Performance Evaluation of the XN-550 Automated Hematology Analyzer Body Fluid Mode — Considerations for Operational Conditions for Cell Counting with Cerebrospinal and Synovial Fluids —

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The performance of the body fluid mode on the Automated Hematology Analyzer XN-550 (XN-550; Sysmex Corporation, Kobe, Japan) was evaluated using cerebrospinal and synovial fluid samples. Correlations with the chamber counting method of cerebrospinal fluid were generally good for white blood cell, mononuclear cell, and polymorphonuclear cell counts (correlation coefficients: r = >0.952). However, drainage samples containing degenerative cells deviated from the chamber counting method. Correlations with the Automated Hematology Analyzer XN-9000 (XN-9000; Sysmex Corporation, Kobe, Japan) were good for white blood cell, mononuclear cell, and polymorphonuclear cell counts (correlation coefficients: r = >0.979). Correlations with the chamber counting method of synovial fluid were good for white blood cell, mononuclear cell, and polymorphonuclear cell counts (correlation coefficients: r = >0.911). The body fluid mode of XN-550 may become useful for tests on off-duty time by setting operational conditions.

Key Words ▷ Cerebrospinal Fluid, Synovial Fluid, Automated Hematology Analyzer, XN-550, White Blood Cell Count

INTRODUCTION

Cell counting in cerebrospinal fluid samples is essential in diagnosis of central nervous system disorders, such as meningitis and encephalitis [1], while synovial fluid cell counts may help in diagnosing inflammatory diseases. Cerebrospinal and synovial fluid cell counts are often performed using the traditional hemocytometer chamber counting method. However, many laboratory technologists who have less experience in body fluid analysis, or those who work off-duty time shifts, may not be as competent in reporting accurate chamber counts. Automated blood cell counters measuring cerebrospinal and body cavity fluids have recently been developed and are now becoming utilized in many facilities during emergency examinations such as on off-duty time [2, 3].
The Automated Hematology Analyzer XN-550 (XN-550; Sysmex Corporation, Kobe, Japan), equipped with a body fluid mode, is a compact device developed for use in clinics and small hospitals. The performance of this device was evaluated using the body fluid mode, including cell counting of cerebrospinal and synovial fluids and the effects of adding hyaluronidase to synovial fluid samples.

SAMPLES AND METHODS

1. Samples

Cerebrospinal and synovial fluid samples submitted to the Department of Clinical Laboratory of The University of Tokyo Hospital were used in the study. The study was conducted with the approval of the Research Ethics Committee of Graduate School of Medicine and Faculty of Medicine, The University of Tokyo under a contract research agreement with Sysmex Corporation.

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2. Analyzer

The XN-550 was evaluated for reporting cell counts in body fluids. The measurement principle of the analyzer is flowcytometry using a semiconductor laser. In this method, cells are irradiated by laser for cell counting and cell differentiation using data of forward scattered light (cell size), side scattered light (intracellular structure) and side fluorescence light (amounts of cellular nucleic acid) (*Fig. 1*).

3. Methods

1) Repeatability and Reproducibility
For repeatability, two concentrations of dedicated control, XN CHECK™ BF levels 1 and 2 (XN CHECK BF; Sysmex Corporation, Kobe, Japan), were measured 10 times consecutively. Reproducibility measurements were conducted once daily for 20 days using the same control as the repeatability test.

2) Effects of adding hyaluronidase to synovial fluid
The effects of hyaluronidase, an enzyme used to reduce the viscosity of synovial fluid, were examined. Hyaluronidase (SIGMA, Inc.) was dissolved in saline to prepare a dilution series (0, 200, 400, 1,200, and 1,600 units/mL). Peripheral blood supplemented with EDTA-2K was diluted with saline to prepare samples. Then 1 mL each of synovial fluid samples and hyaluronidase solution were mixed and measured in triplicate. The mean values of the measurement results were calculated.

3) Correlation
Correlation between the chamber counting method (as per "Cerebrospinal Fluid Testing Textbook [4]") and Automated Hematology Analyzer XN-9000 (XN-9000; Sysmex Corporation, Kobe, Japan), also equipped with a body fluid mode, were examined for white blood cell, mononuclear cell, and polymorphonuclear cell counts in cerebrospinal fluid.
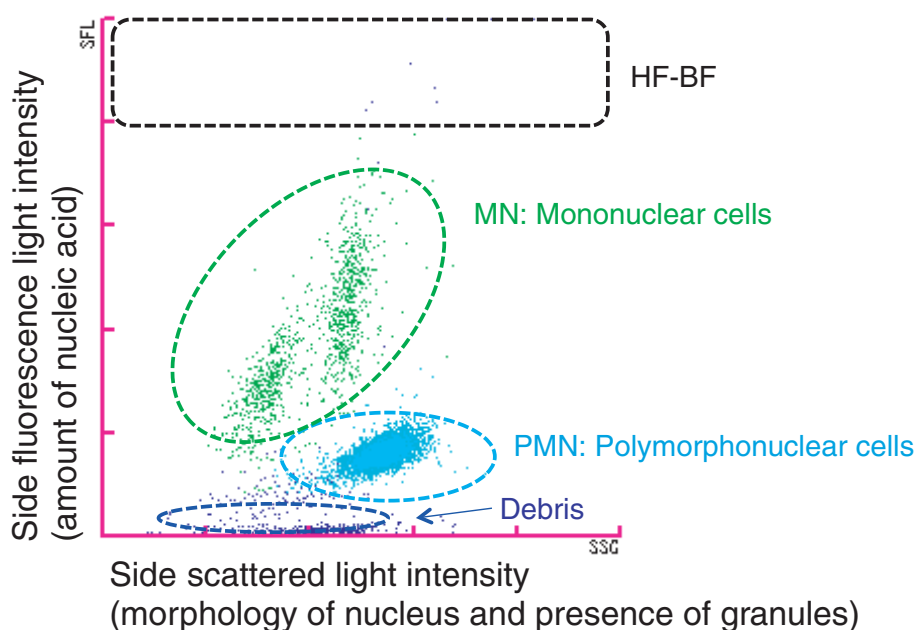
Fig. 1   *Scattergram in body fluid mode*

