Evaluation of Assay Performance of the Body Fluid Mode on the Automated Hematology Analyzer XN-2000

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We evaluated the basic assay performance of the body fluid (BF) mode on the automated hematology analyzer XN-2000 (Sysmex), which is used for analysis of body and punctured fluids. We examined the within-run reproducibility study with 5 or 10 continuous measurements. The coefficients of variation (CV%) of nucleated cell count were 2.7–16.0% for cerebrospinal fluid (CSF), 5.6% for pleural fluid, 2.0% for ascites, 3.8% for synovial fluid, 0.8–25.1% for continuous ambulatory peritoneal dialysis (CAPD) effluent and 5.0% for bronchoalveolar lavage fluid (BALF). The within-run reproducibility was comparatively good in the differential cell counts, although the CV% was relatively high in samples with low number of nucleated cells and for cells with a low appearance ratio. Dilution linearity confirmed a wide range of 0.01-100 × 10^3/μL. The correlation between the manual method and XN-2000 analysis for nucleated, polymorphonuclear and mononuclear cells was also studied using samples of CSF (63 samples), pleural fluid (16), synovial fluid (7), and CAPD effluent (21). The coefficients of correlation (r) for CSF with the nucleated cell count ≥ 100/μL were respectively 0.99, 0.87 and 0.86 for nucleated, polymorphonuclear and mononuclear cells, and those for CSF with the nucleated cell count < 100/μL were respectively 0.85, 0.77 and 0.84. These coefficients of correlation were respectively 1.00, 0.94 and 0.95 for pleural fluid, 0.93, 0.86 and 0.82 for synovial fluid, and 0.99, 0.71, and 0.62 for CAPD effluent. The correlation was high in order of nucleated, polymorphonuclear, and mononuclear cells. The WDF scattergrams for differentials of samples containing abnormal cells showed leukemic cells plotted in the mononuclear cell (MN) area, and cancer cells of epithelial cell origin appeared in the high fluorescence intensity (HF-BF) area. The nucleated cell count and cell classification of body and punctured fluids by the BF mode on XN-2000 were thought to be useful for examination of routine laboratory, as well as for that on day and night duty.

Key Words Body Fluids, Punctured Fluids, Automated Hematology Analyzer, XN-2000, Body Fluid Mode

INTRODUCTION

Most medical laboratories employ a visual method for cytological examination of body and punctured fluids [1)]. Various types of body and punctured fluids are submitted for testing on day and night duty also. When medical technologists who do not routinely work with body fluids, problems related to accuracy of results, experience level of the technologist and the burden of manual examination often crop up. Now, however, such cell count and differential cell count analysis of body and punctured fluids have become possible using automated hematology analyzers [2-4)]. The recently launched automated hematology analyzer XN-2000 has the BF mode used for analysis of body and punctured fluids. We evaluated the basic assay performance of the BF mode, and report the results here.

MATERIALS

1. Patient samples

The samples used were body and punctured fluids submitted to the clinical laboratory of our hospital. These were cerebrospinal fluid (CSF) of 63, pleural fluid of 16, ascites of 3, synovial fluid of 7, continuous ambulatory peritoneal dialysis (CAPD) effluent from 21, and bronchoalveolar lavage fluid (BALF) of 4 subjects. The samples were obtained in EDTA or heparin-containing sampling tubes or sterile Spitz tubes. Strictly speaking, CAPD effluent and BALF are not body fluids, but they are often ordered for the BF analysis.

Note: This article is translated and republished from the Sysmex Journal Web Vol. 22 No. 1, 2012.

2. Instruments and the manual method

We used the newly developed Sysmex automated hematology analyzer XN-2000 (XN) [5]. XN uses a sheath flow DC (direct current) detection method and flow cytometry using a semiconductor laser as its measurement principles. Red blood cells are measured by the former method (RBC channel), and white blood cells by the latter in the WDF channel (***Fig. 1a***). White blood cells are irradiated with the laser beam after being stained with the fluorescent dye Fluorocell WDF to obtain the side-scattered light intensity (reflects intracellular information) and side fluorescent light intensity (reflects the content of nucleic acid and cell organelles), which are used for counting nucleated cells and classifying white blood cells into mononuclear cells (MN) and polymorphonuclear cells (PMN), and, as research parameters, for differential counts of lymphocytes (LY-BF), monocytes (MO-BF), neutrophils (NE-BF) and eosinophils (EO-BF). The results of analysis are displayed as scattergrams (***Fig. 1b***) in addition to the cell number. Cells other than blood cells (mesothelial cells and abnormal cells like epithelium-derived tumor cells) are also stained, and they are mainly plotted in the high fluorescence intensity area (HF-BF) (***Fig. 1b***). The principle of measurement used in BF mode is the same as used in the whole blood (WB) mode. But the volume of samples analyzed is greater, about 10 times in the WDF channel and 3 times in the RBC channel, compared to the WB mode. It is sufficient to simply switch the system to the BF mode to make measurements on body fluids. There is no need to change the reagents and to make any preparation of samples. Also, as the reagents used are the same as the WB mode, there is no influence of intermixed red blood cells on the differential white blood cell counts.

The manual counting of nucleated cells was done by the micropipetting method, following Methods of Analyzing Cerebrospinal Fluid 2002 [1], for comparing with the results of XN. For manual differential counting, wedge smear or cytospin samples were prepared and May-Giemsa stained.

