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A comparison of the analysis of 3 types of body fluids using the XN-350 hematology analyzer versus light microscopy assessment

Jiwon Lee, MD, Younggeun Cho, MD, PhD, Han-Sung Kim, MD, PhD, Hee Jung Kang, MD, PhD, Miyoung Kim, MD, PhD*, Young Kyung Lee, MD, PhD

Abstract

We evaluated the capacity of the XN-350 instrument to analyze 3 different types of body fluid samples under "body fluid mode."

The performance of XN-350 was evaluated in terms of precision, carryover, limit of blank, limit of detection, limit of quantification, and linearity. Cell enumeration and differential data produced by the XN-350 were compared to manual chamber counting results in 63 cerebrospinal fluid (CSF), 51 ascitic fluid, and 51 pleural fluid (PF) samples. Comparisons between XN-350 versus Cytospin data were also performed in PF samples.

The precision, carry-over, limit of blank, and linearity of the XN-350 were acceptable. The limits of detection for white blood cells (WBCs) and red blood cells were 1.0/μL, and 1,000.0/μL, respectively; the corresponding limits of quantitation (LOQs) were 5.0/μL and 2,000.0/μL, respectively. The XN-350's cell enumeration and differential counting correlated well with those of manual chamber counting for all 3 sample types (except for differential counting in CSF samples), particularly parameters involving monocytes ($r = 0.33$) and mononuclear cells (MO- body fluid [BF]; $r = 0.26$), as well as total cell (TC-BF) enumeration ($r = 0.50$) and WBC-BF ($r = 0.50$) in PF samples. The MO-BF in CSF samples differed significantly from manual chamber counting results, but neither TC-BF nor WBC-BF in PF samples did. The XN-350 also showed good correlations with Cytospin analyses for differential counting of neutrophils, lymphocytes, and monocytes in PF samples. The differential counting of eosinophils via the XN-350 and Cytospin were not significantly correlated, but the difference between them was not significant.

The XN-350 is an acceptable alternative to manual fluid analysis. Samples with low cellularity around the LOQ should be checked manually. Moreover, manual differential counting should be performed on CSF samples, particularity those with low cell numbers.

Abbreviations: AF = ascitic fluid, AHA = automated hematology analyzer, BF = body fluid, CI = confidence interval, CLSI = Clinical and Laboratory Standards Institute, CSF = cerebrospinal fluid, CV = coefficient of variation, ICSH = International Council for Standardization in Haematology, LOB = limit of blank, LOD = limit of detection, MN = mononuclear cells, PF = pleural fluid, PMN = polymorphonuclear cells, RBC = red blood cell, WBC = white blood cell.

Keywords: automated hematology analyzer, body fluid, chamber counting, correlation, cytospin, performance, XN-350

1. Introduction

Body fluid (BF) analysis, especially total white blood cell (WBC) count (with differential) and malignant cell detection, is considered a cornerstone test in patients with inflammatory, infectious, and neoplastic diseases.[1–3] Different types of BFs have distinct parameters used for identifying underlying clinical conditions. In pleural fluid (PF), approximately 80% of transudates will have cell counts less than 1000/μL, whereas counts above 10,000/μL are usually associated with parapneumonic effusions.[1] Exudative PF with a predominance of neutrophils reflects acute inflammation or parapneumonic effusion, whereas that with a predominance of lymphocytes

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Department of Laboratory Medicine, Hallym University Sacred Heart Hospital, Hallym University College of Medicine, Anyang, Republic of Korea.

** Correspondence: Miyoung Kim, Department of Laboratory Medicine, Hallym University Sacred Heart Hospital, Hallym University College of Medicine 22, Gwanpyeong-ro 170 beon-gil, Dongan-gu, Anyang-si, Gyeonggi-do, 14068, Republic of Korea (e-mail: rabbit790622@gmail.com).*

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suggests the presence of a tubercular infection, metastatic disease, lymphoproliferative disorder, or chylous effusion.[1,3] Eosinophilia is observed in certain conditions such as pulmonary emboli, hemothorax, immunoallergic reaction to chest tubes, parasitic diseases, and Churg-Strauss syndrome.[1] In ascitic fluid (AF), an absolute neutrophil value of >250/μL is indicative of spontaneous bacterial peritonitis;[1,3,4] in contrast, tuberculous peritonitis is usually characterized by a total nucleated cell count of >1000/μL with a predominance of lymphocytes.[1,3] In cerebrospinal fluid (CSF), the predominance of polymorphonuclear cells (PMNs) with increased WBCs suggests bacterial meningitis, whereas a predominance of mononuclear cells (MNs) suggests aseptic meningitis [3,5]. Moreover, it is particularly important to measure low-level cell counts in CSF accurately given that the upper limits of the WBC reference ranges in CSF samples are only 5/μL, 7/μL, and 27/μL in adults, children up to 16 years of age, and neonates, respectively.[1]

The gold standard protocol for enumerating and differentiating cells in BFs is the combination of manual chamber counting with improved Neubauer rules and Cytospin analysis;[1,3,6] nevertheless, these methods have some drawbacks. The accuracy of manual chamber counting depends on the sample volume, dilutions, and number of squares and cells counted.[3] Counting samples with low cellularity can be highly imprecise, while accuracy in enumeration requires highly trained laboratory personnel and is time-consuming.[6] The cytocentrifugation process involved in Cytospin analysis may result in the loss of cells during centrifugation,[7,8] aberrant cell morphology,[9] and clustering of macrophages or mesothelial cells in PF and AF.[10] Despite these drawbacks, the accurate and timely analysis of the cellular compositions of BFs is critical for facilitating prompt patient management.

