

Validation of Improved Body Fluid Mode in Sysmex UF-5000, an Automated Urine Particle Analyzer: Comparison with XN-1000

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The UF-5000 fully automated urine particle analyzer (hereafter referred to as UF-5000; Sysmex Corporation, Kobe, Japan) is equipped with a body fluid mode. The UF-5000 body fluid mode was compared with the body fluid mode on the XN-1000 automated hematology analyzer (hereafter referred to as XN-1000; Sysmex). The previous UF-5000 model measured low WBC counts due to poor stainability in samples with high WBC counts. Given the inconsistency, the sample dilution rate on the system was subsequently changed. The comparison and analysis of discrepant samples using the improved XN 1000, showed good correlation with differential WBC counts, at a coefficient of $r = 0.982$ to 0.997 . 15/29 samples showed a discrepancy using the pre-improved method with WBC counts of $\geq 1,000/\mu\text{L}$ on the XN-1000. Of the 42 samples evaluated using the improved system, no discrepancies were reported with WBC count of $\geq 1,000/\mu\text{L}$ on the XN-1000. Based on the study findings, WBC stainability was confirmed.

Key Words Body Fluid, Body Fluid Mode, UF-5000, XN-1000, Cell Count

Received 30 September, 2021; Accepted 18 October, 2021

Introduction

In recent years, an increasing number of laboratories have obtained automated hematology analyzers equipped with a function for measuring body fluid cell counts. It is expected that body fluid cell counting automation will reduce turn-around time, enable testing in daytime work hours, and save on labor costs.¹⁾ The XN series of automated multiparameter hematology analyzers have shown good correlation when compared with visual microscopy.^{2,3)}

The UF-5000 fully automated urine particle analyzer (hereafter referred to as UF-5000; Sysmex Corporation, Kobe, Japan) has been further improved by the addition of a body fluid (BF) mode to count cells in spinal, pleural effusion, and ascitic fluids. Comparison of the UF-5000 with the XN-1000 automated hematology analyzer (hereafter referred to as XN-1000; Sysmex) has shown good correlation, although discrepancies have been reported on samples with low values.⁴⁾ In these samples, inaccurate counts occurred due to poorly stained WBCs which caused the discrepancy. As a result of this finding, the sample dilution rate was increased to improve the staining capability of cells.

This paper reports the findings of a study comparing the BF modes of the improved UF-5000 with the XN-1000.

1. Subjects and Methods

1.1 Subjects

Deidentified samples were submitted to the Department of Clinical Laboratory for testing in the study. A total of 111 samples (55 pleural effusion fluid samples, 55 ascites fluid samples, 1 unknown sample) were analyzed using the original UF-5000 system. 121 different samples (81 pleural effusion fluid samples, 40 ascites fluid samples) were then analyzed using the updated UF-5000 system. This study was conducted under a research contract agreement with Sysmex Corporation and the Graduate School of Medicine and Faculty of Medicine at the University of Tokyo following the approval by their Research Ethics Committee (approval No. 3333-99, approved on March 1, 2015).

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1.2 Measuring principle of UF-5000

In the BF mode of the UF-5000, similar to the urinary particle analysis mode, components are classified using a combination of particle size and nucleic acid content data obtained by flow cytometry with a blue semiconductor laser. A polymethylene fluorescent dye is used which stains intracellular nucleic acids in the WBC counting channels. Epithelial cells (ECs) and WBCs are classified – using a combination of signal information, including: a. a forward-scattered light signal width (an index of particle length), b. side-scattered light signal waveform area (an index of size in view of internal structure complexity), c. side fluorescent light signal waveform area (an index of the amount of nucleic acid), d. side fluorescent light intensity (an index of nucleic acid stainability), and e. side-scattered light intensity (an index of internal structure complexity and thickness). WBCs are further classified into mononuclear cells (MNs) and polymorphonuclear cells (PMNs) (**Fig. 1**). In the newer specification of the UF-5000, the sample dilution rate was increased from four-fold to eight-fold to improve WBC stainability. **Table 1** shows the UF-5000 BF mode specifications.