Correlation with the chamber counting method using a Burker-Turk hemocytometer was examined for white blood cell, mononuclear cell, and polymorphonuclear cell counts in synovial fluid. Equal amounts of viscous synovial fluid and hyaluronidase solution (100 units/mL) dissolved in saline were mixed, and after confirming the absence of viscosity, these samples were measured. Samples with high white blood cell counts were diluted with saline before measurement.

RESULTS

1. Repeatability and Reproducibility

The repeatability (CV values; %) of white blood cell counts (WBC-BF), mononuclear cell counts (MN#), and polymorphonuclear cell counts (PMN#) was 4.4 to 8.1% in the low concentration area (level 1) and 3.1 to 5.7% in the medium concentration area (level 2), respectively. The CV (%) of each parameter for reproducibility were 3.7 to 7.5% in the low concentration area and 2.2 to 3.4% in the medium concentration area, respectively (***Table 1***).

Table 1 *Results of repeatability and reproducibility*

Repeatability

(n=10)

/μL	XN CHECK BF L1 (Low concentration area)			XN CHECK BF L2 (Medium concentration area)		
	WBC-BF (White blood cell count)	MN# (Mononuclear cell count)	PMN# (Polymorpho-nuclear cell count)	WBC-BF (White blood cell count)	MN# (Mononuclear cell count)	PMN# (Polymorpho-nuclear cell count)
MEAN	84.6	29.0	55.6	326.2	109.0	217.0
Min	79.0	26.0	50.0	309.0	99.0	198.0
Max	92.0	34.0	61.0	343.0	118.0	225.0
Range	13.0	8.0	11.0	34.0	19.0	27.0
SD	3.75	2.36	3.60	10.18	6.25	85.5
CV (%)	4.4	8.1	6.5	3.1	5.7	3.9

Reproducibility

(n=20)

/μL	XN CHECK BF L1 (Low concentration area)			XN CHECK BF L2 (Medium concentration area)		
	WBC-BF (White blood cell count)	MN# (Mononuclear cell count)	PMN# (Polymorpho-nuclear cell count)	WBC-BF (White blood cell count)	MN# (Mononuclear cell count)	PMN# (Polymorpho-nuclear cell count)
MEAN	85.5	28.8	56.8	329.5	111.2	218.3
Min	79.0	25.0	53.0	316.0	105.0	207.0
Max	91.0	33.0	60.0	341.0	118.0	233.0
Range	12.0	8.0	7.0	25.0	13.0	26.0
SD	3.19	2.15	2.45	7.17	3.82	6.76
CV (%)	3.7	7.5	4.3	2.2	3.4	3.1