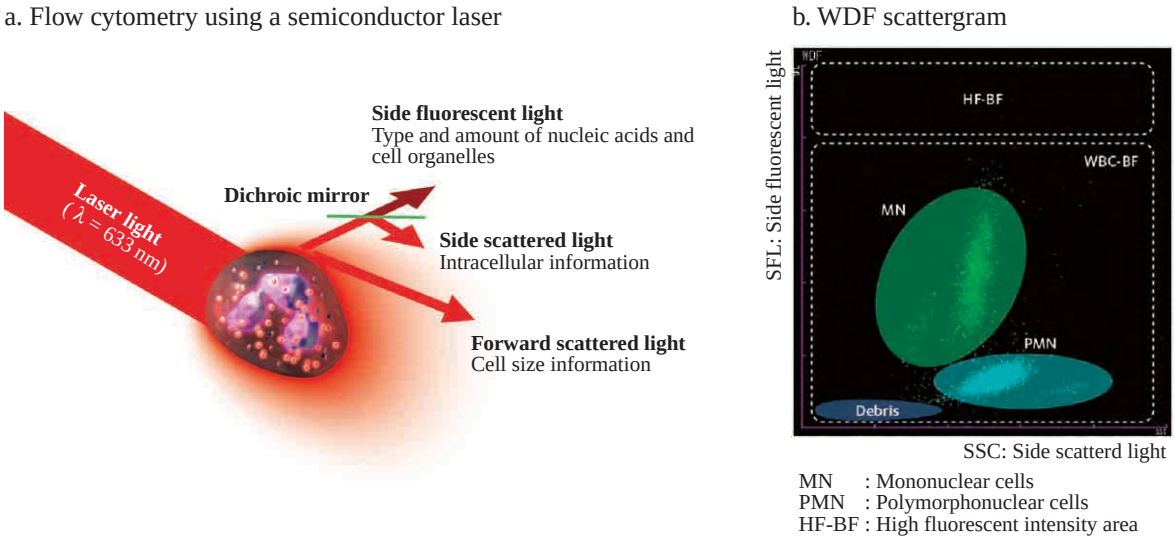


Fig. 1 *Principle of measurement in the BF mode of XN-Series analyzers*

METHODS

1. Within-run reproducibility

Within-run reproducibility was evaluated using three concentrations (low, medium and high) of CSF and CAPD effluent, and one concentration each of pleural fluid, ascites, synovial fluid, and BALF samples. The reproducibility of total nucleated cell count (TC-BF), white blood cell count (WBC-BF) and red blood cell count (RBC-BF), and the differential cell counts (PMN, MN, NE-BF, LY-BF, MO-BF, and EO-BF) were examined. The measurement of each sample was continuously repeated 5 times or 10 times.

2. Dilution linearity

A peripheral blood sample with a high white blood cell count was centrifuged at first, and then the white blood cell fraction was suspended in CELLPACK DCL, the special diluent used with XN, to prepare a high concentration sample, and a dilution series was prepared using the same diluent. The linearity of analysis was studied with samples having WBC counts in ranges: (a) 0.01-0.1 × 10^3/μL, (b) 0.1-1.0 × 10^3/μL, (c) 1.0-10 × 10^3/μL, and (d) 10-100 × 10^3/μL. Three replicate measurements were made at each concentration and the mean value used for studying linearity.

3. Correlation between the manual method and XN analysis

The correlation of nucleated cell counting and cell classification (counting of mononuclear and polymorphonuclear cells) between the manual method and XN were examined using CSF of 63, pleural fluid of 16, ascites of 3, synovial fluid of 7, CAPD effluent from 21, and BALF of 4 samples. In the CSF testing in our hospital, the results of differential cell counting were reported as percentages when the nucleated cell count ≥ 100/μL, and as actual numbers were reported if the nucleated cell count <100/μL. Therefore, for examining the correlation also CSF samples were grouped according to this reporting form. There were 21 samples with the nucleated cell count ≥ 100/μL and 42 with < 100/μL. The macrophages in pleural fluid, synovial fluid, and CAPD effluent were included as mononuclear cells on the manual method. The total of these 114 samples were grouped into 53 with the nucleated cell count ≥ 100/μL and 61 with < 100/μL, and the correlation of the differential white blood cell (NE-BF, LY-BF, MO-BF and EO-BF) between the manual method and XN was examined. In the case of monocytes count, the sum of macrophage and monocyte counts of the manual method was compared with MO-BF of XN. In the group of samples with the nucleated cell count < 100/μL, the actual cell counts were compared, following the reporting mode used for CSF. In each examination, when necessary, the samples were also grouped into by presence of cells other than blood cells, such as macrophages, tumor cells and other atypical cells. When a sample had about a 2-fold difference in the results of the manual method and XN, the reason for the difference was investigated by cell images and patterns of WDF and WDF (EXT) scattergrams which extended the high fluorescence intensity area of WDF scattergram.

4. Samples with abnormal cells

XN measurements and the cell morphology were compared, and WDF and WDF (EXT) scattergram patterns were investigated for CSF samples with blastoid cells and CSF, pleural fluid and ascites samples with abnormal cells.

5. Statistical analysis

The significance test of the Pearson's product moment correlation coefficients were examined. In samples with a size of 10 or less, these were tested by the Spearman's rank correlation coefficient test.

RESULTS

1. Within-run reproducibility

The CV for nucleated cell counting was 16.0% for a CSF sample with TC-BF 2.8/μL and 15.2% for a sample with 3.4/μL. With CAPD effluents, the CV was high at 25.1% in a sample with TC-BF 5.2/μL (a low range). But the CV was at an acceptable level of less than 10% for samples with TC-BF ≥ 10/μL. As for differential cell counts, the CV was high when TC-BF count was low and also when the percentage of a respective cell type was low, whereas the CV was acceptably low in samples with high TC-BF (***Table 1***).

2. Dilution linearity

The linearity of WBC count measured by XN was confirmed in low to high WBC concentration ranges, i.e., (a) 0.01-0.1 × 10^3/μL, (b) 0.1-1.0 × 10^3/μL, (c) 1.0-10 × 10^3/μL, and (d) 10-100 × 10^3/μL (***Fig. 2***).

