Research Article

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Cell counting chamber vs. Sysmex XN-1000 for determining white blood cell count and differentiation for body fluids

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Abstract

Objectives: Sterile body fluids (BFs) include key information for the diagnosis and monitoring of a variety of diseases. A cornerstone test is the total white blood cell (WBC) count, which comprises the differential of WBC of body fluid analysis. It is important to test automated hematology analyzers that should be verified using patient samples. The aim of this study is to compare both the performance of the Sysmex XN-1000 system's body fluid module for cell counting and differentiation to the results of a cell counting chamber.

Methods: This study was performed on 200 routinely laboratory sent BFs. Cell counts and differentiation were determined with both bright-lined Neubauer Cell Counting Chamber and Sysmex XN-1000 system body fluid mode.

Results: The correlation coefficients of WBC count by two methods indicated very strong correlation ($r \geq 0.90$, $p < 0.0001$) for any specimen except pleural fluid. According to Passing Bablok regression analysis, Sysmex XN-1000 showed acceptable performance according to bias of the slope criteria (<±20).

Conclusions: The XN-1000 hematological analyzer's body fluid mode can rapidly count and identify cells, and it can be used as a simple and rapid screening method for laboratory testing of sterile body fluids, particularly in laboratories with a massive quantity of biological fluids.

Keywords: cell counting chamber; cerebrospinal fluid; sterile body fluids; synovial fluid; Sysmex XN-1000.

Introduction

Biological or sterile body fluids (BFs) provide crucial information for the diagnosis and monitoring of a wide range of disorders, as well as for determining the best therapy options. The total white blood cell (WBC) count, including differential of WBC and malignant cell characterization of body fluid analysis, is regarded a cornerstone test in patients with inflammatory, infectious, and neoplastic disorders. Significant treatment parameters include the amount of white blood cells (WBCs), as well as their differentiation into mononuclear (MN) or polymorphonuclear leukocytes (PMNL) [1, 2].

WBCs play a crucial role as a part of immune system against pathogens. Neutrophils are the first line defense mechanisms especially against bacterial infections. Antigens are recognized by lymphocytes, and monocytes have a variety of activities in the immune system, such as transforming into phagocytic macrophages. Numerous cells can be found in BFs and these cells reflect important informations for inflammatory diseases. Currently, manual microscopy, automated flow cytometry, and automated impedance technology for counting and differentiating WBCs in BFs are used. Conventional manual microscopy is still the gold standard, although automated analyzers have become prevalent in clinical laboratories in the recent decade. The hemocytometer, commonly known as a "counting chamber" is commonly used to determine the total WBC and RBC concentrations in BFs. For determining the MNs and PMNs

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of WBC a stained slide is prepared and investigated under light microscope [3, 4].

Although conventional manual microscopy is still the gold standard, and commonly performed in medical microbiology laboratories, it has some limitations such as samples showing low cellularity may be highly inaccurate. While the accuracy of the count depends on highly qualified laboratory staff and as a con it is time consuming [4].

For the cytometric analysis of biological fluids, a variety of commercial automated hematological analyzers that can also count sterile body fluids which requires special modules have been on the market in recent years. Automated systems provide two key benefits over manual systems: faster response times and reduced interobserver variability. Additionally, their main advantage is that they can even analyze small sample sizes [5].

The International Standards Council for Hematology (ICSH) pointed out that the performance of an automated hematology analyzers (AHA) should be verified using patient samples [1, 6].

Although studies on cell counting for blood are widely performed, studies evaluating cell count and differentiation especially in body fluids (other than blood and urine) in automated hematology analyzers are limited [7, 8].

The aim of this study is to compare both the performance of the Sysmex XN-1000 system's body fluid module for cell counting and differentiation to the results of a cell counting chamber.

Materials and methods

Samples

This study was performed on 200 routinely collected sterile body fluid samples from hospitalized- and out-patients. These samples comprised of 93 (46.5%) synovial fluid, 78 (39%) CSF, 22 (11%) peritoneal fluid, 7 (3.5%) pleural fluid samples. CSF samples were collected in sterile tubes while others were collected in tubes coated with K_2EDTA (Becton Dickinson, USA). Macroscopically clotted, viscous, mucoid, cloudy, and bloody samples were not enrolled in this study. The BFs were transported to the laboratory at ambient temperature in 1 h. All samples were examined within 1 h after being received to the laboratory.