2. Effects of adding hyaluronidase to synovial fluid

Adding hyaluronidase (0, 100, 200, 400, and 800 units/mL) to diluted peripheral blood samples caused no change in the white blood cell counts, differentiations, scattergrams, or microscopic images (***Fig. 2***).
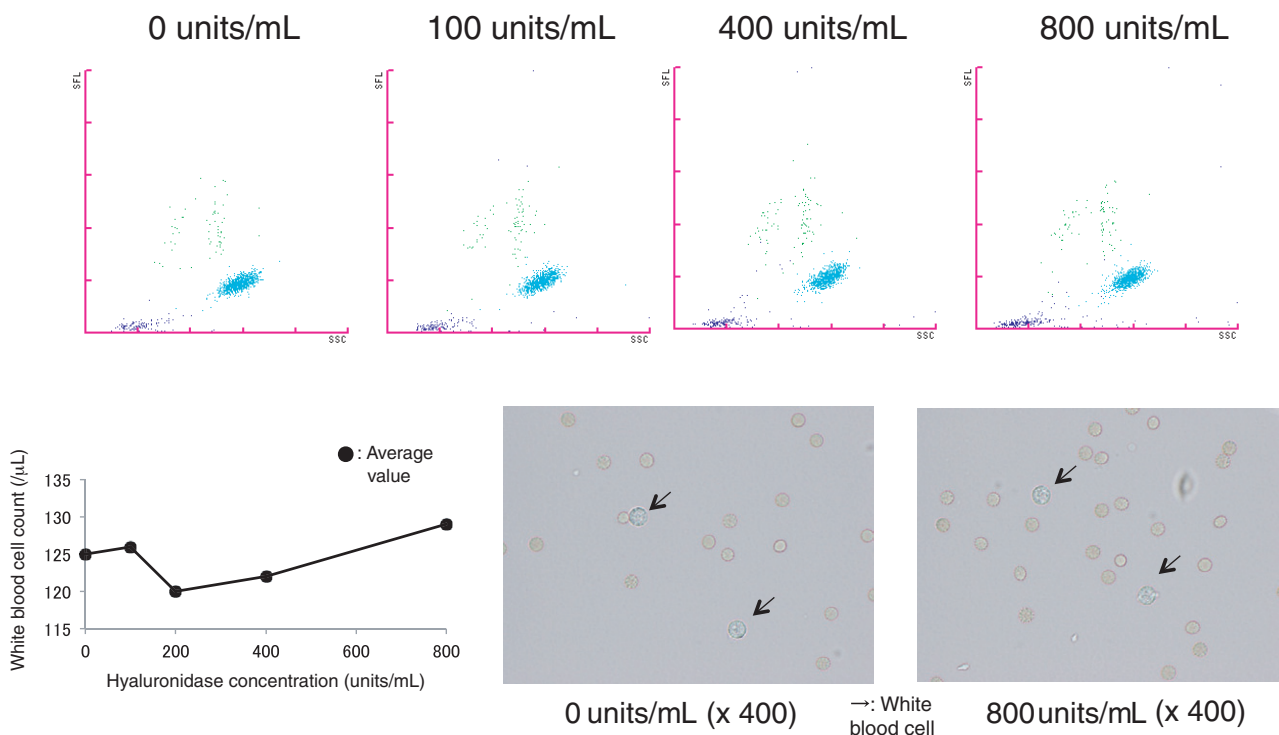
3. Correlations

1) Cerebrospinal fluid

Correlation coefficients between chamber counting method and XN-550 in all samples (n = 88) were r = 0.996, 0.992, and 0.952 for white blood cell, mononuclear cell, and polymorphonuclear cell counts, respectivel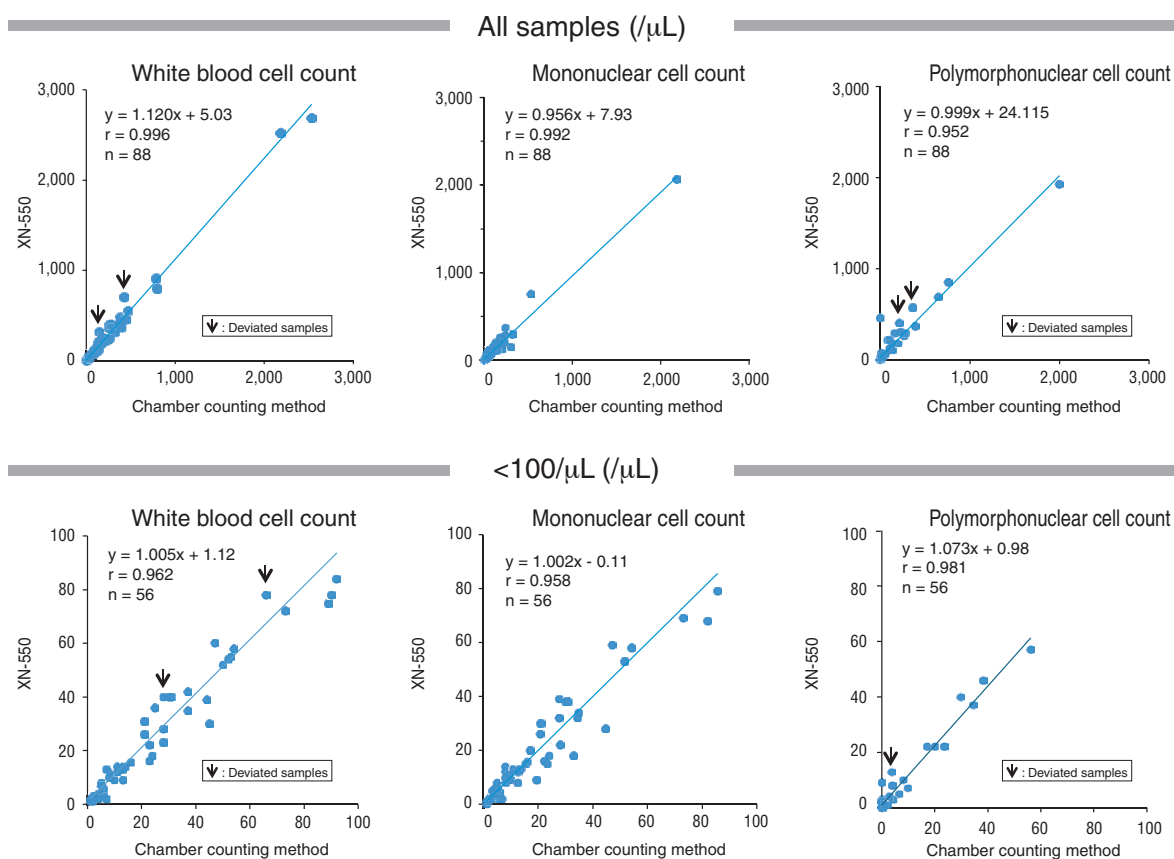y. Correlation coefficients in samples with <100/μL white blood cell counts (n = 56) were r = 0.962, 0.958, and 0.981 for white blood cell, mononuclear cell, and polymorphonuclear cell counts, respectively. However, white blood cell counts and differentiations deviated in some samples (***Fig. 3***). Correlation coefficients between XN-9000 and XN-550 in all samples (n = 73) were r = 0.999, 0.994, and 0.998 for white blood cell, mononuclear cell, and polymorphonuclear cell counts, respectively. Correlation coefficients in samples with <100/μL white blood cell counts (n = 52) were r = 0.985, 0.979, and 0.989 for white blood cell, mononuclear cell, and polymorphonuclear cell counts, respectively (***Fig. 4***).