Table 1 *Within-run reproducibility in the BF mode*

BF mode		TC-BF#	WBC-BF	RBC-BF	PMN#	MN#	PMN%	MN%	NE-BF#	LY-BF#	MO-BF#	EO-BF#	NE-BF%	LY-BF%	MO-BF%	EO-BF%
		/μL	/μL	/10^3	/μL	/μL	%	%	/μL	/μL	/μL	/μL	%	%	%	%
CSF (low concentration 1)	MEAN (/μL)	2.8	2.6	0.0	0.6	2.0	23.3	76.7	0.6	2.0	0.0	0.0	23.3	76.7	0.0	0.0
	SD	0.45	0.55	0.00	0.55	0.71	22.35	22.35	0.55	0.71	0.00	0.00	22.35	22.35	0.00	0.00
	CV (%)	16.0	21.1	0.0	91.3	35.4	95.9	29.2	91.3	35.4	0.0	0.0	95.9	29.2	0.0	0.0
CSF (low concentration 2)	MEAN (/μL)	3.4	3.4	0.0	0.7	2.7	20.0	80.0	0.7	2.3	0.4	0.0	20.0	67.5	12.5	0.0
	SD	0.52	0.52	0.00	0.67	0.67	18.50	18.50	0.67	0.48	0.52	0.00	18.50	7.29	16.30	0.00
	CV (%)	15.2	15.2	0.0	96.4	25.0	92.6	23.1	96.4	21.0	129.1	0.0	92.6	10.8	130.5	0.0
CSF (middle concentration 1)	MEAN (/μL)	9.2	8.8	0.0	2.8	6.0	31.8	68.2	2.8	5.0	1.0	0.0	31.8	56.7	11.4	0.0
	SD	0.45	0.84	0.00	0.45	0.71	4.65	4.65	0.45	0.71	0.00	0.00	4.65	4.79	1.07	0.00
	CV (%)	4.9	9.5	0.0	16.0	11.8	14.6	6.8	16.0	14.1	0.0	0.0	14.6	8.4	9.3	0.0
CSF (middle concentration 2)	MEAN (/μL)	10.6	10.4	0.2	2.6	7.8	25.3	74.7	2.6	7.0	0.8	0.0	25.3	67.0	7.7	0.0
	SD	0.89	0.89	0.45	0.55	1.30	6.49	6.49	0.55	1.22	0.45	0.00	6.49	6.71	4.34	0.00
	CV (%)	8.4	8.6	223.6	21.1	16.7	25.6	8.7	21.1	17.5	55.9	0.0	25.6	10.0	56.7	0.0
CSF (middle concentration 3)	MEAN (/μL)	22.2	21.6	0.0	6.4	15.2	29.5	70.5	6.4	11.0	4.2	0.0	29.5	51.1	19.4	0.0
	SD	1.79	1.52	0.00	1.14	0.84	3.75	3.75	1.14	0.71	0.45	0.00	3.75	4.15	1.01	0.00
	CV (%)	8.1	7.0	0.0	17.8	5.5	12.7	5.3	17.8	6.4	10.6	0.0	12.7	8.1	5.2	0.0
CSF (high concentration)	MEAN (/μL)	320.6	319.1	1.0	278.5	40.6	87.3	12.7	278.2	21.2	19.4	0.3	87.2	6.7	6.1	0.1
	SD	8.55	8.46	0.00	7.00	4.43	1.24	1.24	6.81	1.81	4.45	0.48	1.31	0.54	1.35	0.14
	CV (%)	2.7	2.7	0.0	2.5	10.9	1.4	9.8	2.4	8.6	22.9	161.0	1.5	8.2	22.3	161.0
Pleural fluid	MEAN (/μL)	111.1	109.9	1.0	83.4	26.5	76.0	24.1	83.4	17.4	9.1	0.0	76.0	15.8	8.3	0.0
	SD	6.17	6.08	0.00	4.60	4.09	3.07	3.07	4.60	3.10	1.29	0.00	3.07	2.47	0.89	0.00
	CV (%)	5.6	5.5	0.0	5.5	15.4	4.0	12.8	5.5	17.8	14.1	0.0	4.0	15.6	10.8	0.0
Ascites fluid	MEAN (/μL)	474.2	461.8	78.4	200.6	261.2	43.4	56.6	177.8	117.0	144.2	22.8	38.5	25.4	31.2	4.9
	SD	9.68	9.44	1.52	7.89	5.07	1.10	1.10	6.61	4.42	5.72	2.77	0.84	1.20	0.97	0.61
	CV (%)	2.0	2.0	1.9	3.9	1.9	2.5	2.0	3.7	3.8	4.0	12.2	2.2	4.7	3.1	12.3
Synovial fluid	MEAN (/μL)	151.6	147.4	0.6	34.8	112.6	23.6	76.4	34.0	47.8	64.8	0.8	23.0	32.5	44.0	0.6
	SD	5.77	5.50	0.55	3.27	3.29	1.60	1.60	2.92	2.28	3.63	0.45	1.39	2.06	1.54	0.31
	CV (%)	3.8	3.7	91.3	9.4	2.9	6.8	2.1	8.6	4.8	5.6	55.9	6.1	6.4	3.5	55.9
CAPD (low concentration)	MEAN (/μL)	5.2	4.4	0.0	2.6	1.8	56.7	43.3	2.6	1.0	0.8	0.0	56.7	25.0	18.3	0.0
	SD	1.30	1.52	0.00	1.34	0.45	14.86	14.86	1.34	0.00	0.45	0.00	14.86	8.30	12.35	0.00
	CV (%)	25.1	34.5	0.0	51.6	24.8	26.2	34.3	51.6	0.0	55.9	0.0	26.2	33.2	67.3	0.0
CAPD (middle concentration)	MEAN (/μL)	55.5	54.7	0.0	14.0	40.7	25.6	74.4	12.4	19.3	21.4	1.6	22.7	35.3	39.1	2.9
	SD	2.80	2.79	0.00	1.41	2.41	2.21	2.21	1.35	2.50	1.35	0.52	2.34	3.87	2.14	0.88
	CV (%)	5.0	5.1	0.0	10.1	5.9	8.6	3.0	10.9	12.9	6.3	32.3	10.3	11.0	5.5	30.3
CAPD (high concentration)	MEAN (/μL)	954.4	952.4	0.0	735.8	216.6	77.3	22.7	728.2	25.4	191.2	7.6	76.5	2.7	20.1	0.8
	SD	8.02	7.73	0.00	5.85	2.61	0.15	0.15	5.93	1.95	4.02	0.55	0.11	0.21	0.35	0.08
	CV (%)	0.8	0.8	0.0	0.8	1.2	0.2	0.7	0.8	7.7	2.1	7.2	0.1	7.8	1.7	10.7
BALF	MEAN (/μL)	296.4	141.1	0.6	33.8	107.3	24.0	76.1	30.2	65.1	42.2	3.6	21.4	46.2	29.9	2.6
	SD	14.75	7.08	0.52	2.15	5.52	0.90	0.90	1.87	2.23	4.32	0.70	0.84	1.86	1.68	0.44
	CV (%)	5.0	5.0	86.1	6.4	5.1	3.8	1.2	6.2	3.4	10.2	19.4	3.9	4.0	5.6	17.1

The measurement of each sample was continuously repeated 5 times or 10 times.

