and 96.97% for ascitic fluid). **Conclusion:** XE-5000 provides accurate and precise count for TC-BF, WBC-BF and polymorphonuclear cells (PMN-BF) in pleural and ascitic fluids in medical decision levels, but the morphological differentiation should continue to be held by OM. Histogram and scattergram displayed on XE-5000 must always be analyzed to assess if there is any interference or flag. The HF-BF parameter is a potential tool for screening.

Key words: body fluids; cell count; pleural effusion; ascitic fluid; laboratory automation.

INTRODUCTION

Total and differential cell counts in body fluid (BF) samples are of enormous diagnostic value. They may guide treatment decisions and follow-up of several illnesses, including those involving body cavities, such as neoplasms, hemorrhages, inflammatory processes and infectious diseases[1-3]. Because of their importance, they must be highly precise and accurate, so as not to under- or overestimate diagnoses of treatable diseases. Such enumerations are traditionally performed using the manual optical microscopy (OM) count as the gold standard method, combining hemocytometer-based total cell counting and differential count on slides prepared by cytocentrifugation and stained with appropriate dyes. The accuracy of these counts depends on variables, such as the correct sample volume loaded into the hemocytometer, preparation of appropriate dilutions, number of counted quadrants and cells, and adequate slide preparation by cytocentrifugation. Nevertheless, they still display high inaccuracy and imprecision in samples with low cellularity, they are arduous and time-consuming, require highly experienced and skilled laboratory staff, besides showing great intra- and inter-operator variability[4-8].

In clinical laboratories, cell count automation in biological fluid samples has evolved considerably in the latest years, and manufacturers offer more and more innovations in their hematology analyzers, including modules dedicated to automated cell counting of BF, such as the cavity fluids (pleural, ascitic and pericardial), synovial and cerebrospinal fluids[4, 5]. Besides hematology analyzers, some urinalysis instruments have also

First submission on 23/09/16; last submission on 25/09/16; accepted for publication on 23/03/17; published on 20/06/17

been used to analyze body fluids by methods such as impedance, and/or flow cytometry[9]. Automation of BF cell counts have provided, most of times, improvements in laboratory exactness, precision, effectiveness and productivity, besides reducing costs and time until result release[10].

The Sysmex XE-5000 hematology analyzer performs BF analysis by means of a dedicated module whose use has been cleared by the Food and Drug Administration (FDA) in the United States. This module enables minimizing analytical interference associated with BF matrix, with the presence of cell types different from those observed in peripheral blood (mesothelial cells, macrophages and tumor cells), and with the detection limit of the hematology analyzer[11].

Although the assessment of XE-5000 hematology analyzer in BF samples has already been described in some studies[4, 11], one must emphasize that the performance evaluation of hematology analyzers for BF analysis must be essentially carried out in the environment where the instrument works, by the team that will operate it and considering the profile of patients being treated.

OBJECTIVES

In order to contribute to existing literature on this subject, the aim of this study was to assess the analytical performance of the Sysmex XE-5000 hematology analyzer for pleural and ascitic fluids in the laboratory routine of a large university hospital, as well as to compare the data obtained by OM according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) document H56-A[12] and of the guidelines of the International Council for Standardization in Haematology (ICSH) for assessment and performance of automated analyzers of biological fluid samples[6].

METHODS

Study setting, samples and patients

This study was conducted at the hematology laboratory of Hospital de Clínicas of Universidade Federal do Paraná (HC-UFPR) after approval by the local Research Ethics Committee. Signed informed consent from patients was not necessary, since the used specimens of pleural and ascitic fluids were those remaining after routine analyses. A total of 56 non-coagulated samples collected in heparin tubes with minimum volume of 400 µl were analyzed. Among them, 35 were ascitic fluids from 30 patients (19 males and 11 females) with mean ages of 52 ±16.3 years (range: 15 days-76 years), and 21 were pleural fluids from 19 patients (10 males and nine females) with mean ages of 52.5 ± 25.4 years (range: 5 days-76 years). Among the total samples of body cavity fluids, four (7.15%) came from outpatients (from the ascites outpatient clinic), and 52 (92.85%) came from inpatients: 12 from the adult intensive care unit (ICU), 10 from the emergency department, 10 from the internal medicine service, five from the surgery service, four from the pediatric and neonatal ICU, three from the high-risk chemotherapy service, two from the infectious disease department, two from the gynecology department, two from the hematology department, one from the liver transplant division and one from the cardiology service. All the manual and automated analyses were conducted within three hours after collection, and all samples met the local criteria for sample acceptance.

Reference manual method for total count of nucleated cells

The count of nucleated cells by OM was performed using a bright-line Neubauer-improved chamber (LaborOptik Ltd., Lancing, United Kingdom) at a depth of 0.1 mm and 0.0025 mm^2 resolution after mixing the sample with fuchsin 0.2% hypotonic solution at 1:20 dilution. In each sample, enumeration was carried out in the four corner squares of the Neubauer chamber (0.4 µl of sample) in duplicate of both sides of the hemocytometer at 400× magnification (BX41 microscope; Olympus, Tokyo, Japan). Manual counts were done before automated counts; results were expressed as the average of counts in duplicate and varied below 20% among themselves. The total of cells counted per microliter of sample was calculated as follows: total of cells counted in the four lateral quadrants (0.4 µl of sample) × 50 [(chamber factor: 2.5) × (dilution factor: 20)][12].