Automated hematology analyzers (AHAs) equipped with a BF mode are a potential alternative to the manual method.[6,11] AHAs are designed to be faster, more precise, and easier to use than manual methods;[3] they also count more cells and are therefore more precise.[12] Among such AHAs, the XN system (Sysmex Corporation, Kobe, Japan) is equipped with a specific module for BF analysis (XN-BF).[13,14] It adopts a flow cytometry system using a semiconductor laser, and its ability to analyze differences in the intensities of scattered and fluorescent light from individual cells enables cell enumeration and identification.[15] The numbers of particles counted in the WBC and red blood cell (RBC) channels in BF mode are approximately 10- and 3-fold higher than those counted in the whole blood mode, respectively.[15] It is also claimed that the XN counts 3–10 times more cells passing through its detector than do previous-generation instruments, and is therefore expected to increase the precision of low WBC counts.[15]

The International Council for Standardization in Haematology (ICSH) [16] emphasized that an AHA's performance should be verified using patient samples, with results compared to the manual method, prior to its use for routine BF analysis.[1] The verification process should include precision, accuracy, sensitivity, specificity, and reportable range.[16] The correlation between AHAs and the manual method should be confirmed for each type of BF separately, as each has a different matrix and cell type composition than whole blood.[16,17] The Clinical and Laboratory Standards Institute (CLSI) guideline recommends that at least 40 samples that cover the analytical range (particularly medical decision-level ranges) should be tested.[16,17] However, according to a survey performed by the ICSH, the extent of verification varies widely among different laboratories that have introduced AHAs for the routine analysis of BFs.[16] Furthermore, only a few investigators have performed correlation analyses for the different types of BF samples in a particular AHA model.[16] Before introducing an AHA into clinical practice, a full-range verification procedure should be performed along with correlation analyses for different types of BFs, considering each type's unique properties, compositions, and values of clinical significance.

In this study, we performed a full-range verification of the XN-350 instrument in BF mode according to the ICSH guidelines and relevant CLSI documents.[1,17–19] We compared XN-350 reference results to those obtained via manual chamber counting and Cytospin analysis using different types of commonly requested BF samples including CSF, AF, and PF.

2. Materials and methods

2.1. Ethics

The study was approved by Institutional Review Board (IRB No.: HALLYM 2020-06-019), and the requirement for written informed consent was waived due to the observational and anonymized nature of the study. The study was performed in accordance with principles of the Declaration of Helsinki.

2.2. Clinical samples

A total of 165 clinical BF samples (63 CSF, 51 AF, and 51 PF samples) submitted for manual chamber counting at Hallym University Sacred Heart Hospital between October 2017 and November 2017 were investigated to compare the limit of detection (LOD), limit of quantification (LOQ), linearity, and methodology. Sample collection was performed according to the CLSI guideline.[1] CSF samples were collected in sterile tubes while AF and PF samples were collected in K_2EDTA tubes (Becton Dickinson, Franklin Lakes, NJ). Clotted, extremely viscous, and mucoid samples were not tested. The samples were processed within 2 hours of arrival.

2.3. Manual chamber counting

Manual cell enumeration and differential counting followed the standard operating procedures of our own laboratory, which are based on the CLSI documents H56-A,[1] H26-A2,[17] EP05-A3,[18] EP06-A,[19] and EP17-A2 [20] as well as the ICSH guidelines.[16] If fewer than 200 cells were present in the area of the 9 squares of the hemocytometer, cells in all 9 squares were counted; if more than 200 cells were present in the 9 squares, only the cells in the 4 corner squares were counted; and if more than 200 cells were present within a single square, cells inside the 5 smaller squares within the larger center square were counted. The standard Neubauer calculation formula was used to determine the number of cells per cubic millimeter.[1] WBCs were classified as either PMNs, lymphocytes, or monocytes according to the CLSI's morphologic criteria after staining with Turk solution.[1] Neutrophils, eosinophils, and basophils were classified as PMN cells because of the varying shapes of their nuclei. Lymphocytes (normal lymphocytes as well as plasma cells and atypical lymphocytes) and monocytes (including histiocytes) were classified as MN cells.

2.4. Cytospin analysis

Differential counting of 30 samples was performed using a Cytospin; the samples were centrifuged (1,500 rpm for 5 minutes)

in a Shandon Cytospin 3 (Thermo Fisher Scientific, Massachusetts) and labeled with Wright-Giemsa staining (RAL diagnostics, Site Montesquieu Martillac, France).

