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LETTER TO THE EDITOR

Utility of the XN-1000 research mode for leukocytes counting in ascitic and pleural fluids

Dear Editors,

The body fluid (BF) module on the XN-1000 (Sysmex Corporation) represents a convenient platform for quantification of white blood cells (WBCs) by flow cytometry in multiple types of BFs.[1,2] The XN-BF mode provides total cell (TC-BF), polymorphonuclear cell (PMN-BF), and mononuclear cell (MN-BF) counts. Research parameters are also provided and include neutrophils (NE-BF), eosinophils (EO-BF), lymphocytes (LY-BF), monocytes (MO-BF), and high-fluorescent cells (HF-BF) classification. These latter cells have a high nucleo-cytoplasmic ratio and a higher nucleic acid content and have been proposed as a useful tool to identify malignant cells.[3] The advantages of BFs analyzers include no sample preparation, reduced turnaround time (TAT), decreased biohazard exposure, low sample volume, low limit of detection (LOD) and of quantification (LOQ), flagging system, and a differential module.[4-6] Excellent linearity, carryover, and imprecision have also been reported.[2,4,7,8]

Several studies have already shown the good performances of the XN-1000 for TC, PMN, and MN compared to reference methods.[2,8-10] However, to the best of our knowledge, only one study specifically focused their method comparison on the research mode provided by the XN-1000 BF analyzer for ascitic fluids (AFs) and pleural fluids (PFs).[7] Research mode parameters may be useful for the rapid diagnosis of various conditions (Table 1).[4,7,11]

Therefore, we aimed at comparing the XN-1000 BF module (including research mode) to optical microscopy (OM). For that purpose, 92 BFs (50 AFs and 42 PFs) were analyzed. Among these BFs, 12 contained malignant cells. The collection and analysis of all BFs were performed according to the CLSI document H56-A.[11] All BFs were collected in K_2-EDTA tubes (Vacuette, Greiner Bio-One, Courtaboeuf) without any dilution.

Samples were first analyzed on the XN-1000 BF mode analyzer. All the analyses were performed according to the manufacturer's recommendations. Thereafter, the manual cell count was performed using Nageotte counting chambers (Marienfeld) by at least two skilled laboratory professionals and following standard conditions, as recommended by ICSH guidelines.[12] Samples were tested within 2 hours following the automated count to avoid sample storage as a variable. For differential count, each BF sample was spun at 160 g for 5 minutes (Cytospin 2 Shandon, Thermo Electron Corporation) followed by May-Grunwald-Giemsa staining (Merck). The differential count was performed with a 40×oil-immersion objective on at least 100 cells by at least two skilled laboratory professionals. Mean of

TABLE 1 Discrepancies observed at particular medical decision cutoffs. FP, false positive; FN, false negative; abs, absolute; resp, respectively; Sp, specificity; Se, sensitivity

Criteria	BF type	Cutoffs	Significance	Sensitivity %	FN (abs.)	Specificity %	FP (abs.)	Agreement
Total cell count	AF and PF	1000×10^6/L	Transudate/exudate	100.0	0	98.4	n = 1 (1,041 vs 948)	0.97 (very good)
	AF	500×10^6/L	Bacterial peritonitis	100.0	0	100.0	0	1.00 (perfect)
PMN or NE count	AF	250×10^6/L	Bacterial peritonitis (Se)	100.0	0	100.0	0	1.00 (perfect)
		500×10^6/L	Bacterial peritonitis (Sp)	100.0	0	100.0	0	1.00 (perfect)
	PF	50% and >1000×10^6/L	Acute inflammation or parapneumonic effusion	100.0	0	100.0	0	1.00 (perfect)
LY count	AF	50% and >1000×10^6/L	Tuberculous peritonitis	100.0	0	100.0	0	1.00 (perfect)
	PF	50% and >1000×10^6/L	Tubercular infection, metastasis, lymphoproliferative disorder or chylous effusion	100.0	0	97.5	n = 2 (1,119 (82.7%) and 1,018 (90.1%) vs 903 (76.3%) and 942 (80.0%), resp.)	0.88 (very good)
EO count	PF	10%	Eosinophilic effusion	100.0	0	100.0	0	1.00 (perfect)