Reference manual method for differential count of nucleated cells

The cytospin slides for differential count of nucleated cells were prepared by cytocentrifugation (Labho®, São Paulo, CT12, Brazil) of 100 µl body cavity fluid samples at 1,200 rpm for 2 minutes, followed by May-Grünwald and Giemsa staining using the automated slide maker/stainer Sysmex SP-1000i (Sysmex Corporation, Kobe, Japan). BF differential cell counts were conducted in all samples using an optical microscope at 1,000× magnification (BX41 microscope; Olympus, Tokyo, Japan) by two experienced observers, and by a third observer in case of discrepancies in the results between the first two[6, 13 14].

Analysis of biological fluids on Sysmex XE-5000 analyzer

The Sysmex XE-5000 hematology analyzer (Sysmex Co., Kobe, Japan) has a mode dedicated to the analysis of biological fluids in which samples do not need previous treatment with additional reagents to be analyzed, and 38 analyses are carried out per hour (one sample each 63 seconds). The switch from whole blood mode to BF mode was preceded by an automatic wash to avoid cross-contamination due to carryover, and an additional washing procedure was carried out if background check result was > 1 leukocyte/µl. All the samples were analyzed in the open mode, and the aspirated sample volume was approximately 130 µl. In order to increase precision in samples with few cells, the biological fluid mode performed an extended cell counting that permitted the passage of three times more cells through the detector than the previous generation hematology analyzer, as the Sysmex XE-2100[15]. The BF mode used a proper software, in which the count of nucleated cells, leukocytes, polymorphonuclear and mononuclear cells was conducted in the differential channel (DIFF channel) count of leukocytes by means of side scatter (internal complexity of the cells) and fluorescence intensity [binds to deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)], after incubation and stain with a fluorescent dye. The light signals were collected and digitized for computer analysis and, next, displayed as scattergrams and histograms to provide information on the cell types within a sample. The BF mode provided 11 parameters: seven reportable [total of nucleated cells (TC-BF), white blood cells (WBC-BF), absolute and relative counts of mononuclear cells (MN-BF), absolute and relative count of polymorphonuclear cells (PMN-BF), and red blood cells (RBC-BF)], which are counted by impedance; and four research parameters for internal use by the laboratory [absolute and relative count of eosinophils (EO-BF), and absolute and relative count of high-fluorescence cells (HF-BF)]. The HF-BF count includes macrophages, mesothelial cells, plasma cells, and malignant cells, and this value is excluded from the WBC count[4, 16]. Quality assurance procedures and internal and external quality control procedures were followed to monitor performance and ensure good working conditions of the Sysmex XE-5000 so as to deliver reliability to the yielded results. All the adjustments and the configurations of hematology analyzers were carried out by the technical and scientific assistance service of the manufacturer.

Analytical measurement range (linearity)

This assay allowed establishing the reportable and linear range for cell counts on XE-5000 by analysis of several dilutions of a BF sample that encompassed the entire operating range of the equipment. The used sample had high count of total cells and was diluted in series with the CellPack diluent (Sysmex) to produce 12 levels of concentration (TC-BF: 13-25,920 cells/µl, WBC-BF: 13-25,825 cells/µl, PMN-BF: 12-24,080 cells/µl, and MN-BF: 1-1,745 cells/µl). Each sample was analyzed three consecutive times. The coefficient of determination, the slope and the intercept between the expected and the obtained values were calculated.

Carryover

Two BF samples – one of ascitic fluid with high cell count (3,410-3,828 TC-BF/µl) and another of pleural fluid with low cell count (52-67 TC-BF/µl) – were selected. Firstly, the sample with high cell count was analyzed in triplicate (H1, H2, H3) on XE-5000, immediately followed by the analysis of the sample with low count, also in triplicate (L1, L2, L3). The carryover was calculated using the Broughton formula: carryover % = (L1 - L3) × 100/(H1 - L3)[17].

Limit of quantitation for leukocyte count

It was defined by the analysis of 10 repetitions of nine pure samples of body cavity fluids in different cell concentrations (average values of WBC-BF were 1-11,416/µl). Mean, standard deviation (SD), and coefficient of variation (CV) of the WBC-BF count were calculated in each sample, and the limit of quantitation was calculated in the concentration in which CV was 20%. This value was then defined as the lower limit of quantitation (LLoQ)[18].

Intra-run imprecision or repeatability

The objective of this assay was to determine the capacity of XE-5000 to reproduce the results of a certain parameter in the analysis of a given sample of body cavity fluid. Analyses were carried out in normal conditions, by the same operator, in the same period of day and with the same reagent lots. The evaluation was conducted by means of nine BF samples that were analyzed 10 consecutive times, allowing to test different levels of concentrations, including points of medical decision (mean values: 1-11,085 TC/µl)[19].