Effects of cell counts by addition of Hyaluronidase

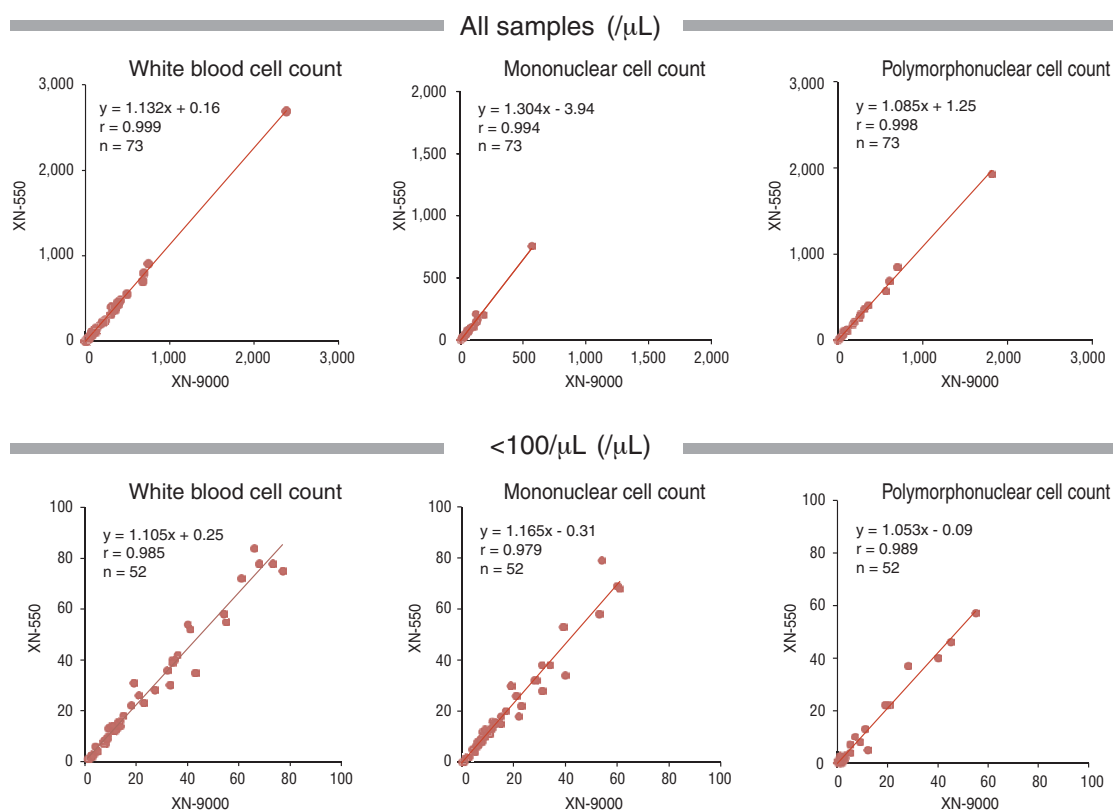0 units/mL   100 units/mL   400 units/mL   800 units/mL

0 units/mL (x 400)   →: White blood cell   800 units/mL (x 400)

*Added 1 mL hyaluronidase solution in each concentration to 1 mL of sample.