1.3 Methods

1.3.1 Correlations

Cell counts from the XN-1000 were compared with visual microscopy. Differential cell counts from the XN-1000 were compared with visual microscopy from smear specimens stained with May-Grünwald-Giemsa stain (hereafter referred to as visual microscopy). Visual microscopy samples expected to contain small numbers of cells based on their color and turbidity, were diluted with Samson stain at a ratio of 10/9 (1.1111) and then counted using a Fuchs-Rosenthal hemocytometer. Samples expected to contain large numbers of cells were diluted 10-fold with Türk solution and analyzed using a Bürker-Türk hemocytometer. Data were statistically analyzed using Deming's regression analysis.

1.3.2 Review of discrepant samples

WBC count samples $\geq 1,000/\mu\text{L}$ on the XN-1000 were considered to be discrepant. On the UF-5000, a result $\leq -20\%$ compared to the XN-1000 was considered a discrepancy. The number of discrepant samples were compared before and after the improvement was made to the analyzer.

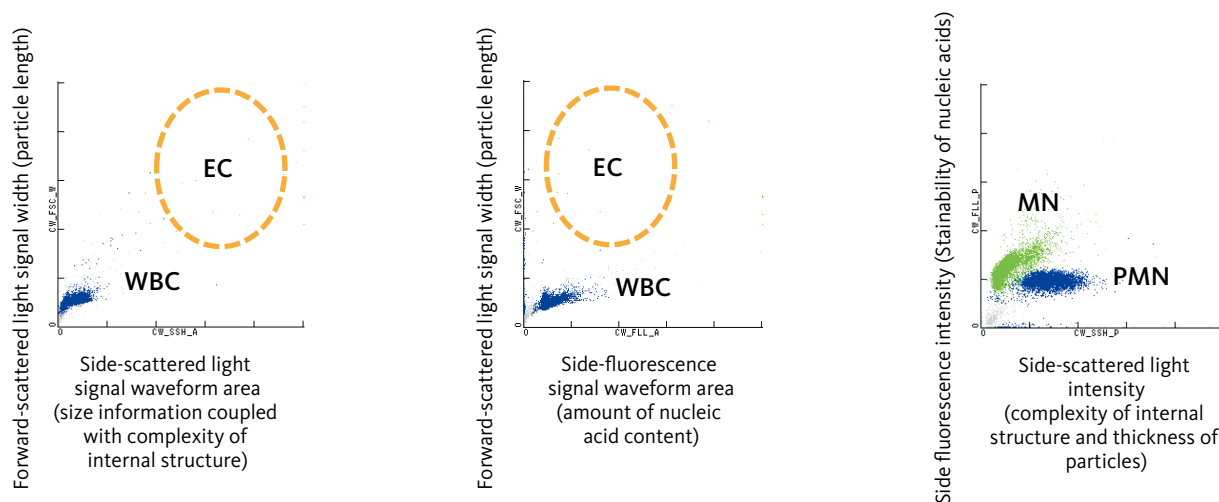



Fig. 1 Scattergram of the BF mode of UF-5000
 EC: epithelial cell (plotted in their region ) , MN: mononuclear cell, PMN: polymorphonuclear cell

Table 1 UF-5000 “BF mode” specifications

Throughput	up to 20 samples/hour
Parameters	RBCs WBCs (mononuclear cell count, mononuclear cell ratio, polymorphonuclear cell count, polymorphonuclear cell ratio)
Sample suction volume	450 μL
Required sample volume	600 μL
Measuring spans	RBC: 15.0–99,999.9 / μL WBC: 2.0–10,000.0 / μL

2. Results

2.1 Correlations

With WBC counts, the correlation between the XN-1000 and the improved UF-5000 was represented by $y = 1.12x + 23.4$ (x: XN-1000, y: UF-5000) and with a correlation coefficient $r = 0.997$ (**Fig. 2A**). The correlation with visual microscopy was represented by $y = 1.32x - 55.0$, $r = 0.978$ (**Fig. 2B**). In MN counting, the correlation between XN-1000 and the improved UF-5000 was represented by $y = 1.20x + 60.7$, $r = 0.993$ (**Fig. 3A**), and the correlation with visual microscopy was represented by $y = 1.30x - 13.2$, $r = 0.969$ (**Fig. 3B**). In PMN counting, the correlation between XN-1000 and the improved UF-5000 was

represented by $y = 1.00x - 65.5$, $r = 0.982$ (**Fig. 4A**), and the correlation with visual microscopy was represented by $y = 1.19x + 5.80$, $r = 0.971$ (**Fig. 4B**).