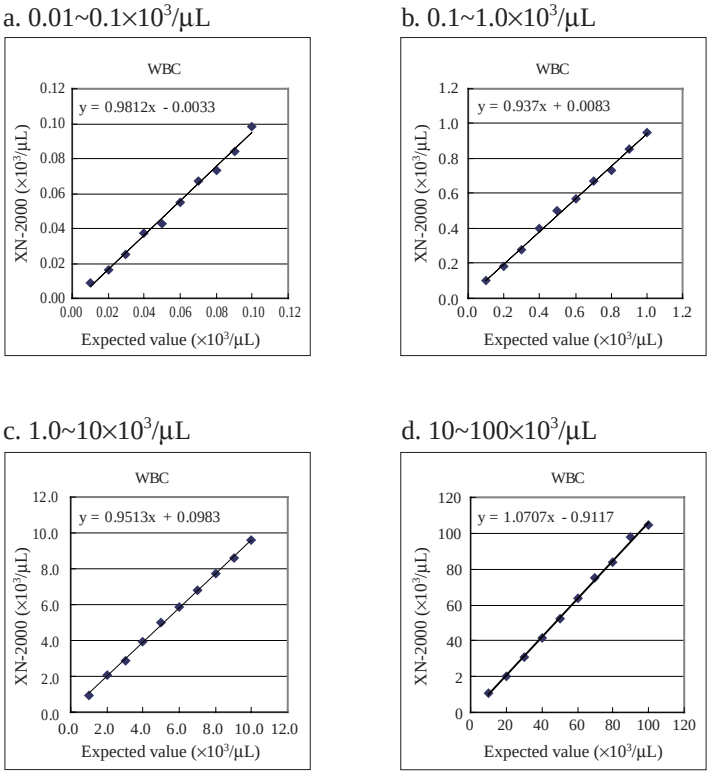
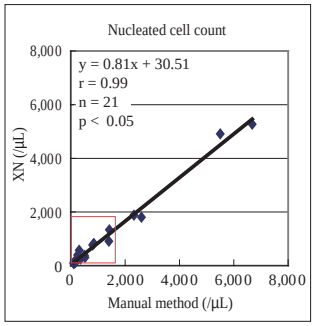


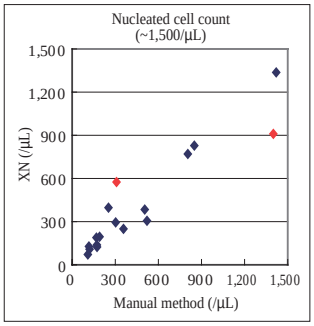
Fig. 2 *Dilution linearity in the BF mode*

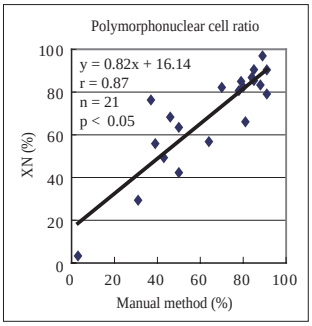
3. Correlation between the manual method and XN

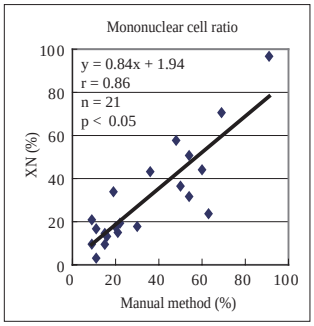
In CSF samples, the correlation coefficients for nucleated, polymorphonuclear and mononuclear cell counts were respectively 0.99, 0.87 and 0.86 for samples with the nucleated cell count ≥ 100/μL (***Fig. 3a***) and 0.85, 0.77 and 0.84 respectively for samples with the nucleated cell count < 100/μL (***Fig. 3b***). The correlation coefficients were respectively 1.00, 0.94 and 0.95 for pleural fluid (***Fig. 3c***), 0.93, 0.86 and 0.82 for synovial fluid (***Fig. 3d***), and 0.99, 0.71 and 0.62 for CAPD effluent (***Fig. 3e***). Thus all types of specimens showed a high level of correlation for nucleated cell counts. Moreover, there was significant correlation for nucleated, polymorphonuclear, and mononuclear cell counts in all types of specimens tested. There were 6 samples with a nearly two-fold difference in the nucleated cell count between the manual method and XN analysis (marked with red dots in ***Figs 3a, 3b*** and ***3c***). Cell degeneration and cell destruction were seen in the smear of 2 CSF samples which had the nucleated cell count ≥ 100/μL, and their XN scattergrams showed poor separation of the Debris area from the WBC area (***Fig. 4a***, circle). In the remaining 2 samples with the nucleated cell count < 100/μL, there was an increased number of debris particles in one sample that showed falsely high in the XN (***Fig. 4b***, arrow) and plots in the high side scatter (SSC) intensity area on the XN scattergram (***Fig. 4b***, circle). Macrophages appeared in the smear of this sample. The other samples showed atypical lymphocytes in the smear, but the cytological features and the scattergrams did not reveal any possible causes for such difference in the nucleated cell count. There were 2 samples of pleural fluid that showed falsely low nucleated cell counts by XN analysis. Aggregates of macrophages appeared in these sample 's smears, and XN scattergrams showed plots in the HF-BF area (***Fig. 4 c***, arrow).

a. CSF (Nucleated cell count ≥ 100/μL) ★

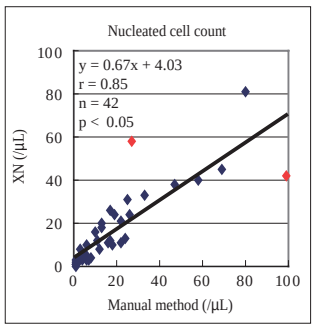


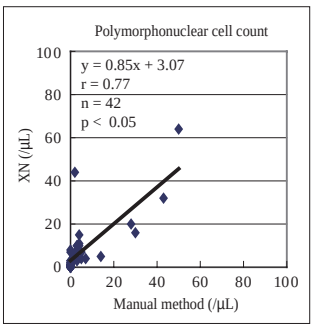


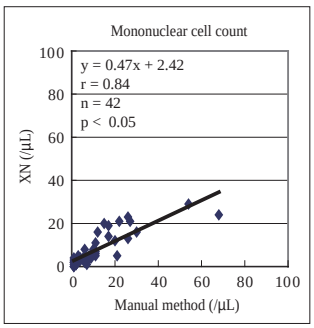




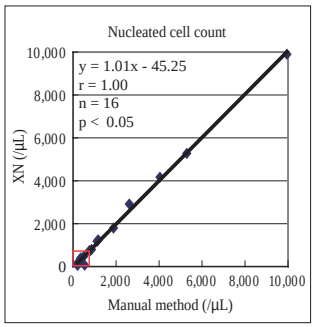
b. CSF (Nucleated cell count < 100/μL)