Cell counts and differentiations were simultaneously determined with both bright lined Neubauer Cell Counting Chamber® (Marienfeld, Germany) and Sysmex XN-1000 (Sysmex, Japan) hematology analyzer using body fluid mode according to the manufacturers' recommendations. Only the samples with cell counts within the analytical limits (0.03–440.00 10^3xcell/μL) were included in the study, and no dilutions were applied. For the Sysmex XN-1000 analyzer, three levels of whole blood internal quality control materials provided by the manufacturer were analyzed daily. The analytical coefficients of variations (CVs) for the study period were as follows: 2.31% for level 1 (mean = 3.07 10^3x cell/μL), 1.56% for level 2 (mean = 7.36 10^3xcell/μL) and 1.19% for level 3 (mean = 16.6 10^3xcell/μL) for WBC count. For neutrophil, lymphocyte, monocyte, eosinophil, and basophil differential percentage CVs were under 8%. During the study period Randox Quality Control (Country Antrim, United Kingdom) external quality assurance (EQA) program was used and all bias values within the limits of the EQA program.

Manual microscopic examination was performed with light microscope using an eyepiece set at 10X and a lens set at 40X, for a total magnification of 400X. All samples were evaluated by the same medical microbiology specialist. Differential counting of 200 samples was performed centrifugation at 300 g, for 5 min and stained with Wright-Giemsa staining. The differential count as polymorphonuclear leukocytes (PMNL) was performed at 400X magnification on 100 cells per each sample. The Sysmex XN-1000 was used in body fluid mode both for the counting of cells and percentage of PMNL. Cell count was evaluated in cells per milliliter and cell distribution was expressed as percentage distribution.

Ethical statement

Ethical approval was waived by the local Ethics Committee of Ankara University School of Medicine in view of the retrospective nature of the study (I07-409-22).

Statistical analysis

Shapiro-Wilk test was performed and showed that the distribution of results departed significantly from normality ($p<0.001$) Therefore, non-parametric tests were used, and the median with 25th and 75th percentile were given for descriptive statistics.

For method comparison, the Spearman correlation coefficient [9] was calculated and Passing-Bablok linear regression analysis was performed. The Bland-Altman plots were also utilized, and absolute mean differences were calculated. Percentage bias of the Passing-Bablok equation slope were used to assess the agreement between the methods and ±20% was used as the agreement limit as suggested by Cho et al. [10]. p values < 0.05 were considered significant. The 95% confidence intervals (CIs) of each test were also calculated.

The Passing-Bablok regression analysis and the Bland-Altman deviation analysis were performed using Microsoft Excel 2010 (Microsoft Corp., Redmond, Washington, USA), and Jamovi software. Descriptive statistics were calculated using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA).

Results

In our study, 200 body fluid samples were counted in the manual method and XN-1000 series body fluid mode. Descriptive statistics of the cell enumerations for each specimen type were given in the Table 1.

Method comparison results for all specimen types are summarized in Table 2. For CSF and synovial fluids, Passing-Bablok and Bland-Altman plots are shown in Figures 1 and 2, respectively. For other fluid types related plots are provided in the Supplementary Material.

Table 1: Descriptive statistics of the cell count and types for each specimen type.

		Manual method					Sysmex XN-1000				
		Min	Max	Median	Percentile		Min	Max	Median	Percentile	
					25th	75th				25th	75th
CSF (n=78)	WBC (/µL)	0	1.200	30	3	100	0	1.270	43	4	116
	PMNL (%)	0	100	10	0	60	0	100	26	0	63
	MNL (%)	0	100	24	0	70	0	100	37	0	74
Synovial fluid (n=92)	WBC (/µL)	20	88.800	2.000	280	5.700	23	73.600	1.890	400	6.360
	PMNL (%)	0	100	65	20	86	2	98	72	27	89
	MNL (%)	0	100	35	14	80	2	98	28	11	70
Peritoneal fluid (n=22)	WBC (/µL)	0	88.000	300	166	1.540	2	78.000	321	242	1.720
	PMNL (%)	0	98	20	10	34	0	95	20	14	40
	MNL (%)	0	100	78	61	90	0	97	76	42	86
Pleural fluid (n=7)	WBC (/µL)	125	1.000	150	129	275	133	1.100	230	200	323
	PMNL (%)	0	97	1	0	50	6	88	8	7	45
	MNL (%)	3	100	99	50	100	12	94	92	55	93

CI, confidence interval; CSF, cerebrospinal fluid; Min, minimum; Max, maximum.