The differential count was performed at ×400 magnification on 100 cells in each sample. Cells were classified into one of the following: neutrophils, lymphocytes, monocytes/histiocytes/macrophages, eosinophils, and basophils. Lining cells (such as mesothelial cells) and malignant cells were classified as "other" and marked separately when detected.

2.5. Automated analysis using the XN-350 instrument

XN series instruments use size (forward scattered light), internal complexity (sideward scattered light), and DNA/RNA content (fluorescence intensity) information to determine the total WBC counts and differentials.[6] The electrical impedance method is used for the measurement of RBCs.[3]

The XN-BF module provides total cell (TC-BF), high-fluorescence cells (HF-BF), and WBC (WBC-BF) data with a 2-part differential count consisting of PMNs (PMN-BF) and MNs (MN-BF) as well as RBCs (RBC-BF).[6] HF-BF included mesothelial cells, which can interfere with the acquisition of WBC-BF counts.[6,21] Additional differential counting research parameters included neutrophils (NE-BF), lymphocytes (LY-BF), monocytes (MO-BF), and eosinophils (EO-BF).[6] TC-BF#, WBC-BF#, PMN-BF%, and MN-BF% were defined as follows: TC-BF# = WBC-BF# + HF-BF#; WBC-BF# = MN# + PMN#; PMN-BF% = NE-BF% + EO-BF%; and MN-BF% = LY-BF% + MO-BF%.

2.6. Performance evaluation of the XN-350

2.6.1. Precision. Low- and high-level control materials (XN check BF levels 1 and 2; Sysmex, Kobe, Japan) were used for precision analysis. The within-run precision was evaluated using 3 patient samples of low, middle, and high levels for each parameter according to the CLSI guideline. [18] Each sample was tested 10 consecutive times within-run, and the coefficient of variation (CV) was calculated.

Short- and long-term precision was evaluated using the low and high levels of the quality control materials "XN check" BF levels 1 and 2, according to the CLSI guideline.[18] Each sample was tested twice per run, 2 runs per day, for 5 days. The short- and long-term CVs were calculated using the equation provided in the CLSI guideline.[1]

2.6.2. Carryover. Samples with high and low counts for each parameter were selected for a carryover study. Each sample was analyzed 3 times consecutively (H1, H2, H3, L1, L2, and L3). The carryover ratio was calculated using the equation introduced in the CLSI guideline.[17] The carryover percentage was calculated as (L1–L3) × 100/(H3–L3).

2.6.3. Limit of blank (LOB), LOD, and LOQ. The LOB, LOD, and LOQ were determined in accordance with the CLSI guideline.[21] For LOB verification, the Cellpack DCL diluent was measured 10 times and the LOB was calculated according to the following equation: LOB = mean (blank) + 1.645 × standard deviation (blank). For LOD verification, patient samples (CSF, AF, and PF) were diluted to the concentrations recommended by the manufacturer, and each diluted sample was analyzed 10 times. The TC, WBC, and RBC were counted, and the LOD was calculated for each parameter according to the following equation: LOD = LOB + 1.645 × standard deviation (low concentration sample).

To measure and verify the LOQ, low level samples (immediately above and below the LOD levels) were obtained and measured 10 times each, and the percentage CV was calculated. The sample with the lowest concentration that met the accuracy specifications suggested by the manufacturer was considered the LOQ.[17]

2.6.4. Linearity (analytical measurement range). Linearity was evaluated by analyzing diluted clinical samples using a dilution solution with known concentrations (1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 128, 256, 512, and 1,024 for WBCs and 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 128, 256, and 512 for RBCs). Diluted samples were tested in duplicate from the lowest to highest concentrations to avoid carryover effects. Each dilution was measured twice. Results were plotted against the expected cell counts, and linearity was then assessed using Pearson's correlation test according to the CLSI guideline.[19]

2.7. Method comparison

Comparisons between the manual method and XN-350 for basic parameters were performed based on the following categorization: TC count using the manual method vs. TC-BF, WBC count using the manual method (i.e., the TC count minus others) vs. WBC-BF using the XN-350, MN using the manual method (the sum of lymphocytes and monocytes/histiocytes) versus MN-BF using the XN-350, and PMN using the manual method (the sum of neutrophils, eosinophils, and basophils) versus PMN-BF using the XN-350.

Research parameters (represented as percentages) obtained using the manual method (lymphocyte, monocyte, neutrophil, and eosinophil counts) via manual chamber counting or Cytospin analysis were compared to their corresponding parameters obtained using the XN-350 (neutrophil [NE-BF], lymphocyte [LY-BF], monocyte [MO-BF], and eosinophil [EO-BF] counts, respectively). Comparing between the eosinophil percentages obtained using the manual method versus EO-BF was performed only for the Cytospin analysis because manual chamber counting does not differentiate eosinophils from neutrophils.