Comparison between XE-5000 and manual optical microscopy

In the method comparison, the 56 samples were separated according to the body cavity of origin (35 ascitic and 21 pleural fluids). These samples were analyzed on a XE-5000 and by OM on a Neubauer chamber to assess the total cell number. For comparison of manual and automated differential counts, cells were grouped into four categories: polymorphonuclear cells (neutrophils + basophils), mononuclear cells (lymphocytes + monocytes), eosinophils and high-fluorescence cells (macrophages,

mesothelial cells, plasma cells and neoplastic cells). Due to the differences in cell classification by using XE-5000 and OM, cells were grouped in homogeneous categories so as to permit a direct comparison between both methods, as follows: total cells on XE-5000 vs. total cells by OM in absolute values (#); total leukocytes on XE-5000 vs. total leukocytes by OM (#); mononuclear cells on XE-500 vs. mononuclear cells by OM (monocytes + lymphocytes) (#); polymorphonuclear cells on XE-5000 vs. polymorphonuclear cells by OM (neutrophils + basophils) (#); eosinophils on XE-5000 vs. eosinophils by OM (#); high-fluorescence cells on XE-5000 vs. sum of macrophages + mesothelial cells + plasma cells + neoplastic cells by OM (#).

Diagnostic agreement between HF-BF count on XE-5000 and by manual optical microscopy

The positive predictive value (PPV) and the positive likelihood ratio (PLR) were calculated in the comparison between HF-BF count on XE-5000 and by OM as follows: PPV = (TP/(TP + FP) × 100); PLR = sensitivity/(1 - specificity). PLR values much higher than 1 demonstrated that the probability of a positive result to be a true positive (TP) (sensitivity) was higher than a positive result to be a false-positive (FP) (1 - specificity). Any positive HF-BF count was considered an indication of presence of high-fluorescence cells due to the clinical importance of identifying malignant cells in biological fluids[10, 14].

Statistical analyses

Statistical analysis was carried out using MedCalc for Windows, version 15.11 (MedCalc Software, Ostend, Belgium) and Excel (Microsoft Corporation, USA). The Shapiro-Wilk test was used to test normality of the set of samples. Linearity was estimated by means of linear regression analysis using the least squares method and by the coefficient of determination (r^2). Agreement between the counts performed on XE-5000 (TC-BF, WBC-BF, MN-BF, PMN-BF, EO-BF and HF-BF) and the manual cell counts by OM was analyzed by means of non-parametric statistical methods: Passing-Bablok regression, Bland-Altman analysis, Spearman's correlation and Mann-Whitney *U* test in which values of $p < 0.05$ were considered statistically significant. In regression analyses, a linear regression equation was created (y = b + mx), in which *b* was called the intercept and *m* was the slope. In Bland-Altman analysis, absolute average differences (y axis) and the arithmetic averages (x axis) of cell count by OM and those obtained on XE-5000 were plotted, with a significant tendency being observed when the average difference was distant from the null value. The 95% confidence intervals (95% CI) were calculated to highlight significant differences between methods. In the Spearman's correlation, the closer the values came to 1 the stronger the positive correlation was between the analyzed variables[10, 14].

RESULTS

Linearity

TC-BF, WBC-BF, MN-BF and PMN-BF counts showed high coefficients of determination (r^2), demonstrating that the linear model resulting from the used dilutions was statistically reliable (**Table 1**). Linearity up to 25,825 WBC/µl and 25,920 TC/µl was observed, values above those specified by the manufacturer (10,000 WBC-BF/µl; 10,000 TC-BF/µl)[20]. Regarding the theoretical dilution values, a positive proportional bias of 5% (1 - slope) was observed for TC-BF and WBC-BF counts, and a constant bias of approximately -100 cells (intercept) was observed for TC-BF and WBC-BF automated counts on XE-5000. Linearity for PMN-BF at medical decision levels (MDL) for diagnosis of spontaneous bacterial peritonitis (250 polymorphonuclear cells/µl) showed $r^2 = 0.988$, and linearity for TC-BF at MDL for diagnosis of pleural effusion (1,000 total cells/µl), $r^2 = 0.998$.

TABLE 1 – Results of linearity of XE-5000 for TC, WBC, MN and PMN counts

	TC	WBC	MN	PMN
r^2	0.999	0.999	0.986	0.999
Slope	1.05	1.05	1.75	0.99
Intercept	-101.44	-100.16	-31.94	-68.64

TC: total cells; WBC: white blood cells; MN: mononuclear cells; PMN: polymorphonuclear cells.

Carryover

Carryover was 0.18% for leukocyte count, a value lower than that specified by the manufacturer (≤ 0.3%)[20].

Limit of quantitation for leukocyte count

The LLoQ for the automated count of leukocytes was established at 22 WBC-BF/µl.

Intra-run imprecision or repeatability

The results from the intra-assay reproduction analysis for nine distinct concentrations are presented in **Table 2**. The samples with low counts of TC and WBC (≤ 10 cells/µl) presented CVs higher than those specified by the manufacturer. The CVs observed for of MN and PMN differential counts were so more elevated as lower were the absolute counts.