Table 2: Method comparison results for each specimen type.

		Spearman's correlation		Passing-Bablok Regression		Bland-Altman plot	
		p	r (95% CI)	Slope (95% CI)	Intercept (%95 CI)	% Bias of the slope	Mean absolute difference
CSF	WBC (/µL)	<0.0001	0.96 (0.94–0.98)	1.13 (1.05–1.23)	0.38 (0.00–2.65)	13.0	–17.3
	PMNL (%)	<0.0001	0.75 (0.64–0.84)	1.00 (0.95–1.07)	0.00 (0.00–2.09)	0	–5.1
	MNL (%)	<0.0001	0.72 (0.60–0.82)	0.97 (0.92–1.00)	0.00 (0.00–1.17)	3.00	–5.4
Synovial fluid	WBC (/µL)	<0.0001	0.95 (0.93–0.97)	0.99 (0.94–1.02)	35.2 (7.77–95.25)	1.00	279
	PMNL (%)	<0.0001	0.95 (0.92–0.97)	0.94 (0.86–0.99)	6.68 (2.96–10.50)	6.00	–4.26
	MNL (%)	<0.0001	0.95 (0.92–0.97)	0.93 (0.87–0.98)	–0.33 (–1.84–1.71)	7.00	4,70
Peritoneal fluid	WBC (/µL)	<0.0001	0.92 (0.83–0.97)	0.89 (0.71–1.09)	52.27 (–0.31–108)	11.0	969
	PMNL (%)	<0.0001	0.90 (0.78–0.96)	1.00 (0.85–1.28)	1.00 (–4.69–4.77)	0	–1.59
	MNL (%)	<0.0001	0.91 (0.79–0.96)	0.98 (0.84–1.13)	1.43 (–8.33–11.00)	2.00	1.14
Pleural fluid	WBC (/µL)	0.0744	0.71 (0.094–0.95)	1.10 (n/c)	18.6 (n/c)	10.0	–60.9
	PMNL (%)	0.0335	0.79 (0.098–0.97)	0.83 (n/c)	6.0 (n/c)	17.0	–1.14
	MNL (%)	0.0335	0.79 (0.098–0.97)	0.82 (n/c)	11.19 (n/c)	18.0	1.14

CI, confidence interval; CSF, cerebrospinal fluid; r, Spearman's correlation coefficient; n/c, confidence intervals cannot be calculated due to low sample count.