The correlation between XN-BF parameters and the manual method/Cytospin analysis was evaluated using Pearson's correlation test. The strength of correlation between the 2 methods for each parameter was defined based on the value of Pearson correlation coefficient (r) as follows: very strong ($0.8 \leq |r| < 1.0$), strong ($0.6 \leq |r| < 0.8$), moderate ($0.4 \leq |r| < 0.6$), weak ($0.2 \leq |r| < 0.4$), or very weak ($0 \leq |r| < 0.2$). The agreement between XN-BF parameters and the manual method/Cytospin analysis was assessed using Passing-Bablok regression and Bland-Altman plot analyses.[22,23] The slope and intercept of the Passing-Bablok regression were calculated with their respective 95% confidence intervals (CIs) to identify statistically significant proportional or systematic differences between the 2 methods. If 0 was in the CI of the intercept, and 1 was in the CI of the slope, the 2 methods were deemed comparable within the investigated range. If 0 is not in the CI of the intercept, a systematic difference was deduced, and if 1 was not in the CI of slope, then a proportional difference between the 2 methods was deemed to exist. In Bland-Altman plots, the absolute and relative differences were plotted against the results obtained with light microscopy. Significant bias was defined as the 95% CI of the mean of differences not containing a 0 value.

2.8. Statistical analysis

Pearson's coefficient was calculated to determine the correlations between methods; Passing-Bablok regression and Bland-Altman

plot analyses were used to compare different methods. *P*-values <.05 were considered significant. Statistical analysis was performed using the MedCalc Statistical Software version 18.9.1 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018) and Microsoft Excel 2007 (Microsoft, Redmond, Washington).

3. Results

3.1. Performance evaluation of the XN-350

3.1.1. Precision. The within-run imprecision rates for low-, medium-, and high-level samples subjected to TC-BF, WBC-BF, and RBC-BF measurements were within the acceptable limits suggested by manufacturer, as were the short-term and long-term imprecision rates for low- and high-level controls. The acceptable limits and test results are presented in Supplementary Table 1, http://links.lww.com/MD/F835.

3.1.2. Carry-over. The carry-overs for WBC-BF (0.06%) and RBC-BF (-0.09%) were negligible and within the manufacturer's specification (less than 0.3%).

3.1.3. LOB, LOD, and LOQ. The LOBs were 0.0/μL, 0.0/μL, and 0.0/μL for TC-BF, WBC-BF, and RBC-BF, respectively. The LODs were 1.0/μL, 1.0/μL, and 1,000.0/μL for TC-BF, WBC-BF, and RBC-BF, respectively. The LOQs were 3.0/μL, 5.0/μL, and 2,000.0/μL for TC-BF, WBC-BF, and RBC-BF, respectively.

3.1.4. Linearity. The linearity of the different parameters was competent. The analytical measurement ranges of WBC-BF and RBC-BF were 5–6,815/μL (equation: $y = 1.012x + 0.0126$; $r^2 = 0.9985$) and 2–1,110 × 10^3/μL (equation: $y = 1.0013x + 0.001$; $r^2 = 1.0000$), respectively.

3.2. Method comparison

3.2.1. Comparison between manual chamber counting and XN-350 results. The results of the Pearson's correlation, Passing-Bablok, and Bland-Altman analyses for all samples as well as CSF samples, AF samples, and PF samples alone are summarized in Tables 1–4 Tables 1, 2, 3, and 4, respectively. The Passing-Bablok and Bland-Altman plots for all samples, CSF samples, AF samples, and PF samples are presented in Supplementary Figures 1, http://links.lww.com/MD/F836, 2, http://links.lww.com/MD/F837, 3, http://links.lww.com/MD/F838, and 4, http://links.lww.com/MD/F839, respectively.

Overall, the XN-350 showed very strong or strong correlations with manual chamber counting in terms of most cell enumeration and differential counting parameters with a few exceptions. Specifically, TC-BF, WBC-BF, and RBC-BF showed very strong or strong correlations with their corresponding manual chamber counting parameters in CSF samples. PMN-BF and MN-BF showed moderate and weak correlations, respectively, with their corresponding manual chamber counting parameters in CSF samples. The research parameters including NE-BF, LY-BF, and

Table 1

Agreement between parameters measured by manual chamber counting and the XN-350 from 161 samples using Passing-Bablok and Bland-Altman regression analyses.