TABLE 2 – Results of precision expressed as CV of Sysmex XE-5000 for TC, WBC, MN and PMN counts

TC/µl	CV%	WBC/µl	CV%	MN/µl	CV%	PMN/µl	CV%
< 1	141.91	< 1	141.91	< 1	210.82	< 1	140.55
2-5	124.57	< 5	124.57	-	-	< 5	124.57
6-10	46.69	6-10	46.69	1-5	105.41	6-10	55.97
40-60	11.57	40-60	12.54	6-10	54.87	40-60	36.37
320-380	5.58	320-380	5.43	20-30	27.34	320-380	5.02
1,000-1,100	2.57	1,000-1,100	2.42	40-60	14.34	1,000-1,100	2.32
4,000-4,300	2.32	4,000-4,300	2.31	150-200	29.84	4,000-4,100	2.21
8,800-9,200	2.24	8,800-9,200	2.22	200-300	44.91	8,800-9,200	2.84
10,000-12,000	5.12	10,000-12,000	5.05	150-200	59.58	10,000-12,000	4.41

CV: coefficient of variation; TC: total cells; WBC: white blood cells; MN: mononuclear cells; PMN: polymorphonuclear cells.

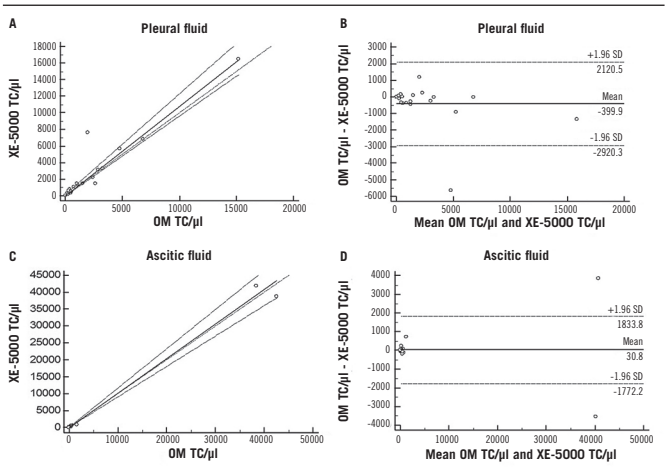

FIGURE 1 – *Comparison of the body fluid total cell counts between the XE-5000 and the manual optical microscopy*

A) Passing-Bablok regression analyses for TC-BF count in pleural fluid. Cusum test for linearity indicates no significant deviation from linearity (p > 0.1); B) Bland-Altman plot for TC-BF count between manual optical microscopy (OM) and the XE-5000 in pleural fluid; C) Passing-Bablok regression analyses for TC-BF count in peritoneal fluid. Cusum test for linearity indicates no significant deviation from linearity (p > 0.1); D) Bland-Altman plot for TC-BF count between MOM and the XE-5000 in peritoneal fluid.

TC-BF: body fluid total cell count; OM: manual optical microscopy; SD: standard deviation; Cusum: cumulative sum.

Assessment between counts on XE-500 and by manual optical microscopy

Pleural fluid

The manual total cell count in the pleural fluid ranged from 50 to 15,150/µl (mean 2,240 ± 3,434/µl) in the 21 analyzed samples. The samples were predominantly sent by the departments of internal medicine (19.05%), chemotherapy (14.29%) and infectious diseases (9.52%). The comparison of total cell count between XE-5000 and OM is shown in **Figure 1** (A and B). A very high Spearman's correlation between both methods was observed for TC-BF ($\rho = 0.94$, **Table 3**). WBC-BF, PMN-BF, MN-BF and HF-BF showed high correlations (ρ between 0.72 and 0.9). For TC-BF, Spearman's correlation at MDL for diagnosis of pleural effusion was high ($\rho = 0.89$, $p < 0.001$). EO-BF did not show statistical correlation ($\rho = 0.17$). Except for EO-BF, for the other parameters the equation coefficients were statistically valid, and the Mann-Whitney *U* test did not show statistical differences in comparisons, since *p* values were ≥ 0.05 (Table 3). Positive proportional biases were observed for TC-BF, WBC-BF, MN-BF and PMN-BF of 8%, 22%, 38% and 14%, respectively. HF-BF showed a

TABLE 3 – Spearman's correlation, Passing-Bablok regression, Bland-Altman bias and Mann-Whitney *U* test for different parameters in XE-5000 × MOM in pleural and peritoneal fluids