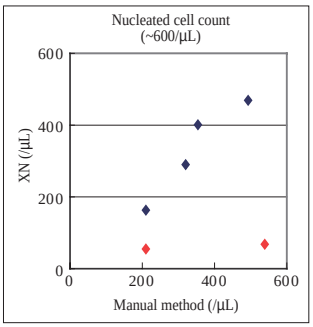


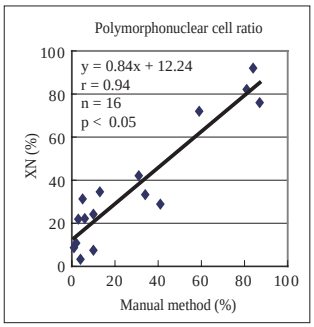


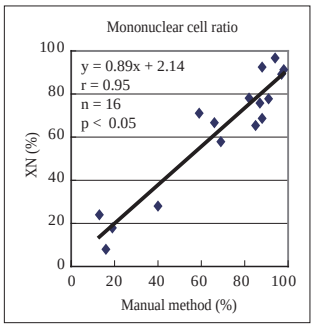


c. Pleural fluid ★

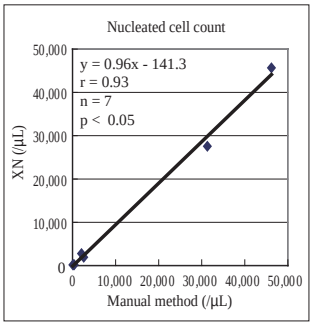


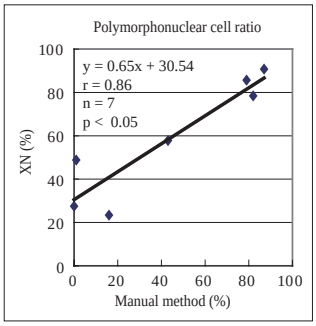


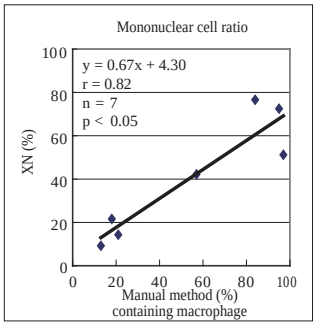




d. Synovial fluid







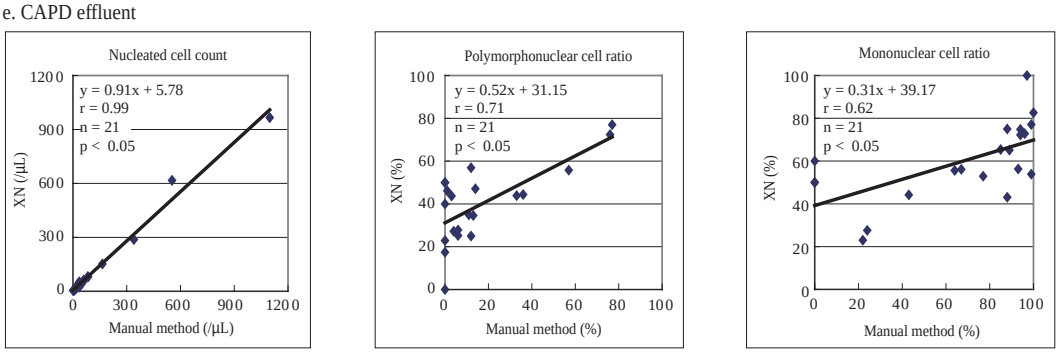


Fig. 3 *Correlation between the manual method and XN in each specimen type*

Marked with red dots were nearly two-fold difference in the nucleated cell count between the manual method and XN analysis.
♦ *showed samples difference in the results of the manual method and XN.*
★ *showed expanded graphs; a. 1500/μL or less of CSF (Nucleated cell count ≥ 100/μL), c. 600/μL or less of pleural fluid*

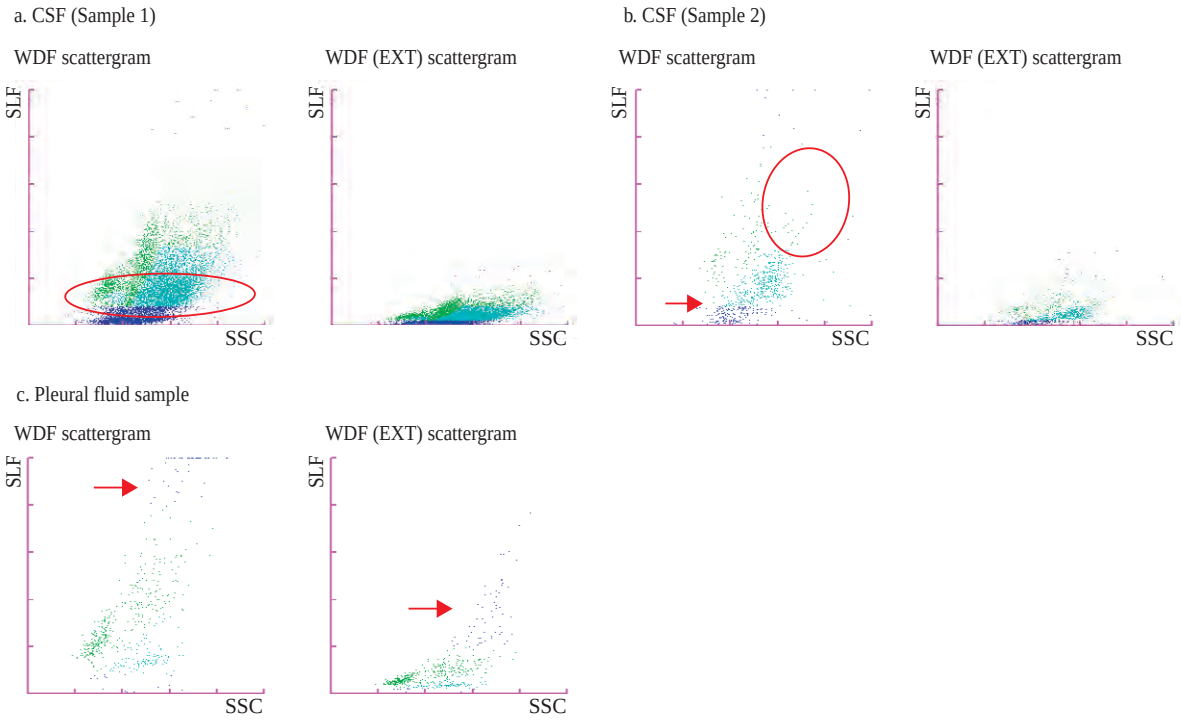
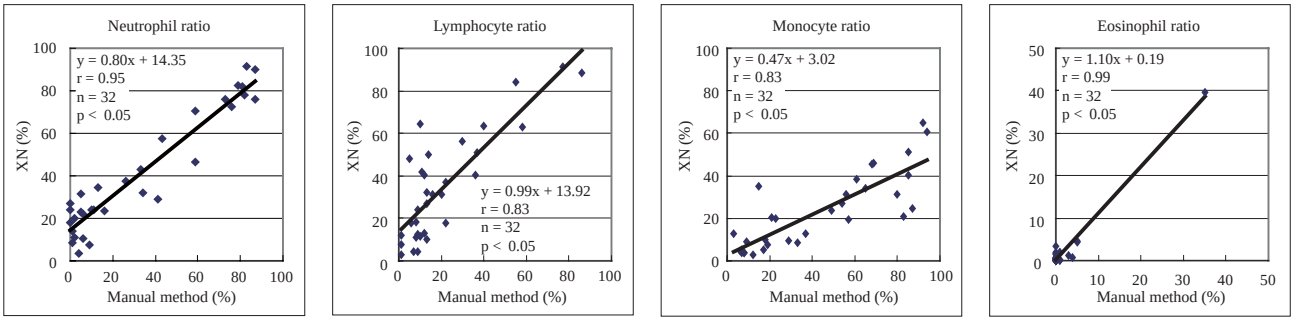