	Manual chamber counting Median 95% CI Range	XN-350 Median 95% CI Range	Pearson's correlation r *P*	Passing-Bablok regression* Equation 95% CI of slope 95% CI of intercept	Bland-Altman regression† Absolute bias 95% CI of bias
Total cell*	190	224	.51	y=1.10x+1.33	−2398
(/μL)	144 to 276	182 to 295	< .001	1.06 to 1.14	−6686 to 1891
	0 to 76000	0 to 387172		−0.28 to 4.00	
WBC*	190	206	.51	y=1.02x+1.00	−2304
(/μL)	144 to 276	165 to 272	< .001	1.00 to 1.04	−6588 to 1980
	0 to 76000	0 to 386773		−0.04 to 3.92	
RBC*,†	1050	2000	.99	y=1.08x+0.00	−5107
(/μL)	733 to 1656	1000 to 3000	<.001	1.04 to 1.13	−9411 to −804
	0 to 1600000	0 to 1576000		0.00 to 0.00	
PMN†	20	32	.78	y=1.01x+0.00	−6
(%)	10 to 30	21 to 40	< .001	0.99 to 1.03	−10 to −3
	0 to 97	0 to 100		0.00 to 0.54	
MN†	51	66	.70	y=0.99x+1.04	−10
(%)	40 to 64	60 to 77	< .001	0.96 to 1.01	−15 to −6
	0 to 99	0 to 100		−0.16 to 3.18	
Neutrophil†	20	31	.81	y=1.00x+0.00	−5
(%)	10 to 31	21 to 39	< .001	0.99 to 1.02	−8 to −2
	0 to 97	0 to 100		−0.35 to 0.24	
Lymphocyte†	24	39	.70	y=1.01x+0.00	−8
(%)	18 to 38	33 to 44	<.001	0.99 to 1.02	−11 to −5
	0 to 97	0 to 100		−0.44 to 0.91	
Monocyte†	11	15	.77	y=0.99x+0.15	−2
(%)	9 to 15	11 to 21	<.001	0.95 to 1.01	−4 to 0
	0 to 87	0 to 100		0.00 to 0.75	

CI = confidence interval, MN = mononuclear cell, PMN = polymorphonuclear cell, RBC = red blood cell, WBC = white blood cell.

Values are shown as percentages (%) and absolute counts.

None of the parameters showed weak or very weak correlations on Pearson's correlation test.

* Proportional difference; otherwise comparable according to Passing-Bablok regression analysis.

† Significant difference; otherwise insignificant on Bland-Altman regression analysis.

Table 2

Agreement between parameters measured by manual chamber counting and the XN-350 from 63 cerebrospinal fluid samples using Passing-Bablok and Bland-Altman regression analyses.

	Manual chamber counting Median 95% CI Range	XN-350 Median 95% CI Range	Pearson's correlation* r *P*	Passing-Bablok regression† Equation 95% CI of slope 95% CI of intercept	Bland-Altman regression‡ Absolute bias 95% CI of bias
Total cell	16	35	.84	y=1.16x+0.84	−17
(/μL)	7 to 40	10 to 49	< .001	−0.48 to 1.00	−36 to 2
	0–587	0–604		−0.48 to 1.00	
WBC†	16	33	.84	y=1.15x+0.00	−16
(/μL)	7 to 40	10 to 45	<.001	1.05 to 1.35	−35 to 3
	0–587	0–597		−0.46 to 1.00	
RBC†,‡	160	1000	.95	y=1.08x+0.00	−7192
(/μL)	5 to 1050	0 to 2000	<.001	1.04 to 1.25	−13804 to −579
	0–551250	0–576000		0.00 to 0.00	
PMN‡	4	41	.50	y=1.02x+0.00	−16
(%)	0 to 23	24 to 56	<.001	0.98 to 1.17	−24 to −8
	0–89	0–100		0.00 to 1.22	
MN*,‡	12	50	.33	y=1.01x+3.80	−27
(%)	0 to 37	42 to 65	<.001	0.94 to 1.39	−36 to −17
	0–99	0–100		0.00 to 6.72	
Neutrophil‡	4	41	.56	y=1.00x+0.00	−14
(%)	0 to 33	23 to 54	<.001	0.95 to 1.09	−21 to −6
	0–89	0–100		0.00 to 2.96	
Lymphocyte*,‡	8	37	.44	y=1.04x+0.00	−18
(%)	0 to 20	24 to 49	<.001	0.99 to 1.54	−26 to −10
	0–97	0–100		0.00 to 1.14	
Monocyte*,†,‡	2	8	.26	y=1.23x+0.00	−8
(%)	0 to 5	6 to 12	<.001	1.04 to 2.08	−12 to −3
	0–50	0–100		0.00 to 0.00	

CI = confidence interval, MN = mononuclear cell, PMN = polymorphonuclear cell, RBC = red blood cell, WBC = white blood cell.

Values are shown as percentages (%) and absolute counts.

* Weak or very weak correlation; otherwise very strong, strong or moderate correlation on Pearson's correlation test.

† Proportional difference; otherwise comparable on Passing-Bablok regression analysis.

‡ Significant difference; otherwise insignificant on Bland-Altman regression analysis.

MO-BF, which comprise PMN-BF and MN-BF, also showed moderate, weak, and very weak correlations, respectively, with their corresponding manual chamber counting parameters. In particular, MO-BF showed a proportional difference and significant difference in the Passing-Bablok and Bland-Altman analyses. In AF samples, all the cell enumeration and differential counting XN-350 parameters showed very strong correlations with their corresponding manual chamber counting counterparts. In PF samples, RBC-BF and all the differential counting parameters obtained via manual chamber counting and the XN-350 were very strongly correlated. TC-BF and WBC-BF showed moderate correlations with their corresponding manual chamber counting parameters; however, they did not show any statistically significant difference according to either Passing-Bablok or Bland-Altman analysis.