2.2 Review of discrepant samples

Before improvements were made to the XN-5000, a comparison study was conducted with the XN-1000. The results showed 15 out of 29 discrepant samples (13 pleural effusion fluid samples, 1 ascitic fluid sample, 1 other sample) of WBC counts $\geq 1,000/\mu\text{L}$ on the XN-1000. (**Fig. 5A**) In contrast, a post improvement comparison showed 0 out of 42 discrepant samples with WBC counts $\geq 1,000/\mu\text{L}$ on the XN-1000. There were no discrepant samples showing decreased WBC cell counts on the UF-5000 (**Fig. 5B**).

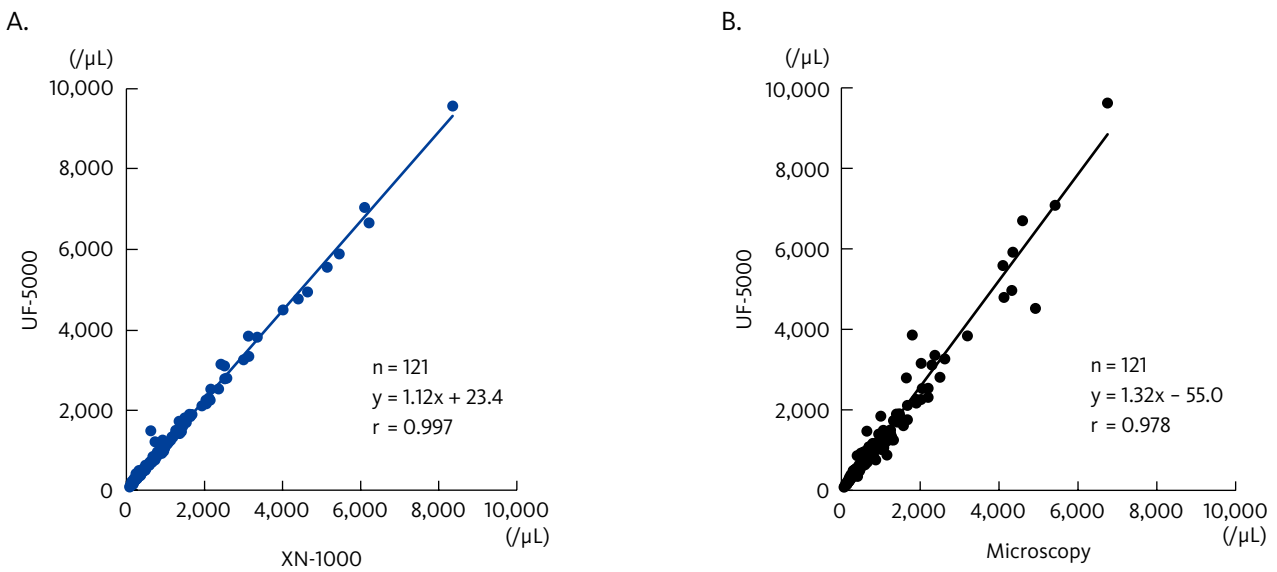


Fig. 2 Post-improvement WBC correlation

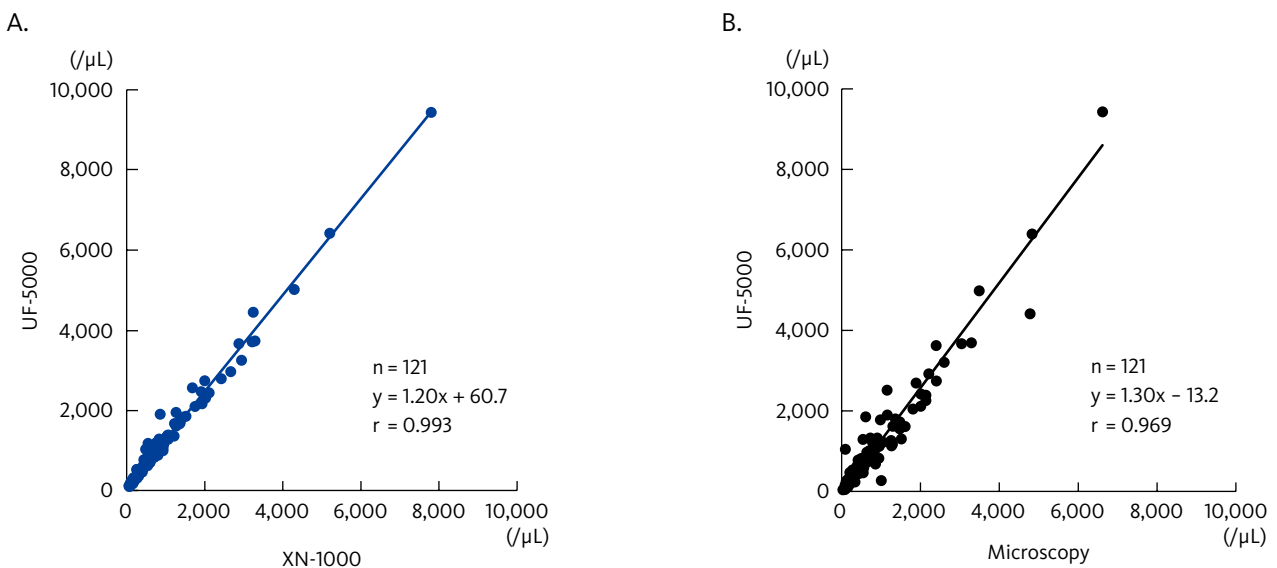


Fig. 3 Post-improvement mononuclear cells (MNs) correlation

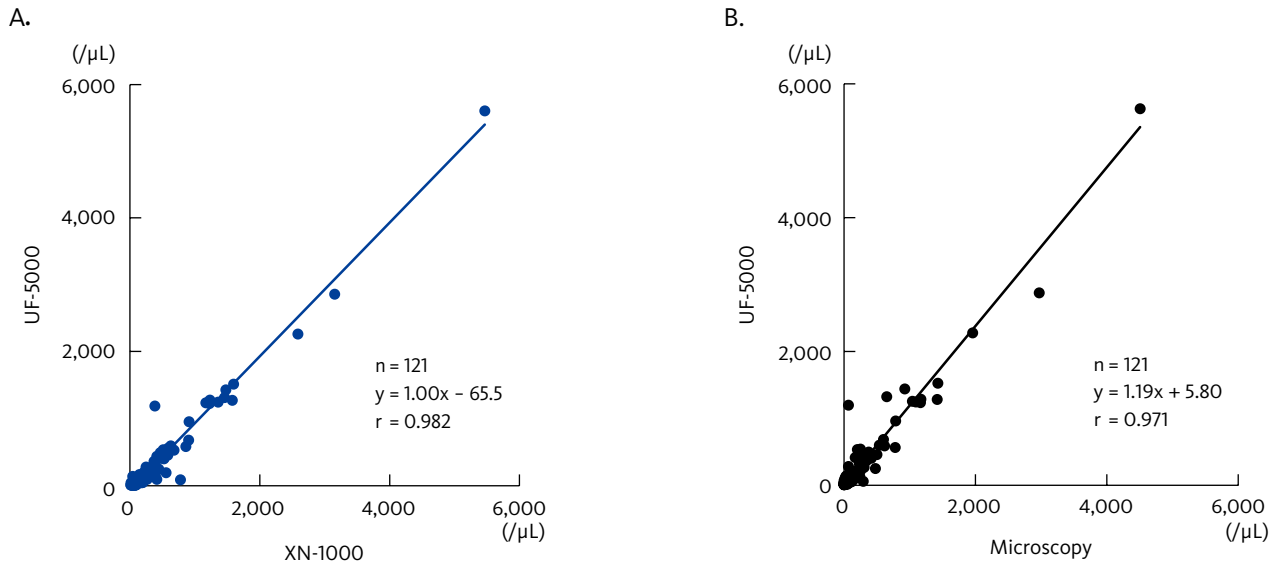


Fig. 4 Post-improvement polymorphonuclear cells (PMNs) correlation

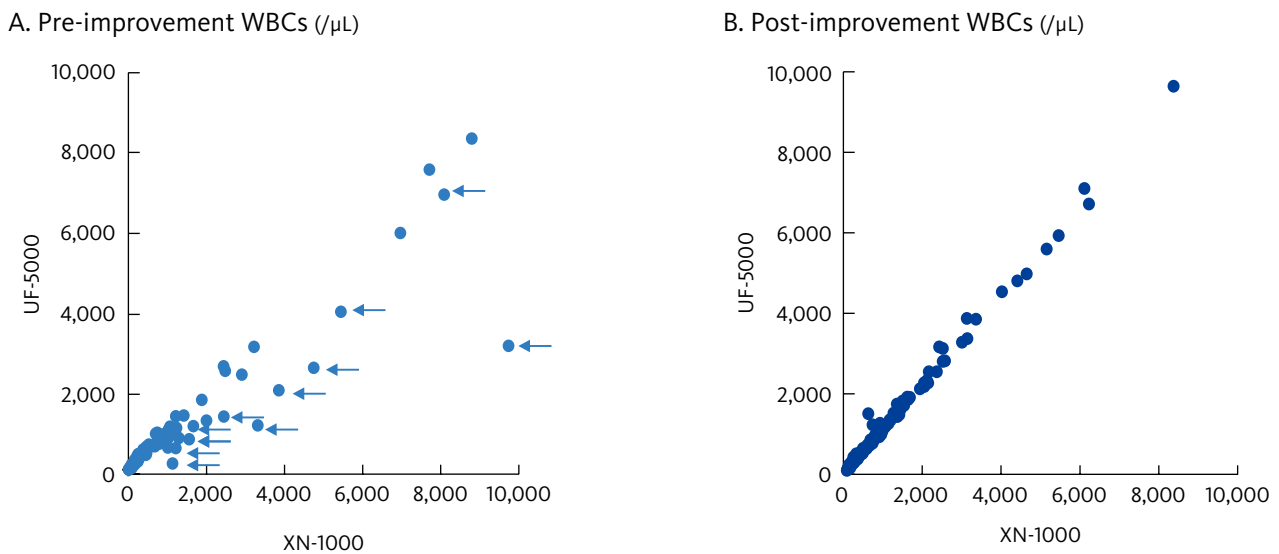


Fig. 5 Review of discrepancies
 A discrepancy was a change of -20% or more when compared with XN samples having a WBC count of $\geq 1,000/\mu\text{L}$.
 A: Before improvement