Fig. 4 *Scattergrams of samples that showed large difference between manual method and XN*

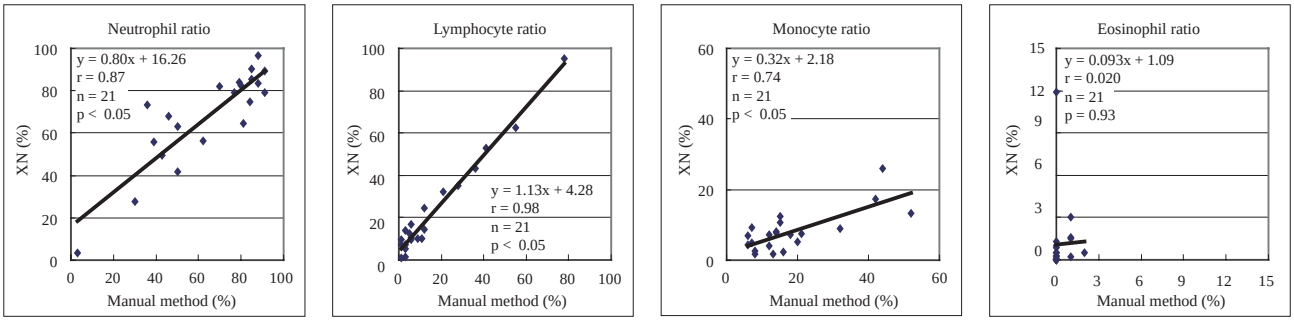
WDF (EXT) scattergram is extended the high fluorescence intensity area of WDF scattergram.
CSF sample 1 and 2 showed falsely high in the XN, and pleural fluid sample showed falsely low in the XN.

The correlation of differential cell counts between two methods was examined using all 114 samples. In samples with the nucleated cell count ≥ 100/μL, the correlation coefficients® for NE-BF, LY-BF, MO-BF, and EO-BF were respectively 0.95, 0.83, 0.83 and 0.99 using 32 samples containing abnormal cells (***Fig. 5a***). All the parameters showed a significant correlation. The correlation coefficients were 0.87, 0.98, 0.74 and 0.02 respectively using 21 samples containing no abnormal cells (only CFS), parameters except for EO-BF showed a significant correlation (***Fig. 5b***). In samples with the nucleated cell count < 100/μL, the correlation coefficients were respectively 0.14, 0.72, 0.98 and 0.56 using 20 samples containing abnormal cells (***Fig. 5c***). Parameters except for NE-BF showed a significant correlation, NE-BF showed a false high tendency in the XN. The correlation coefficients were 0.90, 0.92, 0.79 and 0.22 respectively using 41 samples containing no abnormal cells (CFS and CAPD effluent). Parameters except for EO-BF showed a significant correlation (***Fig. 5d***). The correlation of differential cell counts was good for samples without abnormal cells, there is no large difference between samples with the nucleated cell count ≥ 100/μL and those with < 100/μL. Macrophages were observed in most samples with abnormal cells. The XN does not have an analysis parameter corresponding to macrophages. But good correlation was seen between the monocyte plus macrophage count of the manual method and the MO-BF of XN, the closest parameter in XN (***Fig. 5a, c***). Irrespective of whether the nucleated cell count was ≥ 100/μL or < 100/μL, the EO-BF was maximum 2% or 2/μL respectively for samples with no abnormal cells, which were not sufficient for correlation analysis.

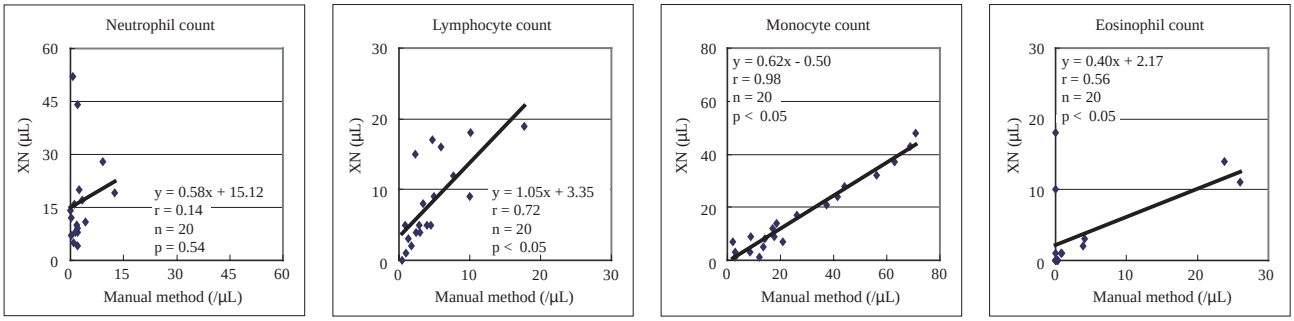
a. Samples with the nucleated cell count ≥ 100/μL containing abnormal cells