TABLE 2 Method comparison performed on 92 BFs

	Samples	Mean count ($\times 10^6$/L)	95% CI ($\times 10^6$/L)	*r*	Slope	95% CI	Intercept	95% CI	Samples
TC-BF# vs TC-OM#	n = 92	913 vs 824	34-4,830 vs 31-3,948	0.99	1.11	1.07-1.12	−3.38	−9.20-1.46	n = 92
TC-BF# vs TC-OM#*	n = 80	812 vs 746	34-3,735 vs 29-3,431	1.00	1.10	1.05-1.12	−2.94	−8.95-3.03	n = 80
PMN-BF# vs PMN-OM#	n = 92	347 vs 321	3-2,266 vs 1-2,041	1.00	1.02	0.98-1.14	3.52	2.08-4.06	n = 92
PMN-BF# vs PMN-OM*	n = 80	382 vs 354	4-3,249 vs 1-2,899	1.00	1.01	0.97-1.14	3.63	2.08-5.12	n = 80
MN-BF# vs MN-OM#	n = 92	452 vs 379	20-1,877 vs 15-1,472	0.98	1.04	0.98-1.08	−6.56	−13.89-1.54	n = 92
MN-BF# vs MN-OM*	n = 80	390 vs 388	20-1,811 vs 16-1,552	0.98	1.01	0.94-1.06	−6.21	−17.50-0.29	n = 80
NE-BF# vs NE-OM#	n = 92	338 vs 317	3-2,250 vs 1-2,041	1.00	1.00	0.96-1.09	3.95	1.95-4.87	n = 92
NE-BF# vs NE-OM*	n = 80	373 vs 350	4-3,238 vs 1-2,899	1.00	0.99	0.95-1.10	3.95	1.90-5.09	n = 80
LY-BF# vs LY-OM#	n = 92	311 vs 256	7-1,666 vs 3-1,461	0.99	1.17	1.12-1.24	4.36	1.18-8.43	n = 92
LY-BF# vs LY-OM*	n = 80	272 vs 230	7-1,664 vs 3-1,410	0.99	1.14	1.09-1.21	4.67	2.08-9.13	n = 80
MO-BF# vs MO-OM#	n = 92	141 vs 176	8-659 vs 9-672	0.88	0.76	0.68-0.86	−3.24	−11.06-2.22	n = 92
MO-BF# vs MO-OM#*	n = 80	118 vs 154	9-518 vs 10-515	0.87	0.75	0.63-0.86	−1.71	−7.36-3.85	n = 80
MO-BF#+HF-BF# vs MO-OM#	n = 92	318 vs 176	11-1,526 vs 9-672	0.91	1.05	0.97-1.13	−6.16	−11.58-1.32	n = 92
MO-BF#+HF-BF# vs MO-OM*	n = 80	158 vs 154	10-575 vs 10-515	0.95	1.02	0.92-1.11	−2.30	−7.92-2.66	n = 80
EO-BF# vs EO-OM#	n = 92	9 vs 2	0-34 vs 0-12	0.13	90.09	-	0.00	-	n = 92
EO-BF# vs EO-OM*	n = 80	9 vs 2	0-38 vs 0-11	0.11	80.96	-	0.00	-	n = 80

*Without malignant cells (n = 80).

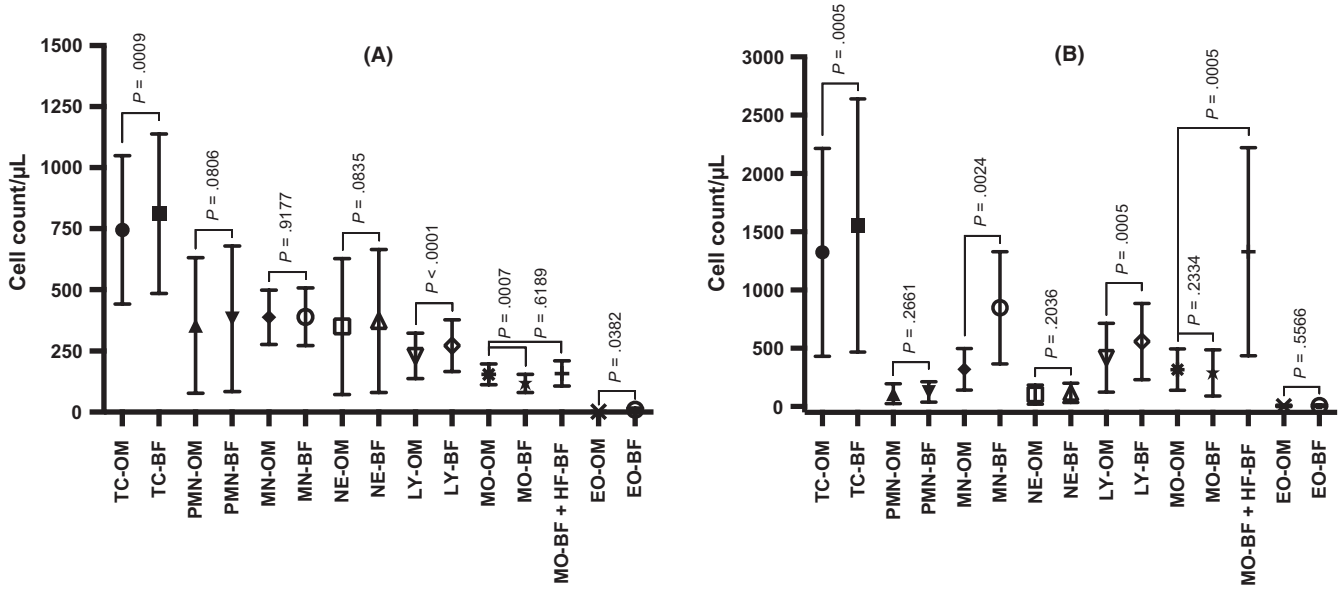

FIGURE 1 Mean and 95% CI of leukocyte counts according to the measurement method used. A, BFs not containing malignant cells; B, BFs containing malignant cells. A *P*-value <.05 was considered as statistically significant