Fig. 2   *Effects by hyaluronidase*

All samples (/μL)

White blood cell count

y = 1.120x + 5.03
r = 0.996
n = 88

Mononuclear cell count

y = 0.956x + 7.93
r = 0.992
n = 88

Polymorphonuclear cell count

y = 0.999x + 24.115
r = 0.952
n = 88

<100/μL (/μL)

White blood cell count

y = 1.005x + 1.12
r = 0.962
n = 56

Mononuclear cell count

y = 1.002x - 0.11
r = 0.958
n = 56

Polymorphonuclear cell count

y = 1.073x + 0.98
r = 0.981
n = 56

Fig. 3 *Correlations of cerebrospinal fluid between chamber counting method and XN-550*

All samples (/μL)

White blood cell count

y = 1.132x + 0.16
r = 0.999
n = 73

Mononuclear cell count

y = 1.304x - 3.94
r = 0.994
n = 73

Polymorphonuclear cell count

y = 1.085x + 1.25
r = 0.998
n = 73

<100/μL (/μL)

White blood cell count

y = 1.105x + 0.25
r = 0.985
n = 52

Mononuclear cell count

y = 1.165x - 0.31
r = 0.979
n = 52

Polymorphonuclear cell count
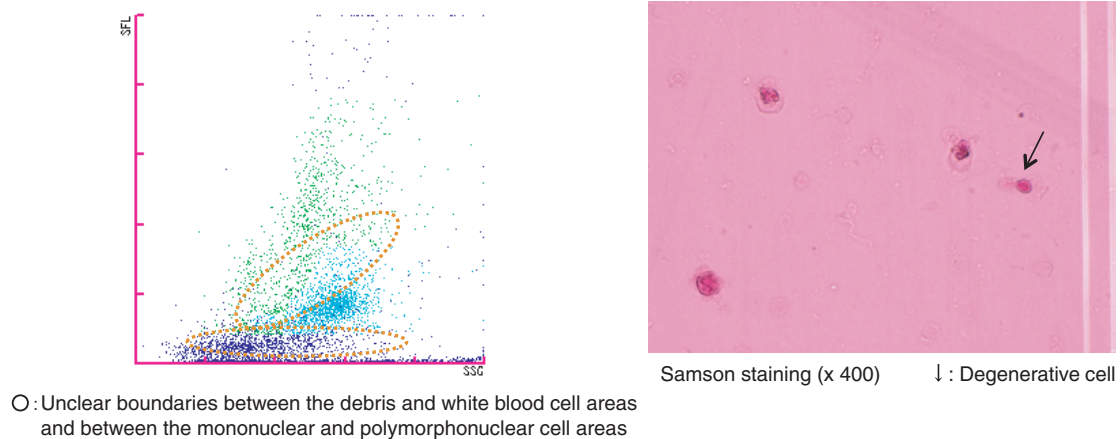
y = 1.053x - 0.09
r = 0.989
n = 52

Fig. 4 *Correlations of cerebrospinal fluid between XN-9000 and XN-550*
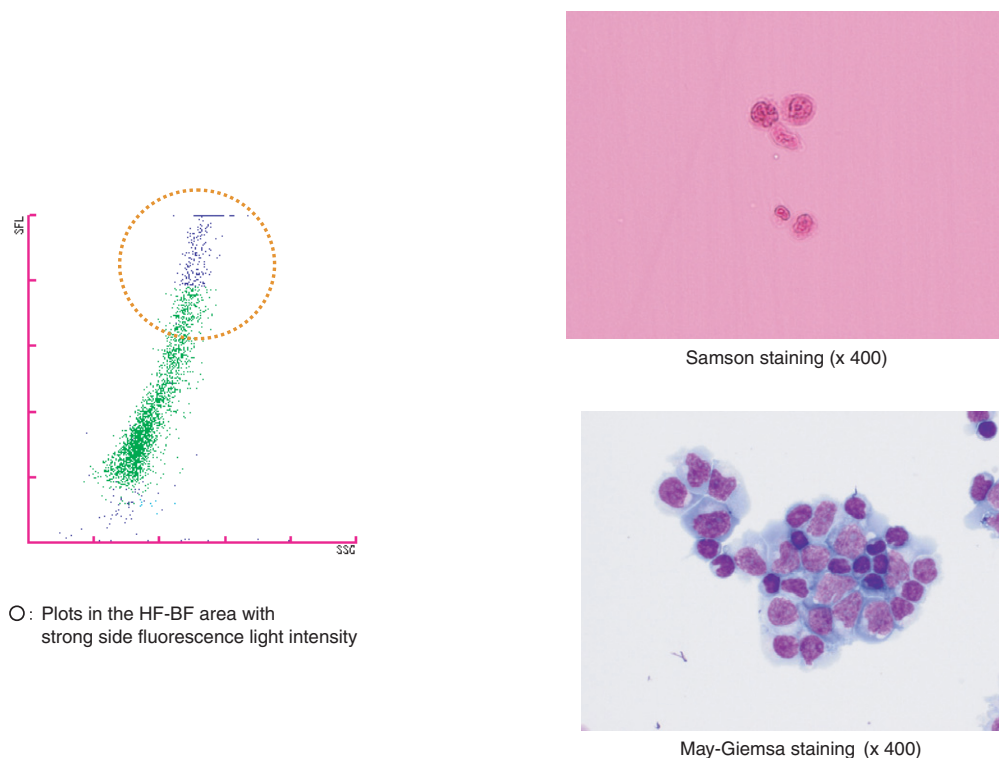
Samples that deviated from chamber counting method showed similar results for both XN-550 and XN-9000. The Samson-stained morphological images of drainage samples contained many degraded cells, including fragments and those with fused fluid or bare nucleus. These discrepant samples included drainage samples. The scattergram showed overlapping clusters of debris, mononuclear cells, and polymorphonuclear cells (○ areas) (*Fig. 5*). In such patterns, the automated white blood cell and polymorphonuclear cell counts were higher compared to results from the chamber counting method.