3.2.2. Cytospin analysis vs. XN-350. A comparison between analyses performed by Cytospin (accompanied by light microscopy) and the XN-350 was performed on 30 of the 51 PF samples based on sample availability (Table 5). None of the samples in the Cytospin analysis contained malignant cells. NE-BF, LY-BF, and MO-BF showed very strong or strong correlations with corresponding Cytospin analysis parameters. EO-BF failed to show any statistically significant correlation with Cytospin analysis; however it also did not show any significant difference in either the Passing-Bablok analysis or the Bland-Altman analysis.

4. Discussion

We evaluated the basic performance of the XN-350 and its correlation with manual chamber counting/Cytospin analysis using 3 different types of BF. The XN-350 showed acceptable precision, carry-over, LOB, and linearity. The LOD and LOQ of the WBC and RBC were not suitable for measuring CSF samples with low cell counts (i.e., near the reference limit). The XN-350 showed strong or very strong correlations with manual chamber counting for most cell enumeration parameters, as well as for 2-part and 4-part differential counting except in CSF samples. The correlations in WBC and RBC cell enumeration with manual chamber counting in PF samples were moderate, with no significant differences. The XN-350 also correlated well with Cytospin analysis results of PF samples for the differential counting of neutrophils, lymphocytes, and monocytes. Differential counting of eosinophils did not show any significant correlation between XN-350 and Cytospin analysis, although the difference was also not significant.

The performance of the XN-350 was acceptable in terms of precision, carry-over, LOB, and linearity. Even though manual chamber counting is the gold standard method for cell enumeration, it is hampered by its high imprecision given that CVs can reach 45% [S56]. Additionally, a major concern with respect to the BF mode in AHAs is poor reproducibility and high background counts, which may lead to falsely elevated cell counts among samples with low cellularity.[25] In our study, the XN-350

Table 3

Agreement between parameters measured by manual chamber counting and the XN-350 from 51 ascitic fluid samples using Passing-Bablok and Bland-Altman regression analyses.

	Manual chamber counting Median 95% CI Range	XN-350 Median 95% CI Range	Pearson's correlation r *P*	Passing-Bablok regression* Equation 95% CI of slope 95% CI of intercept	Bland-Altman regression† Absolute bias 95% CI of bias
Total cell*	280	292	1.00	y=1.05x+10.85	−241
(/μL)	161 to 400	218 to 448	< .001	1.01 to 1.12	−548 to 67
	14 to 76000	12 to 76201		−0.62 to 18.04	
WBC	280	266	1.00	y=1.00x+3.70	−38
(/μL)	161 to 400	189 to 422	< .001	1.00 to 1.02	−86 to 11
	14 to 76000	12 to 76153		0.00 to 5.44	
RBC*	900	1000	.98	y=1.06x+43.38	−1559
(/μL)	457 to 1647	1000 to 3000	<.001	1.00 to 1.12	−10956 to 7838
	0 to 1200000	0 to 1056000		0.00 to 148.70	
PMN	10	13	.98	y=1.00x–0.01	0
(%)	8 to 25	8 to 26	<.001	0.97 to 1.04	−2 to 1
	1 to 90	2 to 93		−0.60 to 0.65	
MN	90	87	.98	y=1.00x–0.43	0
(%)	75 to 92	74 to 92	<.001	0.97 to 1.04	−1 to 2
	10 to 99	7 to 98		−3.05 to 2.35	
Neutrophil	10	12	.98	y=1.00x–0.19	1
(%)	8 to 25	6 to 26	<.001	0.96 to 1.03	−1 to 2
	1–90	2–92		−1.34 to 0.21	
Lymphocyte†	43	43	.97	y=0.99x+0.51	−2
(%)	30 to 55	34 to 56	< .001	0.95 to 1.03	−3 to 0
	2–90	1–90		−0.90 to 2.51	
Monocyte†	28	29	.95	y=0.96x+0.91	2
(%)	24 to 32	23 to 35	<.001	0.90 to 1.00	0 to 4
	2–87	2–88		−0.01 to 2.30	

CI = confidence interval, MN = mononuclear cell, PMN = polymorphonuclear cell, RBC = red blood cell, WBC = white blood cell.

Values are shown as percentages (%) and absolute counts.

None of the parameters showed weak or very weak correlations on Pearson's correlation test.

* Proportional difference; otherwise comparable on Passing-Bablok regression analysis.

† Significant difference; otherwise insignificant on Bland-Altman regression analysis.

showed excellent precision and LOB while carry-over was negligible; the latter could be attributable to the technical aspects of the instrument since it performs a rinse cycle after each run followed by a background check.[6] The linearity was also competent.