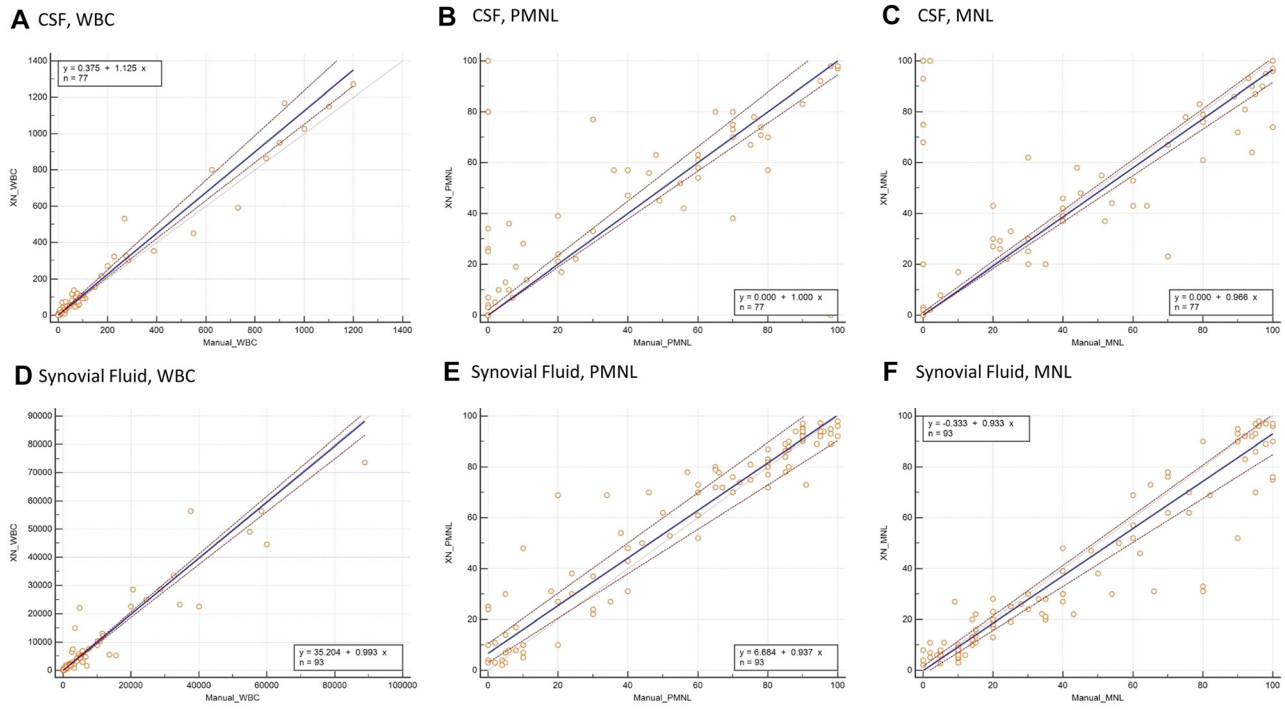


Figure 1: Passing-bablok plots for the CSF and synovial fluids.
CSF, cerebrospinal fluid; MNL, mononuclear leukocytes; PMNL, polymorphonuclear leukocytes; WBC, white blood cells. Identity lines (y=x) are gray, confidence intervals are dashed red and regression lines are blue.