The scattergram of malignant lymphoma showed a straight extension from mononuclear cell area to HF-BF area (*Fig. 6*). The morphological images obtained by Samson staining showed mononuclear cells larger in size than lymphocytes, a high N/C ratio, and prominent nucleoli. The images obtained by May-Giemsa staining showed large cells, basophilic cytoplasm, rough nuclear nets, and prominent nucleoli.

○：Unclear boundaries between the debris and white blood cell areas and between the mononuclear and polymorphonuclear cell areas

Samson staining (x 400)　　　↓ : Degenerative cell

Fig. 5　*Samples deviating from chamber counting method*

○ : Plots in the HF-BF area with strong side fluorescence light intensity

Samson staining (x 400)

May-Giemsa staining (x 400)

Fig. 6　*Case of malignant lymphoma*

2) Synovial fluid
Correlation coefficients between traditional chamber counting and the automated XN-550 counts in all samples (n = 19) were r = 0.977, 0.911, and 0.957 for white blood cell, mononuclear cell, and polymorphonuclear cell counts, respectively. Correlation coefficients in samples with <5,000/μL cell counts (n = 11) were r = 0.992, 0.976, and 0.993 for white blood cell, mononuclear cell, and polymorphonuclear cell counts, respectively (*Fig. 7*).
The case in *Fig. 8* showed a pattern of many cell signals plotted from the debris to white blood cell area (○ area). The cell morphologies obtained by the chamber counting method showed many neutrophils, degenerative cells and histiocytes.
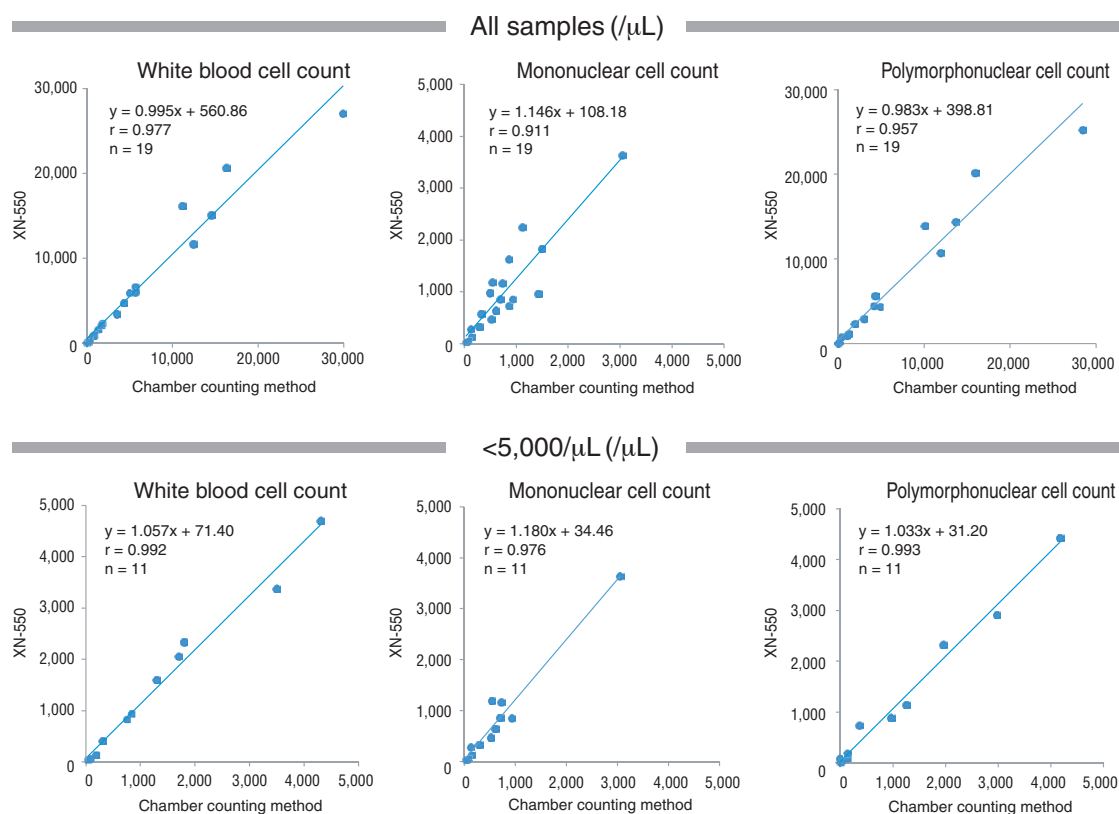
DISCUSSION