Pleural fluid				
Parameter	Spearman's correlation (ρ)	Passing-Bablok regression (95% CI intercept; slope)	Bland-Altman bias (95% CI)	Mann-Whitney (*p* value)
TC-BF/µl MOM × XE-5000	0.94	y = 15.98 + 1.08× (-116.53 to 122.5; 0.97 to 1.22)	-399.9 (-2920.3 to 2120.5)	0.65
WBC-BF/µl MOM × XE-5000	0.89	y = 49.3 + 1.22× (-31.97 to 301.95; 1 to 1.49)	-706.9 (-3539.2 to 2125.5)	0.23
MN-BF/µl MOM × XE-5000	0.83	y = 45.14 + 1.38× (-58.89 to 175.57; 1.1 to 1.67)	-585.9 (-2483.5 to 1311.7)	0.05
PMN-BF/µl MOM × XE-5000	0.9	y = 22 + 1.14× (-6.05 to 30; 0.75 to 1.94)	-157.7 (-1314.1 to 998.8)	0.05
EO-BF/µl MOM × XE-5000	0.17	-	3.7 (-65.1 to 72.6)	0.03
HF-BF/µl MOM × XE-5000	0.72	y = 2 + 0.14× (-23.62 to 7; 0.07 to 0.7)	271.1 (-1191.5 to 1733.8)	0.06
Ascitic fluid				
Parameter	Spearman's correlation	Passing-Bablok regression (95% CI intercept; slope)	Bland-Altman bias (95% CI)	Mann-Whitney (*p* value)
TC-BF/µl OM × XE-5000	0.93	y = -2 + 1.02× (-22.04 to 10.5; 0.9 to 1.16)	30.8 (-1,772.2 to 1,833.8)	0.94
WBC-BF/µl OM × XE-5000	0.95	y = 9.19 + 1.14× (-1.05 to 19.94; 1 to 1.22)	-305.4 (-2,729.5 to 2,118.7)	0.55
MN-BF/µl OM × XE-5000	0.81	y = 9.13 + 1.05× (-8.78 to 24; 0.72 to 1.31)	-21.8 (677.8 to 634.3)	0.07
PMN-BF/µl OM × XE-5000	0.76	y = 5.56 + 1.11× (3.04 to 8.09; 0.98 to 1.23)	-283.3 (-2,683.6 to 2,117)	0.001
EO-BF/µl OM × XE-5000	-0.04	-	-6.2 (-47.2 to 34.8)	0.001
HF-BF/µl OM × XE-5000	0.53	y = 0.86 + 0.15× (-1.95 to 2.25; 0.06 to 0.26)	339 (-2,781.1 to 3,459.1)	0.005

OM: manual optical microscopy; TC-BF: total nucleated cell; WBC-BF: body fluid white blood cell count; MN-BF: body fluid mononuclear cell count; PMN-BF: body fluid polymorphonuclear cell count; EO-BF: body fluid eosinophil count; HF-BF: body fluid high-fluorescence cell count; CI: confidence interval.

negative proportional bias of 86%. At MDL for diagnosis of pleural effusion, XE-5000 counted approximately 1,096 TC-BF/µl (y = 15.98 + 1.08×, with × = 1,000; Figure 1A and Table 3), what corresponds to a systematic error of 9.6%. One can note by the Bland-Altman analysis that XE-5000 counted on average approximately 400 cells more than OM at the counting magnifications (Figure 1B and Table 3). The PPV to identify high-fluorescence cells (macrophages + mesothelial cells + plasma cells + neoplastic cells) found for the HB-BF parameter was 94.74% (95% CI – 73.97-99.87), and the PLR found for HF-BF demonstrated low accuracy, because it indicated that the occurrence of HF-BF positive values (> 0) in samples containing any high-fluorescence cell observed by OM was just 1.89 (95% CI – 0.47-7.61) times higher than in samples with HF-BF = 0. Malignant cells were detected in three of the analyzed samples, what was confirmed by cytology. In these three samples, HF-BF count was higher than three cells per microliter on XE-5000. The diagnosis of lung cancer was confirmed in two samples, and the third sample was confirmed as plasma cell leukemia. **Figure 2** shows a scattergram of the sample from the patient with plasma cell leukemia, exhibited on XE-5000.Those malignant cells showed high fluorescence and were displayed at the high part of the Y axis.

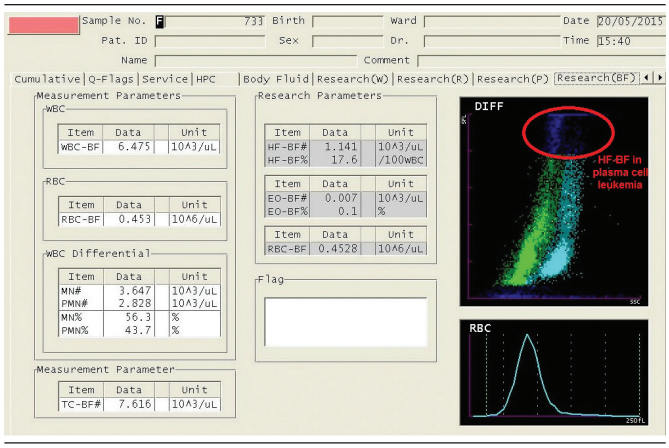

FIGURE 2 – *Scattergram displayed on XE-5000 from a pleural effusion with plasma cells in plasma cell leukemia*

Ascitic fluid

Thirty-five samples were analyzed, with a manual count of total cells that ranged from 50 to 42,600/µl (mean 2,605 ± 9,627/µl). The samples were sent to laboratory predominantly by emergency (22.85%), followed by the internal medicine department (17.14%), and ICU (14.29%). Figure 1 (C and D) shows a comparison of TC-BF counts on XE-5000 with OM for ascitic fluid samples. A very high correlation between both methods was observed just for TC-BF and WBC-BF; for the other parameters, including PMN-BF, statistically significant differences were observed, as can be seen in Table 3. For PMN-BF, Spearman's correlation at MDL for diagnosis of spontaneous bacterial peritonitis was moderate ($\rho = 0.66$, $p < 0.001$), and XE-5000 counted approximately 283 PMN-BF/µl (y = 5.56 + 1.11×, with × = 250; Figure 1C and Table 3), what corresponds to a systematic error of 13.2%. One can note by Bland-Altman analysis that XE-5000 counted on average approximately 31 cells less than OM at the counting magnifications (Figure 1D and Table 3). PPV for the HF-BH parameter was 96.97% (95% CI – 84.24-99.92), and PLR was 1.88 (95% CI – 0.47-7.55).