b. Samples with the nucleated cell count ≥ 100/μL containing no abnormal cells



c. Samples with the nucleated cell count < 100/μL containing abnormal cells



d. Samples with the nucleated cell count < 100/μL containing no abnormal cells

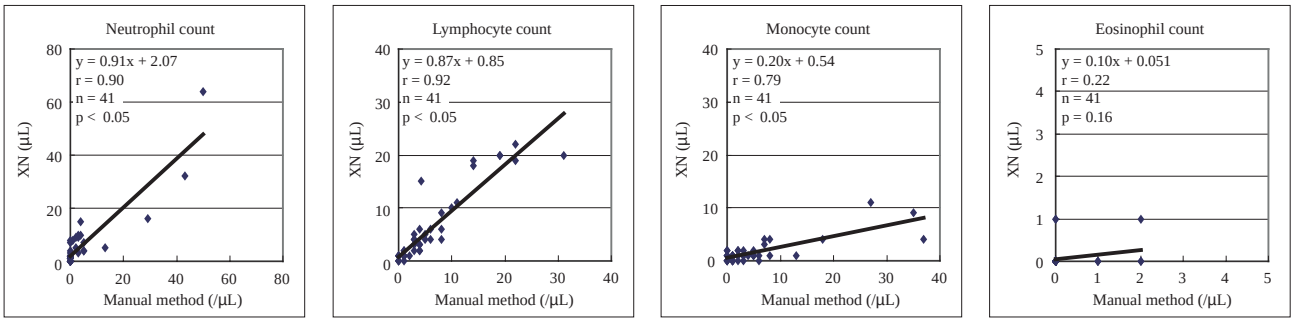


Fig. 5 *Correlation between the manual method and XN in all the samples*

Monocyte contained macrophage in the manual method.

4. Cases with abnormal cells

*1) Case1: Acute lymphocytic leukemia (CSF) (**Fig. 6a**)*
There were blastoid cells with a high N/C ratio, fine nuclear chromatin and nucleoli in the smear. These cells were classified as mononuclear cells (LY-BF in research parameters) in XN, and the area where they were plotted matched with the area of blasts in the whole blood mode scattergrams.

*2) Case 2: Brain tumor (CSF) (**Fig. 6b**)*
The smear showed large atypical oval cells with an irregular lamellar cytoplasm. These were classified as mononuclear cells (MO-BF in research parameters) by XN. Cells with a wide cytoplasm and folded structures, and macrophages with vacuoles tended to spread towards the high side scattered light (SSC) intensity area.

*3) Case 3: Lung cancer (pleural fluid) (**Fig. 6c**)*
Aggregated tumor cells and anisocytic mucinous tumor cells appeared in the smear. In XN analysis, the cells appeared in the HF-BF area of the WDF scattergram. In the WDF (EXT) scattergram also the cluster extended to an upper area. This was probably because the aggregated cells were detected as giant cells with a high nucleic acid content.

*4) Case 4: Gastric cancer (ascites) (**Fig. 6d**)*
In the smear, the tumor cells were relatively low in the N/C ratio and anisocytic, and some cells were polymorphonuclear. In the WDF scattergram, the cells were plotted in the HF-BF area. The cluster extended to an upper area in WDF(EXT) scattergram also. However, some tumor cells were classified as mononuclear cells (MO-BF in research parameters), therefore the results differed from the manual method.