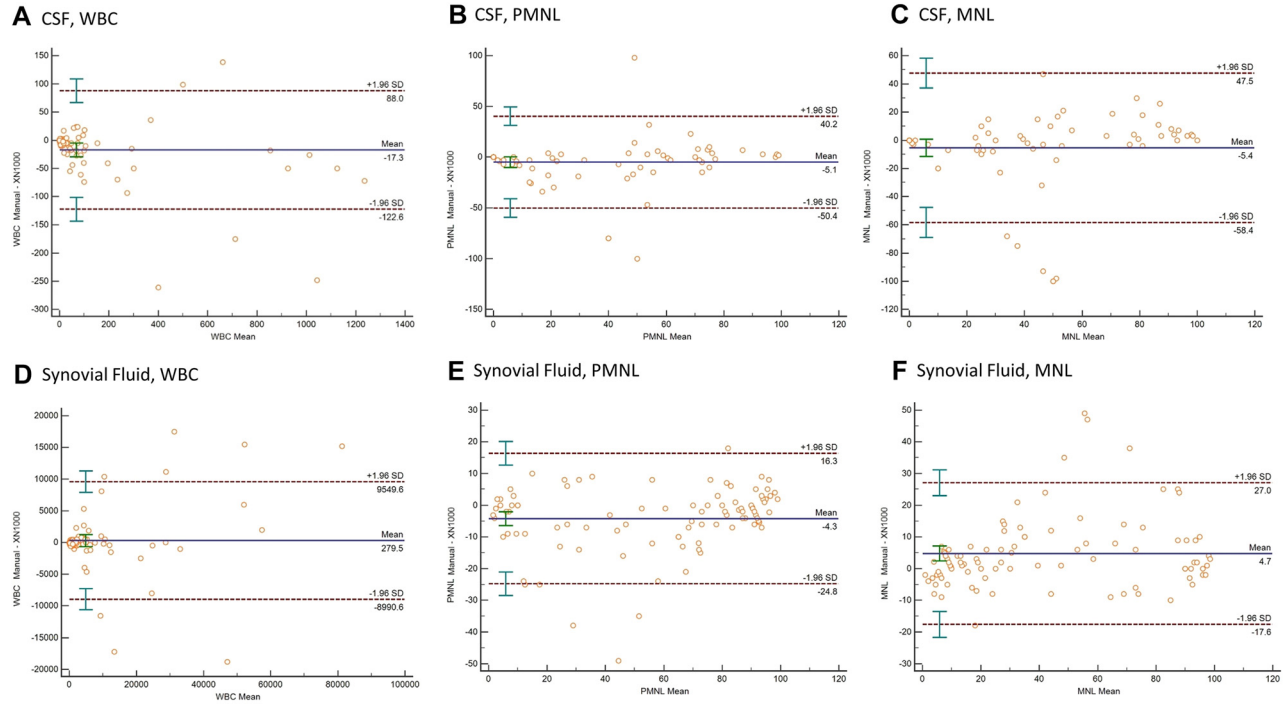


Figure 2: Bland-altman plots for the CSF and synovial fluids.
CSF, cerebrospinal fluid; MNL, mononuclear leukocytes; PMNL, polymorphonuclear leukocytes; WBC, white blood cells. Mean absolute differences are blue, 95% limits of agreement are dashed red, confidence intervals are cyan.

According to the results of Spearman's correlation results for WBC cell enumeration, there were statistically significant ($p<0.0001$) and very strong correlation ($r\geq0.90$) between the manual chamber counting and the XN-1000 BF mode for all BF types except pleural fluid samples. The correlation for WBC count for pleural fluid is strong although p value is >0.05 due to possible unsufficient sample size ($r=0.71$, $p=0.0744$). When Passing-Bablok regression results were evaluated, Sysmex XN-1000 demonstrated acceptable performance according to bias of the slope criteria and all percentage bias of the slope values were under 20%. Moreover, for CSF and peritoneal fluids PMNL and MNL results met the Passing-Bablok method agreement criteria (95% CI for the intercept, covers zero, and CI for the slope, covers one). However, for the synovial fluid WBC count results CIs covered one for the slope but does not cover zero for the intercept.

The Bland-Altman plots show that Sysmex XN-1000 presented slightly higher cell count results compared to the manual method and for the CSF samples. Additionally, there was no apparent increasing or decreasing trend for any BF type in Bland Altman plots.

Discussion

In the current study, the cell count and differentiation of PMNL in significant clinical sterile BFs other than urine and blood were examined using the Sysmex XN-1000 BF mode and a conventional cell counting chamber. Our results showed that Sysmex XN-1000 had comparable results with manual WBC and differential count which is considered as gold standard method for cell enumeration.

This finding is supported by evaluation of the Passing-Bablok regression results and Bland-Altman analysis. For all BF types and test results, % bias of the regression analysis slope values were lower than predefined acceptability criteria (within 20%). Furthermore, Bland-Altman analysis showed relatively low bias for differential counting (1.14–5.4%).

Owing to lack of data in this field, it is critical to assess the performance of various automated cell counting and differential technologies [7]. Lee et al. reported that for cerebrospinal fluid, ascitic fluid, and pleural fluid sample types the XN-350s cell enumeration and differential counting correlated well with manual chamber counting (except for differential counting in CSF samples). For WBC count in CSF, they reported –16 cell/μL absolute bias with the 95% CI of –36 to 2 [5]. These results were similar with our finding (Absolute bias for WBC = –17 cell/μL). Our cell differential count for PMNL and MNL had similar but lower absolute bias compared to their results (–5.1% vs.–16% for PMNL and –5.4 vs. 27 for MNL).

Lohajaroensub et al. has also reported that except for synovial fluid, the Sysmex XT-4000i performed well in terms of basic body fluids assessment [11]. Evren et al. have reported that for macroscopically purulent samples, counting chambers would still be the best choice for WBC counting, however for macroscopically non-purulent samples Sysmex UF-1000i is a good alternative instrument for rapid workflow [12]. Moreover, these systems even enable the detection of malignant cells in serous body fluids. However, it is emphasized that research on this area still needs to be evaluated [13].

Total WBC and differential count by XN-1000 had a high correlation with the conventional manual microscopy approach. Due to the high cellular content of the CSF samples evaluated in this study, drawing inferences about low cellularities was challenging. Cell differentiation may be insufficient in general due to the low cellularity of CSF samples, particularly for samples with low cellular content.