DISCUSSION

The latest generation automated analyzers, both hematology and urine analyzers, offer laboratories an alternative to increase speed, precision and exactness in BF analysis, besides enabling decreased costs. Although imprecision in low counts is high using automated analyzers (30% or less for counts from 15 to 30 cells/µl), it is still lower than that observed in manual counts done by hemocytometers (as high as 45% for a wide range of values). Moreover, there is wide variability between observers (2.5%-116% for leukocytes > 300/µl). Manufacturers of each analyzer must inform which types of biological fluids have been approved by a regulatory agency to be analyzed, as well as the parameters that were validated to be reported[4, 8, 10, 12, 21-25].

Biological fluids have a matrix different from whole blood and may contain different cell types. Therefore, providing evidence that an analyzer is able to report reliable results for samples of biological fluids is a good laboratory practice, besides being a clinical laboratory duty according to regulatory health care entities in the world (FDA, Health Canada, Japan Ministry of Health, CE Marking) and in Brazil [Agência Nacional de Vigilância Sanitária (Anvisa) RDC no. 302/2005). The first step before introducing automation in laboratory routine is the validation and/or verification of performance characteristics of analyzers. Generally validation is performed by the manufacturer, but in case it has not been carried out for a certain method, it must be done by the laboratory, following adequate recommendations[6, 10]. This study verified the analytical performance of XE-5000 in samples of pleural and peritoneal fluids. Automated body fluid analysis on XE-5000 is an easy procedure, without the need for pre-treatment of the sample, and the swift from whole blood mode to biological fluid mode is fast (< 2 min), allowing the interruption of routine blood counts in order to perform an urgent BF analysis.

Data showed extended linearity for TC-BF and WBC-BF during the counting interval of XE-5000, which was higher

than the specified by the manufacturer[20] and by Williams *et al*. (2011)[26] (13,900 WBC-BF/µl) and Boer *et al*. (2009)[7] (10,000 WBC-BF/µl). The encountered linearity of up to 25,825 WBC-BF/µl and up to 25,920 TC-BF/µl covers, with exactness and without necessity of previous dilutions, most samples of BF analyzed in our laboratory. Linearity for TC-BF and PMN-BF was very satisfactory at MDL. Both proportional and constant bias observed were not critical in assessing linearity and possibly resulted from imprecision in automated counts of hypocellular samples, possible presence of cellular debris, of small dilution errors, and matrix effect. The carryover observed in this study (0.18%) is important to ensure that a high-count sample does not cause falsely raised results in a subsequently analyzed sample, and that the possible presence of bubbles in the analyzer tubes after a background check does not provide a falsely decreased result due to dilutional effect. Although the carryover for WBC-BF was lower than that specified by the manufacturer, it was higher than the encountered by Bottini *et al*. (2015)[24] and Williams *et al*. (2011)[26]. The LLoQ for leukocyte count on XE-5000 was established as 22 WBC-BF/µl and represents the lowest leukocyte count performed with acceptable precision and exactness. However, this result was higher than those found by Jonge *et al*. (2010)[11] (10 WBC/µL), Paris *et al*. (2010)[4] (20 WBC/µl), and Boer *et al*. (2009)[7] (20 WBC/µl).

For repeatability assessment, nine BF samples with different concentrations were used, so samples had the same characteristics as clinical samples. Besides, they were analyzed 10 consecutive times, what allowed for a valid statistical analysis. The general performance in this item was very satisfactory, but in low cell counts a high CV was observed, as expected[24, 26]. As XE-5000 loses precision in the low counting range, samples with low leukocytes (< 10 WBC-BF/µl) should be recounted by OM, and in samples containing < 40 WBC-BF/µl differential automated count should not be reported.

The good correlation found between the methods for total count of nucleated cells and leukocytes in body cavity fluids in this study confirms previous studies[4, 11, 23, 24, 26, 27]. The automated analysis for TC-BF and WBC-BF in cavity fluids is an excellent alternative, because it can avoid the errors inherent in manual cell count[10]. Since including high concentrations of cells in the data may hide the actual performance in the upper limits of medical decision, TC-BF was tested to the MDL for diagnosis of pleural effusion, which showed a very satisfactory performance. As described by other authors[24, 27], we also observed significant difference between the methods in the differentiation of mononuclear cells in both assessed body cavity fluids, with XE-5000 displaying tendencies towards nonlinearity and overestimation (Table 3). This can be attributed to differences in categorization of cells, once in the MN-BF parameter lymphocytes, monocytes, and macrophages with low fluorescence are included, and at OM only lymphocytes and monocytes were included[24].