The LOD and LOQ values were suitable for the analysis of AF and PF, but were not sufficiently sensitive for the analysis of CSF samples with low cellularity. The LOD and LOQ for WBCs were 1/μL and 5/μL, respectively. AF and PF samples are classified into transudates and exudates, with the cut-off value for the WBC count generally being 1000/μL.[25] CSF samples commonly show low cellularity; therefore, the upper limits of TC and WBC are much lower than those of AF and PF samples. The WBC counts and differentials in CSF samples determine the patient's diagnosis and/or type of meningitis. The upper limits of the TC count reference ranges in CSF are 7/μL in children and 5/μL in adults,[26] whereas WBC counts in CSF range from 0 to 5/μL in adults and up to 30/μL in neonates.[25] The LOD and LOQ of RBCs were 1000/μL and 2000/μL, respectively; these were too high when considering that the upper limits of the reference ranges of RBC in CSF are 50/μL in neonates and 5/μL in adults,[26] whereas RBC counts (particularly in the range of 0–1000/μL) are of little significance in PF and AF.[26] Furthermore, the unit of RBC measurement used by the XN-350 is "$\times 10^3$/μL," which is not sufficiently sensitive for estimating small values.

In light of these data, each laboratory needs to evaluate the basic performance of the XN-350, including LOD and LOQ, when using this instrument for BF analysis and to establish a protocol for how to handle samples with low cellularity around the LOQ. Samples with low cell counts around the LOQ could either be evaluated via manual chamber counting, with the results provided accordingly, or could otherwise be labeled "Below LOQ" (such as <5/μL for WBCs) if the value is not critical for clinical decision-making. At the same time, the manufacturer of the XN-350 instrument should improve the LOD and LOQ of RBCs and provide a unit of measurement that is clinically relevant.

The XN-350 showed strong or very strong correlations with manual chamber counting or Cytospin analysis in most cell enumeration parameters and differential counting. One exception was the parameters related to both 2-part and 4-part differential counting in CSF samples using the XN-350, which showed moderate, weak, or even very weak correlations with manual chamber counting. In particular, differential counting for parameters involving monocytes (MN-BF and MO-BF) showed weak correlations with their corresponding manual chamber counting parameters ($r=0.33$ and $r=0.26$, respectively), which might also have weakened the correlation with LO-BF ($r=0.44$). The MO-BF also differed significantly from the corresponding manual chamber counting parameters according to both Passing-Bablok and Bland-Altman analyses. This could be partly due to

Table 4

Agreement between parameters measured by manual chamber counting and the XN-350 from 51 pleural fluid samples using Passing-Bablok and Bland-Altman regression analyses.

	Manual chamber counting Median 95% CI Range	XN-350 Median 95% CI Range	Pearson's correlation *r* *P*	Passing-Bablok regression* Equation 95% CI of slope 95% CI of intercept	Bland-Altman regression Absolute bias 95% CI of bias
Total cell	580	1181	0.50	y = 1.08x–0.22	−7494
(/μL)	361 to 1595	748 to 1821	< .001	1.00 to 1.19	−21596 to 6607
	0 to 72500	96 to 387172		−43.63 to 61.69	
WBC	580	1106	.50	y = 1.06x–8.60	−7396
(/μL)	361 to 1595	426 to 1664	< .001	0.99 to 1.18	−21485 to 6694
	0 to 72500	75 to 386773		−58.60 to 9.54	
RBC*	2850	5000	1.00	y = 1.02x + 395.35	−6081
(/μL)	1112 to 7193	2000 to 12898	< .001	1.00 to 1.19	−12856 to 693
	0 to 1600000	0 to 1576000		0.00 to 549.48	
PMN	40	38	.96	y = 1.07x + 0.23	−1
(%)	23 to 75	23 to 81	< .001	0.97 to 1.04	−3 to 2
	0 to 97	3 to 99		−0.74 to 1.74	
MN	54	62	.96	y = 1.01x–0.58	−1
(%)	20 to 71	19 to 77	< .001	0.97 to 1.04	−4 to 2
	0–97	1 to 97		−3.16 to 1.16	
Neutrophil	40	38	.96	y = 1.01x–0.37	0
(%)	23 to 75	23 to 81	< .001	0.98 to 1.04	−3 to 3
	0–97	3–99		−1.83 to 0.82	
Lymphocyte	24	33	.97	y = 1.01x–0.56	−1
(%)	10 to 48	11 to 47	< .001	0.99 to 1.05	−3 to 1
	0–96	0–96		−2.26 to 0.29	
Monocyte	10	10	.89	y = 0.99x–0.09	0
(%)	7 to 15	8 to 19	< .001	0.89 to 1.08	−2 to 2
	0–68	0–53		−0.87 to 0.67	

CI = confidence interval, MN = mononuclear cell, PMN = polymorphonuclear cell, RBC = red blood cell, WBC = white blood cell.

Values are shown as percentages (%) and absolute counts.

None of the parameters showed weak or very weak correlations on Pearson's correlation test.

* Systematic difference; otherwise comparable on Passing-Bablok regression analysis.

None of the parameters showed significant differences on Bland-Altman regression analysis.

Table 5

Agreement between parameters measured in 30 pleural fluid samples via Cytospin analysis and the XN-350 as determined using Passin-Bablok and Bland-Altman regression analyses.