A differential cell count for sterile body fluids indicates the proportion of each type of white blood cell and aids in guiding therapy. For this purpose, modern hematology analyzers combine multiple techniques to evaluate cell counting and subgroups percentage of leucocytes, as well. Identifying the types of cells present in sterile body fluids such as joint fluid, CSF, pleural and peritoneal fluids except urine and blood provides valuable diagnostic information. For this reason, the analysis of sterile body fluids is important for rapid presumptive diagnosis. The laboratory analysis of these samples should include both cell counting and a differential cell count. The gold standard method for cell counting is the cell counting chambers while the differential cell count should be performed on a stained smear [5, 14].

Hematology analyzers were designed to measure cells in whole blood at first. Automated cell counters have led to significant improvements in BF analysis despite the fact that their use is limited and not widely preferred since their inception in clinical laboratories, and these have received increasing acceptance as a crucial alternative to manual microscopy. WBCs in blood samples, on the other hand, are at least 1,000 times higher than in BF samples, making these analyzers unsuitable for BF analysis due to high imprecision in the lower concentration range [15, 16].

However, in recent years there are a lot of manufacturers on the market with BF cell counting tools (hematology and urine), and several manufacturers have increased their technological capabilities by incorporating specific

BF applications into their automated cell counters. Sysmex's latest hematology analyzer, the XN-Series, was launched in 2011. Sysmex analyzers such as XE-5000 and XN-series include separate BF modes and are fully automated systems that measures a variety of BFs and counts. Urinalysis analyzers, such as the Sysmex UF-Series and Iris iQ200, have risen in favor for BF analysis in addition to hematological analyzers. The Sysmex XN-1000 hematology analyzer is a fully automated flow cytometric system with validated body fluid modules for the evaluation of cellular contents of sterile body fluids. In this study, we have evaluated the performance of the body fluid module of Sysmex XN-1000 system in comparison with manual methods.

To date, there is a debate concerning whether to utilize automated cell counters or manual microscopy for BF cell counts and evaluate percentage of cellular subgroups. Many of their advantages, such as improved proficiency and workflow productivity, advantage of being able to assess small sample quantities like CSF have been proven in many studies, and they are recommended as acceptable alternative to the conventional microscopy [3, 14, 17–21].

Despite the benefits of hematology analyzers, considerable quantities of epithelial cells and macrophages in the PMNL count, making automated differentiation unreliable depending on the brand of the analyzer [18, 22, 23]. Additionally, overestimation of PMNL counts with low WBC counts was shown to be associated with certain cell debris or fragments in CSF samples that usually contain very few cells. We shouldn't evaluate the low counts for CSF in our study since the mean cell count was around 200 cells/microliter. In routine practice, these tests can be performed both by clinical biochemistry and microbiology experts. On the biochemistry side, the tests are mostly performed by automated systems such as hematology analyzers, while manual procedures are most often used on the microbiology side. Although manual procedures are mostly preferred, it is well known that these methods are time consuming and labor intensive. The use of automated systems in clinical microbiology laboratories has recently gained attention as an alternative method to microscopic examination.

One limitation of this study is related with evaluation of the calculated biases. In contrast to complete blood count analysis, body fluids do not have well defined analytical performance specifications. This is the most important challenge related to the body fluid method comparison studies. To overcome this problem, we used the predefined criteria recommended by Cho et al. [10]. Other limitation is, in the study body fluid specific control materials was not used and only commercial materials were evaluated. Final limitation of this study was the number of BF samples. Only 7 pleural fluid and 22 peritoneal fluid samples were included in the study which is lower than minimal requirement of 40 samples [23].

As a conclusion, clinical laboratories want to use automated and standardized analysis methods to reduce turnaround time and variability between laboratories. The body fluid mode on the XN-1000 hematological analyzer can count and identify cells immediately and can be used as a simple and rapid screening approach for laboratory testing of sterile body fluids, especially in laboratories with massive quantities of biological fluids. The widespread use of BF modes in automated hematology analyzers can reduce manual methods' high inter-observer variability, bias, and low reproducibility. For widespread usage, expanded samples including various BFs should be tested and verified for automated hematology analyzers. There is still need further investigations.

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