As opposed to what was demonstrated in another study[27], we did not find good general correlation between methods for polymorphonuclear cell counts in the pleural fluid, just in the ascitic fluid. However, in both body cavity fluids, XE-5000 counted on average more polymorphonuclear cells than OM, what represents higher sensitivity of automation to indicate infectious and inflammatory diseases in body cavities. It should be noted that the discrepancy observed in the correlation of counts of polymorphonuclear cells in the pleural fluid may have occurred due to the design of the study as to data analysis, since we compared the counts in pleural and ascitic fluids separately, a fact that may have made the statistical analysis less robust due to low sampling. Technical limitations of manual differential count [cell loss during cytocentrifugation, variation in cell shape and size due to centrifugation forces and these cells forming clusters on the slide[25]] must also be considered possible sources of error. In spite of that, XE-5000 provides a fast two-part differential count, which can be useful in cases of urgency[24].

EO-BF count did not show good correlation with the manual method as well, and this may have happened due to a low number of these cells in these types of samples. The HF-BF count on XE-5000 did not present good correlation when compared with OM. However, a high PPV was observed in both cavity fluids. As the HF-BF parameter can represent malignant cells, it is essential to carefully investigate the samples with positive HF-BF by OM, since high mortality occurs in these diseases. Thus, the number of false-negative cases must be minimized, and with this purpose HF-BH may present the highest possible sensitivity. All samples with malignant cells demonstrated increased HF-BF count, confirming other authors[4, 26]. Thus, the presence of HF-BF can be used as a potential screening tool[1, 24, 26, 27]. Moreover, the histogram and the scattergram displayed at XE-5000 must be always analyzed to evaluate if there was any interference or flag. In such cases, OM must be employed to confirm cell count.

CONCLUSION

XE-5000 perfectly fits the routine of a large hospital to analyze body cavity fluids. All the peritoneal and ascitic fluids in our service can be analyzed on XE-5000 and have TC-BF and WBC-BF reported. TC-BF and PMN-BF parameters may be trustingly

reported at MDL for diagnosis of pleural effusion and spontaneous bacterial peritonitis, respectively. Histograms and scattergrams must always be critically analyzed so as to detect abnormalities that need a microscopic review, which continues to be the leading test to enable more appropriate medical approaches, especially in cases of suspected malignancy. HF-BF provides invaluable information for screening, as well as for the definition of rules of validation of automated counts. The adequate implementation of automated body cavity fluid analysis may exert positive impacts on laboratories, especially in relation to human and financial resources, and time until result release.

ACKNOWLEDGEMENTS

The authors thank the team of the hematology laboratory of HC-UFPR for the aid in the conduction of this study.

RESUMO

Introdução: *A análise de líquidos corporais é fundamental para o diagnóstico e o acompanhamento de várias condições patológicas. Devido às limitações da contagem manual de células, tem ocorrido maior interesse no desenvolvimento da análise automatizada de líquidos corporais.* ***Objetivo:*** *Verificar o desempenho analítico do analisador hematológico Sysmex XE-5000 na análise de fluidos pleurais e ascíticos na rotina laboratorial de um hospital universitário de grande porte.* ***Métodos:*** *Um total de 56 amostras (35 de líquidos ascíticos e 21 de líquidos pleurais) foi analisado por microscopia ótica manual (MOM) e pelo XE-5000. O estudo de verificação incluiu linearidade,* carryover*, limite de quantificação e comparação de amostras de pacientes.* ***Resultados:*** *O estudo de verificação mostrou linearidade de até 25.825 WBC-BF/ml (*$r^2 = 0,999$*); WBC-BF,* carryover *de 0,18%; e o menor limite de quantificação foi fixado em 22 WBC-BF/ml. Boas correlações entre o método manual e o automatizado foram observadas apenas para as contagens de total de células nucleadas (TC-BF) e glóbulos brancos (WBC-BF) em líquidos pleurais e ascíticos. A contagem de células de alta fluorescência (HF-BF) mostrou correlação fraca, porém valor preditivo positivo (VPP) elevado para ambos os líquidos corporais (94,74% pleural; 96,97% ascítico).* ***Conclusão:*** *O XE-5000 fornece contagem confiável para TC-BF, WBC-BF e células polimorfonucleares (PMN-BF) em fluidos pleurais e ascíticos em níveis de decisão médica, contudo a diferenciação morfológica deve continuar a ser realizada por MOM. O histograma e o diagrama de dispersão exibidos no XE-5000 devem ser sempre avaliados quanto à existência de qualquer interferência ou alerta suspeito. A contagem de HF-BF constitui uma potencial ferramenta para triagem.*

Unitermos: *líquidos corporais; contagem de células; derrame pleural; líquido ascítico; automação laboratorial.*

REFERENCES

1. Hussong JW, Kjeldsberg CR. Kjeldsberg's body fluid analysis. Chicago, IL: ASCP Press; 2015.

2. Comar SR, Machado NA, Schulz T, França FS, Haas P. Análise citológica do líquido pleural no Hospital de Clínicas da Universidade Federal do Paraná (UFPR). Estud Biol. 2008; 30(70/71/72): 17-25.

3. Comar SR, Schulz T, Machado NA, França FS, Haas P. Análise citológica do líquido peritoneal. Estud Biol. 2010/2011; 32/33(76-81): 73-9.

4. Paris A, Nhan T, Cornet E, Perol JP, Malet M, Troussard X. Performance evaluation of the body fluid mode on the platform Sysmex XE-5000 series automated hematology analyzer. Int J Lab Hematol. 2010; 32(5): 539-47.