	Cytospin analysis Median 95% CI Range	XN-350 Median 95% CI Range	Pearson's correlation* r *P*	Passing-Bablok regression† Equation 95% CI of slope 95% CI of intercept	Bland-Altman regression Absolute bias 95% CI of bias
Neutrophil	36	36	.93	y = 0.91x + 5.66	−2
(%)	20 to 55	22 to 73	< .001	0.77 to 1.07	−7 to 3
	0 to 99	3 to 97		−1.74 to 9.77	
Lymphocyte	41	43	.65	y = 1.03x–2.33	2
(%)	23 to 53	11 to 57	< .001	0.88 to 1.21	−4 to 7
	0 to 96	0 to 96		−8.53 to 3.64	
Monocyte†	14	11	.89	y = 0.94x + 0.27	0
(%)	5 to 22	8 to 21	<.001	0.66 to 1.45	−5 to 4
	0 to 55	0 to 48		−4.07 to 3.75	
Eosinophil*	0.5	0	.07	y = 0.40x + 0.00	1
(%)	0 to 2	0 to 1	.723	0.15 to 2.20	0 to 1
	0 to 11	0 to 7		0.00 to 0.10	

CI = confidence interval.

Values are shown as percentages (%).

* No statistically significant correlation on Pearson's correlation test.

† Proportional difference; otherwise comparable on Passing-Bablok regression analysis.

None of the parameters showed significant differences on Bland-Altman regression analysis.

the low cellularity of the CSF samples as well as the low proportion of monocytes, which often results in poor correlations between the manual methods and AHA or between different AHAs in studies of whole blood with much higher WBC counts than BF samples.[14,27,28] This limitation was not observed in AF or PF samples, which had higher cell numbers in our study. This indirectly indicates that our results may be attributed to the low cell numbers in our CSF samples, and implies that each laboratory ought to establish a threshold value for manual differential counting of samples with low cellularity such as CSF. Nevertheless, the strong correlations between differential counting via the XN-350 and manual chamber counting in AF and PF samples as well as Cytospin analysis in PF samples demonstrate that differential counting using the XN-350 is reliable and can replace manual methods.

Another discrepancy was observed in the enumeration of cells in PF samples. TC-BF and WBC-BF showed only moderate correlations with their corresponding manual chamber counting parameters ($r=0.50$ and $r=0.50$, respectively). Both parameters also showed a negative absolute bias compared to manual chamber counting (i.e., higher values on XN-350 analysis), even though the difference was not statistically significant. Similar phenomena were consistently observed in previous studies, particularly for WBC counting;[21,29,30] this could be attributed to the presence of cell debris or interfering fragments that may be counted as WBCs, particularly PMNs.[3] Therefore, some investigators established reference values for AHAs that were separate from (and slightly higher than) those used for the manual method.[21,24,31] This phenomenon was only observed in PF samples; it was not present in AF, which had lower median TC and WBC counts in our study. It remains unclear if this was attributable to sample characteristics or to the cell number range; therefore, further verification is required with a greater number of samples that encompass different ranges of TC and WBC counts across different sample types. Simultaneously, this finding suggests that laboratories should consider separate reference intervals for AHAs in BF mode when using these instruments in routine practice.

The strength of our study was that we evaluated the XN-350 using CSF, AF, and PF, which are the 3 most commonly requested BF samples. By evaluating the similarities and differences in 3 types of samples separately, we were able to assess the applicability of the XN-350 in BF mode to each sample type. A limitation of our study was that our samples did not include any malignant cells; therefore, we were unable to establish reliable criteria for Cytospin analysis reflex testing or for second-level testing such as flow cytometry. Further studies that include samples with malignant cells would be helpful for setting XN-350 reflex testing rules.

5. Conclusion

Our data showed that the performance of the XN-350 was excellent, although there were notable exceptions: the LOD and LOQ were not sensitive enough for CSF samples with very low cellularity, and the WBC differential counting results in CSF samples (particularly parameters involving monocytes) obtained using the XN-350 and manual method were weakly correlated. Taken together, the XN-350 may be regarded as a sensitive and reliable alternative to the manual method for routine BF analysis and could contribute to the timely management of patients. A thorough evaluation of the performance of AHAs for each type of BF, as well as devising policies for managing and reporting samples with low cellularity, are required before their deployment in clinical practice. Separate reference intervals for BF samples measured by AHAs that are independent of those obtained by manual methods should be considered when necessary.

Author contributions

J Lee collected the data, performed the statistical analysis, and wrote the manuscript. Y Cho performed the statistical analysis. H-S Kim and HJ Kang collected the data, reviewed the statistical analysis, and provided expert opinions. M Kim and YK Lee designed and supervised the study.

Conceptualization: Miyoung Kim, Young Kyung Lee.
Data curation: Jiwon Lee, Han-Sung Kim, Hee Jung Kang.
Formal analysis: Jiwon Lee, Younggeun Cho, Han-Sung Kim, Hee Jung Kang.
Project administration: Miyoung Kim.
Supervision: Han-Sung Kim, Hee Jung Kang, Miyoung Kim, Young Kyung Lee.
Writing – original draft: Jiwon Lee, Young Kyung Lee.
Writing – review & editing: Miyoung Kim, Young Kyung Lee.

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