The performance of the body fluid mode on the XN-550 was evaluated using cerebrospinal and synovial fluids. Repeatability and reproducibility was less than 10% CV for white blood cell, mononuclear cell, and polymorphonuclear cell counts. The minimum detection sensitivity was 2/μL white blood cell as determined in the previous study by authors [5].
Correlation coefficients of white blood cell, mononuclear cell, and polymorphonuclear cell counts between XN-550 and the traditional chamber counting method in cerebrospinal fluid were r = 0.952 to 0.996. The results

were acceptable, although some samples showed deviations. Correlation coefficients between XN-550 and XN-9000 in white blood cell, mononuclear cell, and polymorphonuclear cell counts were r = 0.979 to 0.999 and the results were good. Samples that demonstrated discrepant results between automated and traditional counting methods were yellow to reddish drainage samples with many degraded cells including fragments. The scattergrams of deviated samples exhibit a pattern of overlapping clusters of debris and white blood cells or mononuclear and polymorphonuclear cells, with unclear boundary. These degenerative cells might be plotted at the boundary of the clusters [6,7]. The pattern of unclear boundary between debris and white blood cell areas might influence white blood cell counts, and the boundary between mononuclear and polymorphonuclear cell areas might influence the differentiation.
In HF-BF area, atypical cells with large amounts of nucleic acid (such as malignant lymphoma or leukemia cells) and histiocytes are plotted [8,9]. In the scattergram, malignant lymphoma cells were plotted from the mononuclear cell to high side fluorescence intensity HF-BF areas. The ALL and AML-M1 cases showed similar patterns. Therefore, in cases with the patterns as shown in *Fig. 6*, manual smear review would be required to check for atypical cells. In addition, cells in HF-BF areas are not counted as white blood cells and thus TC-BF (HF-BF + WBC-BF) values should be reported. However, in some cases, malignant lymphoma cells were not plotted in the HF-BF area using XN-9000 which

All samples (/μL)

White blood cell count
y = 0.995x + 560.86
r = 0.977
n = 19

Mononuclear cell count
y = 1.146x + 108.18
r = 0.911
n = 19

Polymorphonuclear cell count
y = 0.983x + 398.81
r = 0.957
n = 19

<5,000/μL (/μL)

White blood cell count
y = 1.057x + 71.40
r = 0.992
n = 11

Mononuclear cell count
y = 1.180x + 34.46
r = 0.976
n = 11

Polymorphonuclear cell count
y = 1.033x + 31.20
r = 0.993
n = 11

Fig. 7   Correlations of synovial fluid between chamber counting method and XN-550
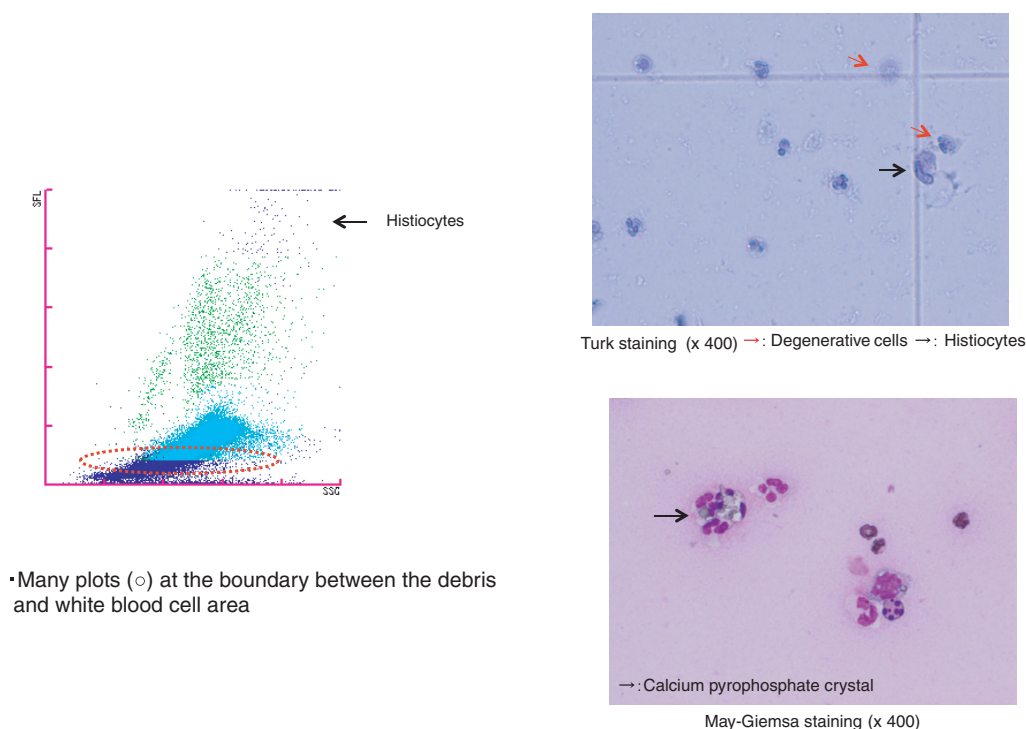
applies the same principle as XN-550. Because of this, it is assumed that the same phenomenon is seen in XN-550. Therefore, it was difficult to detect all atypical cells using scattergram patterns at times.