5. Van Acker JT, Delanghe JR, Langlois MR, Taes YE, De Buyzere ML, Verstraete AG. Automated flow cytometric analysis of cerebrospinal fluid. Clin Chem. 2001; 47(3): 556-60.

6. Bourner G, De la Salle B, George T, et al. ICSH guidelines for the verification and performance of automated cell counters for body fluids. Int J Lab Hematol. 2014; 36(6): 598-612.

7. Boer K, Deufel T, Reinhoefer M. Evaluation of the XE-5000 for the automated analysis of blood cells in cerebrospinal fluid. Clin Biochem. 2009; 42(7-8): 684-91.

8. Sandhaus LM, Ciarlini P, Kidric D, Dillman C, O'Riordan M. Automated cerebrospinal fluid cell counts using the Sysmex XE-5000: is it time for new reference ranges? Am J Clin Pathol. 2010; 134(5): 734-8.

9. Buoro S, Gustinetti R, Dominoni P, et al. Analytical evaluation of Sysmex UF-1000i for flow cytometric analysis of peritoneal fluid. Clin Biochem. 2012; 45(15): 1263-5.

10. Sandhaus LM. Body fluid cell counts by automated methods. Clin Lab Med. 2015; 35(1): 93-103.

11. de Jonge R, Brouwer R, de Graaf MT, et al. 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(5): 665-75.

12. Clinical and Laboratory Standards Institute (CLSI). Body fluid analysis for cellular composition: approved guideline. Wayne, PA: CLSI Document H56-A; 2006.

13. Clinical and Laboratory Standards Institute (CLSI). Reference leukocyte (WBC) differential count (proportional) and evaluation of instrument methods: approved standard. 2nd ed. Wayne, PA: CLSI Document H20-A2; 2007.

14. 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 leukocyte and reticulocyte counting. Int J Lab Hematol. 2014; 36(6): 613-27.

15. Perné A, Hainfellner JA, Womastek I, Haushofer A, Szekeres T, Schwarzinger I. Performance evaluation of the Sysmex XE-5000 hematology analyzer for white blood cell analysis in cerebrospinal fluid. Arch Pathol Lab Med. 2012; 136(2): 194-8.

16. Zimmermann M, Otto C, Gonzalez JB, Prokop S, Ruprecht K. Cellular origin and diagnostic significance of high-fluorescent cells in cerebrospinal fluid detected by the XE-5000 hematology analyzer. Int J Lab Hematol. 2013; 35(6): 580-8.

17. Broughton P. Carry-over in automatic analyzers. J Autom Chem. 1984; 6(2): 94-5.

18. Clinical and Laboratory Standards Institute (CLSI). Evaluation of the linearity of quantitative measurement procedures: a statistical approach: approved guideline. Wayne, PA: CLSI document EP06-A; 2003.

19. Clinical and Laboratory Standards Institute (CLSI). Evaluation of precision of quantitative measurement procedures: approved guideline. 3rd ed. Wayne, PA: CLSI Document CLSI EP05-A3; 2014.

20. Sysmex Corporation. Operator's manual. Automated hematology analyzer XE-5000. Main Unit. Kobe, Japan: North American Edition; 2011.

21. Aulesa C, Mainar I, Prieto M, Cobos N, Galimany R. Use of the Advia 120 hematology analyzer in the differential cytologic analysis of biological fluids (cerebrospinal, peritoneal, pleural, pericardial, synovial, and others). Lab Hematol. 2003; 9(4): 214-24.

22. Butch AW, Wises PK, Wah DT, Gornet TG, Fritsche HA. A multicenter evaluation of the Iris IQ200 automated urine microscopy analyzer body fluids module and comparison with hemocytometer cell counts. Am J Clin Pathol. 2008; 129(3): 445-50.

23. Lippi G, Cattabiani C, Benegiamo A, et al. Evaluation of the fully automated hematological analyzer Sysmex XE-5000 for flow cytometric analysis of peritoneal fluid. J Lab Autom. 2013; 18(3): 240-4.

24. Bottini PV, Pompeo DB, Souza MI, Garlipp CR. Comparison between automated and microscopic analysis in body fluids cytology. Int J Lab Hematol. 2015; 37(2): e16-8.

25. 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(11): 1689-1706.

26. Williams JE, Walters J, Kabb K. Gaining efficiency in the laboratory – automated body fluid cell counts: evaluation of the body fluid application on the Sysmex XE-5000 hematology analyser. Lab Med. 2011; 42(7): 395-401.

27. Buoro S, Appassiti Esposito S, Vavassori M, et al. Reflex testing rules for cell count and differentiation of nucleated elements in pleural and ascitic fluids on Sysmex XE-5000. J Lab Autom. 2016; 21(2): 297-304.

CORRESPONDING AUTHOR

Samuel Ricardo Comar

Laboratório de Hematologia; Unidade de Laboratório de Análises Clínicas; Complexo Hospital de Clínicas; Universidade Federal do Paraná; Rua Padre Camargo, 280; Alto da Glória; CEP: 80060-240; Curitiba-PR, Brasil; Phone: +55 (41) 3360-7973; e-mail: srcomar@ufpr.br.