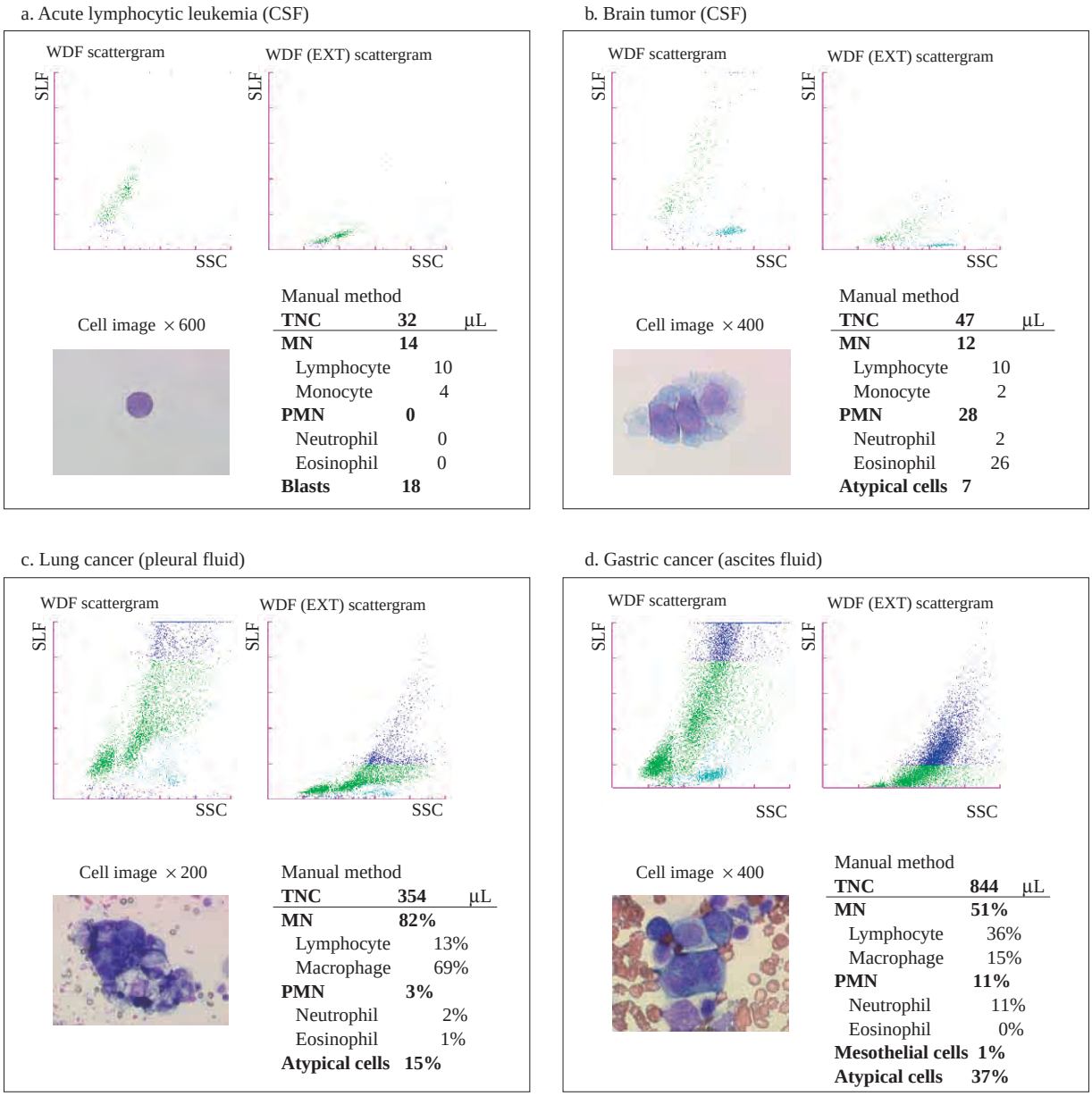


Fig. 6 *Cases of appearance of abnormal cells*

DISCUSSION

The basic assay performance of the BF mode of XN, which is used for analysis of body and punctured fluids, was evaluated. In the within-run reproducibility, samples with the nucleated cell count ≥ 10/μL had a satisfactory CV which was lower than 10%, and CV of samples with a fewer nucleated cell count was a higher tendency. It was thought that it was necessary to retest by the manual method when nucleated cell count by XN is lower than 10/μL. Although the CV of the cell differential was high when the nucleated cell count or the differential cell percentage was low, it was acceptably low when the nucleated cell counts were high. Because body and punctured fluids examinations are ordered frequently on day and night duty, it is desirable to further improve the accuracy of the XN in the low cell count range, so that it can be used for all samples in the future.

The specified upper limit for linearity with XN is 10 × 10^3/μL. However, when using samples prepared from peripheral blood with a high white blood cell count, dilution linearity was confirmed in a range of 0.01-100 × 10^3/μL. This suggests that accurate analysis was possible over a wide range of cell counts. Nevertheless, various types of specimens are to be analyzed in the testing of body and punctured fluids. It is not easy to evaluate assay linearity using specimens of various types, because the measurement is affected by different factors regarding sample properties such as sample storage, sample viscosity, cell destruction and aggregated cell masses. Therefore, various properties of the sample need to be taken into consideration in evaluating the assay linearity. This is one of the issues to be studied in the future.

The correlation of nucleated cell count between the manual method and XN was good for all specimen types. The correlation was somewhat lower in CSF samples with nucleated cell count < 100/μL compared to samples with nucleated cell count ≥ 100/μL, but the accuracy comparable to the manual method was confirmed. In pleural fluid, synovial fluid, and CAPD effluent, good correlation was confirmed when macrophages were included in the count of mononuclear cells in the manual method. This result suggested that although XN did not have an analysis parameter corresponding to macrophages, the macrophages were classified as mononuclear cells in many cases of XN analysis. Six samples showed a two-fold difference between two methods for the nucleated cell counts. The XN scattergrams showed poor separation of the Debris from the WBC area in CSF samples whose smears showed a number of cell degeneration or destruction. Because of cell destruction, nucleated cell count by XN was falsely decreased. Alternatively, because some cell fragments formed by cell destruction were counted as cells, the nucleated cell count was falsely increased. Such cell destruction is often seen in samples with significant increased number of cells, or long time passed after collected. Therefore, it is preferable to measure body fluids as quickly as possible after they are collected. Protein concentration of the sample with plots in the strong SSC area of WDF scattergram was high, and it was thought that a fluorescent dye nonspecifically bound to precipitated protein particle, and then it was counted as a cell. There was a case that the cause was not able to be specified from the scattagram and the cytological analysis. Two samples of pleural fluid showed falsely low nucleated cell counts by XN. Aggregations of macrophages were seen in these samples smears and plots appeared in the HF-BF area of WDF scattergram. Such macrophage aggregates were counted as individual large cells resulting in falsely low cell counts. As discussed above, in body and punctured fluids analysis by XN, not only the numerical values but also scattergrams need to be carefully observed, and the results need to be comprehensively interpreted taking the properties of the sample, particularly degenerative changes during sample storage, etc also into account.

In the differential cell counts of all samples, correlation between the manual method and XN was good when the nucleated cell count ≥ 100/μL. The correlation coefficients were slightly low for samples with the nucleated cell count < 100/μL that contained abnormal cells also. This was probably because some of abnormal cells were often counted as mononuclear cells (monocytes or lymphocytes), depending on their type and size. Macrophages were suggested to appear mainly in the monocyte area of WDF scattergram, because correlation was good between the MO-BF count by XN and the sum of monocyte and macrophage counts by the manual method. However, a part of macrophage was counted as neutrophil, and it suggested that neutrophil showed false high tendency. This information would be useful for interpreting the differential cell counts during on day and night duty, where smears are not prepared. When abnormal cells appeared, it was possible to detect in the HF-BF area of the WDF and WDF (EXT) scattergrams, which extended the high fluorescence intensity area of WDF scattergram. In such cases, it is particularly important to classify the cells by the manual method, and the information from scattergrams can be utilized for it as a reference. It is also possible to set the analyzer to output a message when the cell count in the HF-BF area exceeds a certain level, and such a flagging function would be useful.

The aspirated sample volume by XN is as little as only 88μL, and the analysis time is fast with only 1.5 minutes per sample. It is sufficient to simply switch the system to the BF mode to make measurements on body fluids. There is no need to change the reagents and to make any preparation of samples. A background check is performed automatically when the analyzer is switched to the BF mode after analysis in the whole blood mode. This background check is done a maximum of 3 times until the WBC count becomes not more than 1/μL and the RBC count becomes not more than 3×10^3/μL, to avoid any influence of the sample carryover. Thus, the body fluid examination by the BF mode of XN was thought to be useful for examination of on routine laboratory, on day and night duty, if it is operated by an appropriate rule including use of a flagging function in the clinical laboratory.

CONCLUSION

Our evaluation of the basic assay performance of the BF mode on the automated hematology analyzer XN-2000 showed that the nucleated cell count and the differential cell counts measured in the BF mode had good within-run reproducibility, linearity and correlation with the manual method. The analyzer requires very small sample volumes, short analysis times, and runs a background check before the analysis to avoid the influence of carryover. It provides accurate results even in a low measured value range, therefore, the analyzer is useful in examination of on routine laboratory, on day and night duty.

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