 →: A discrepancy of -20% or more compared with XN
 B: After improvement

3. Discussion

Cell count results using the improved UF-5000 were compared with the XN-1000. The improved UF-5000 showed good correlation with XN-1000/visual microscopy for WBC, MN and PMN counts. Although the correlations for differential counts was good, higher WBC counts were obtained using the improved UF-5000.

Before improvements were made to the UF-5000, some samples showed lower WBC values due to count failure because of poor staining. The cause of the decreased stain capacity remains unknown. Nakahira et al. reported a

dilution linearity study involving the pre-improved UF-5000. The study found WBC counts with decreased sample concentrations of $\geq 80\%$ showed good correlation with 10-fold diluted samples using the XE-5000 automated multiparameter hematology analyzer (Sysmex). Additionally, the proportion of discrepancies rose with increasing Lactate Dehydrogenase (LD) values.⁵⁾ Hence, the UF-5000 was improved to enhance cell stainability by changing the sample dilution rate from four to eight. Comparisons were done on discrepant sample data using the XN-1000 and the UF-5000, pre and post-improvement. Results showed discrepancies involving 15 out of 29 samples with WBC counts of

$\geq 1,000/\mu\text{L}$ on the XN-1000 when compared to measurements from the pre-improved UF-5000. Post-improvement, 42 samples were evaluated and no discrepancies or low values were reported. Although the WBC stainability improved following the dilution rate change in the present study, further investigation is needed to determine the underlying cause of the discrepancies.

Conclusion

The findings from this study showed the sample dilution change made to the BF mode on the UF-5000 enhanced the WBC stainability. Prior to the improvement, the UF-5000 analyzed samples with lower values that showed discrepancies when compared to results from the XN-1000. Post-improvement, the UF-5000 showed better correlation with the XN-1000. The change made to the analyzer improved WBC stainability, enabling our laboratory to report out test results with higher clinical utility.

References

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