operators was used for the method comparison. The mean interobserver agreement was very good (kappa 0.85). Counted cells were classified as NE, LY, MO/macrophages (MA), EO, basophils (BA), mesothelial cells (MESO), and other cells including blasts and neoplastic cells. MO and MA were not considered independently since it is often difficult to distinguish them.[11] Furthermore, the specific count of MESO may also be difficult to apprehend and their specific count is not clinically significant.[11] Therefore, we decided to cluster MO, MA, and MESO together.

To enable direct comparison between manual and automated methods, cells were gathered in different categories as follows:

1. TC-BF vs TC-OM
2. MN-BF vs MN-OM (=LY + MO/MA + MESO)
3. PMN-BF vs PMN-OM (=NE + EO + BA)
4. NE-BF vs NE-OM
5. LY-BF vs LY-OM
6. MO-BF vs MO-OM (including MO/MA + MESO)
7. MO-BF + HF-BF vs MO-OM (including MO/MA + MESO)
8. EO-BF vs EO-OM

For results comparison, the Passing-Bablok regression analysis was used.[11,13] Slope and intercept were calculated with their 95% confidence interval (95% CI) to emphasize potential discrepancies between methods.[13] As recommended by ICSH guidelines,[12] we also studied potential discrepancies at medical decision cutoffs found in the literature.[4] For that purpose, sensitivity, specificity, and agreement (kappa) were determined (manual count considered as the reference). Means of each leukocyte type (after excluding malignant cells and according to the method tested) were also compared by a paired *t* test. The impact of BFs that contained malignant cells (n = 12; mean manual malignant cells numeration/L = 474 × 10^6) was evaluated by a Wilcoxon matched-pairs signed rank test. A *P*-value <.05 was considered as statistically significant. MedCalc® (version 12.7.0) and GraphPad Prism® (version 6.0e) softwares were used to perform statistical analysis.

Overall, the XN-1000 BF mode slightly overestimated TC-BF and LY-BF as compared to OM, with or without exclusion of malignant cells. The counting of PMN-BF and NE-BF was not significantly different from OM, even in the presence of malignant cells. In BFs without malignant cells, MN-BF was not significantly different from MN-OM, and MO-OM was more closely correlated with MO-BF + HF-BF as compared to MO-BF only (Table 2, Figure 1A). However, if focusing on samples containing malignant cells (Figure 1B), MN-BF was significantly higher as compared to MN-OM and MO-OM was more closely correlated with MO-BF. The EO-BF was found to be inaccurate (Table 2). The HF-BF count was also higher in samples containing malignant cells (mean = 1,039 × 10^6/L) compared to samples without malignant cells (mean = 41 × 10^6/L).

Based on clinical cutoff found in the literature,[4,11,14] no false-negative results for clinical diagnosis were found when reporting automated parameters and only one and two false-positive results (ie, 2.8% of all BFs) were found on TC-BF and LY-BF counts, respectively (Table 1). Given that, it is currently still recommended to use manual methods in case of doubtful results or suspicion of malignant cells,[4] some still recommend slide review for the detection of malignant cells.[15] However, the major drawback for reviewing all BFs is that it requires experienced cytologists and may lead to a prolonged TAT. Therefore, reflex testing rules in case of WBC Abn. Scattergram, elevated EO-BF (ie, >5%), and HF-BF (ie, >68 × 10^6/L [7]) have been proposed to identify samples that need to be reviewed or not.[7] The HF-BF cutoff of 68 × 10^6 cells/L of Buoro et al[7] should however be verified locally. In case where rules are all negative, we might therefore consider to directly import the BF data (TC-BF, NE-BF, LY-BF, MO-BF + HF-BF) in the laboratory information system to decrease the overall TAT.

In conclusion, our results confirm the good performances of the XN-1000 BF mode for TC-BF, PMN-BF, MN-BF, NE-BF, LY-BF, and MO-BF + HF-BF as compared to OM, especially when BFs containing malignant cells are excluded from the analysis. Given the drawbacks of reference methods and the absence of analytical performance specifications for BFs, the number of misclassifications obtained at clinical cutoff values was acceptable. It is also critical to remind that the correct interpretation of BF does not only rely on the leukocyte count but also on biochemical parameters (ie, lactate dehydrogenase and proteins).[4] Further studies focusing on the integration of reflex testing rules and the use of BF research mode parameters should be undertaken to find an optimal workflow that could be used in hematology laboratories.

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CONFLICTS OF INTEREST

None declared.

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