For cell counting in synovial fluids, viscosity needs to be reduced to prevent clogging in analyzer. The effects of adding hyaluronidase (0 to 800 units/mL) to reduce viscosity were examined. White blood cell counts and scattergram patterns did not change when using different hyaluronidase concentrations. According to the Body Fluid Analysis for Cellular Composition; Approved Guideline [10] of the Clinical and Laboratory Standards Institute (CLSI), standard laboratory practice includes adding 400 units of hyaluronidase per 1 mL of synovial fluid. However, no guideline is available in Japan, and dosage varies among articles. Therefore, there is a need for practice standardization.

The correlation coefficients between XN-550 and chamber counting methods for white blood cell, mononuclear cell, and polymorphonuclear cell counts were as high as r = 0.911 to 0.993, almost comparable with those reported by Hoshina et al. The scattergram patterns (*Fig. 8*) showed many plots from the debris to polymorphonuclear cell areas and some plots in the HF-BF area. Many neutrophils and degenerative cells were contained in the samples. These cells were plotted from the debris to polymorphonuclear cell areas (○ areas). The plots in the HF-BF area were considered to be histiocytes. Pseudogout was suspected in this case because synovial fluid testing was conducted for the swelling of the knee joint and calcium pyrophosphate crystals were also detected. In this study, no deviation from chamber counting method was observed. However, considering the study results for cerebrospinal fluid, samples with overlapping cluster patterns on the scattergram should be confirmed by the chamber counting method. Further data collection is planned in the future for further validation due to small sample size of this study.

The XN-550 automated cell counts for cerebrospinal and synovial fluids demonstrated good correlation with the traditional chamber counting method for white blood cell counts and differentiations. However, if cluster overlapping patterns exist in the scattergram, the automated results may differ from the chamber count results. In addition, since atypical cells and histiocytes may be plotted in the HF-BF area, such results should be interpreted carefully. Laboratory technologists who perform fluid analysis less frequently should be well trained on scattergram interpretation. It is imperative that these technologists understand the differences between reliable normal and unreliable abnormal patterns. It is recommended that a simplified schematic diagram and actual scattergram (*Fig. 9*) be presented during training to facilitate the understanding of important points for differentiation. From the above results, the XN-550 is considered to be useful for tests on off-duty time if operational conditions are appropriately set.

• Many plots (○) at the boundary between the debris and white blood cell area

Turk staining (x 400) →: Degenerative cells →: Histiocytes

→:Calcium pyrophosphate crystal

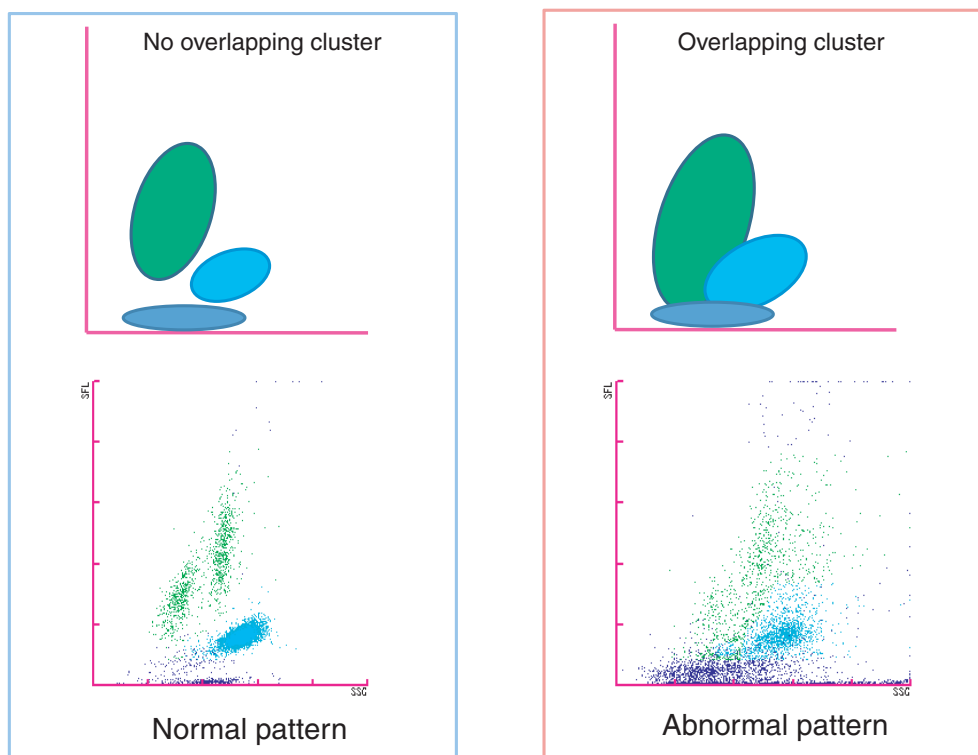May-Giemsa staining (x 400)

Fig. 8   Synovial fluid

Fig. 9 *Examples of educational slide*

CONCLUSIONS

Cell counting in cerebrospinal and synovial fluids samples using XN-550 showed good repeatability, reproducibility and correlations with the traditional chamber counting method. To use XN-550, operational conditions, such as sample properties and scattergram patterns, as well as measurement results, need to be examined. This analyzer is easy to operate and considered to be useful for many laboratory technologists who have less experience in body fluid analysis.

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