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Letter to the Editor

Elimination of interference by lipids in the low WBC mode in the automated hematology analyzer XN-2000

Sir, The dissemination of chemotherapy in various malignancies and expanded application of stem cell transplantation have currently made more chances for the physicians to take care of the patients with leukopenia [1, 2]. There is a demand for immediate and reliable data for the low counts of white blood cells (WBC) in automated hematology analyzers. However, the measurement of WBC counts and differentials has a limitation in the accuracy in leukopenia. Particularly spuriously high counts by the contamination of nucleated erythrocytes (NRBC) and lipids are problematic in the care of the leucopenia patients [3]. Conventional analyzers usually use scattered lights or direct-current electricity to count WBC, and thus, the intensities are subject to interference from such a non-WBC particle.

The newly developed automated hematology analyzers XN-series (Sysmex Corp., Kobe, Japan) have several modifications for the reliability of measurement by optimization of the reagent reactions, the signal processing, and the analysis algorithms: The contamination of NRBC in the measurement of WBC counts is eliminated using WNR channel, and the accurate WBC differentials are provided using WDF channel [4]. Using fluorescence staining for nucleic acid and scattered light, both measurement channels exclude the interference such as lipid, nonlysed RBCs, and so on. Furthermore, using the WDF channel, the XN-series are also equipped with the low WBC (LW) mode, which is intended for measurement of a low range of WBC count and differentiation by counting threefold sample volume [4].

We have evaluated the assay performance of WBC counting of XN-2000 in a low range by comparing the LW and the normal measurement mode (Whole blood mode: WB mode), the conventional analyzer XE-2100 (Sysmex Corp.), and the manual method.

Clinical samples used in the study were submitted to the clinical laboratory of Tokai University Hospital for a complete blood count test and sampled during a 6-month period from September 2010 to March 2011. Peripheral blood was taken with addition of EDTA-2K as an anticoagulant. The study was approved by Institutional Review Board for Clinical Research of Tokai University Hospital (12R116) and Sysmex Corp.

When the within-run reproducibility in 10 samples with a LW count ($<1.40 \times 10^9$/L, three of them were $<0.13 \times 10^9$/L) was determined in 5 or 10 replicates, the LW mode had better within-run reproducibility of WBC counting than the WB mode, with the coefficients of variation (CV%) being 0.6–7.7% and 1.6–11.2%, respectively. The LW mode also provided better within-run reproducibility than the WB mode in the analysis of the absolute numbers of each cell fraction of WBC in neutrophil and lymphocyte, with the CV being <15% in the LW mode, as long as the absolute count of each differential fraction was more than 0.10×10^9/L (data not shown). This improvement in the measurement reproducibility in LW mode is confirmatory of WBC counting using threefold more cells in the samples in comparison with the WB mode. The LW mode showed the linearity of WBC counting in a range $0.01–1.15 \times 10^9$/L ($y = 0.990x + 0.002$, $r = 0.9997$), when evaluated with an eleven-step dilution series using the diluent CELLPACK (Sysmex Corp.). The linearity was also seen in each WBC differential measured by the LW and WB modes: $0.01–0.33 \times 10^9$ and $0.02–0.33 \times 10^9$/L for neutrophils, $0.01–0.61 \times 10^9$ and $0.05–0.56 \times 10^9$/L for lymphocytes.

Then, we evaluated the interference of lipid particles for WBC counting by the addition of fat emulsion to a sample from a healthy volunteer. XN-2000 WNR and WDF channel for WB and LW mode could accurately count WBC by distinguishing it from the lipids (Figure 1a and b). On the other hand, XE-2100 showed false elevation of WBC counts, with a curved strand of 'Lissajous-like pattern' plots in the scattergram (Figure 1b) [5, 6].

The method comparison for WBC counting between the LW and WB modes in XN-2000 and XE-2100 showed good correlation in the range of $<1.40 \times 10^9$/L (293, 298 and 294 samples, respectively; Figure 2a). However, 8 of the samples showed discrepancy in the

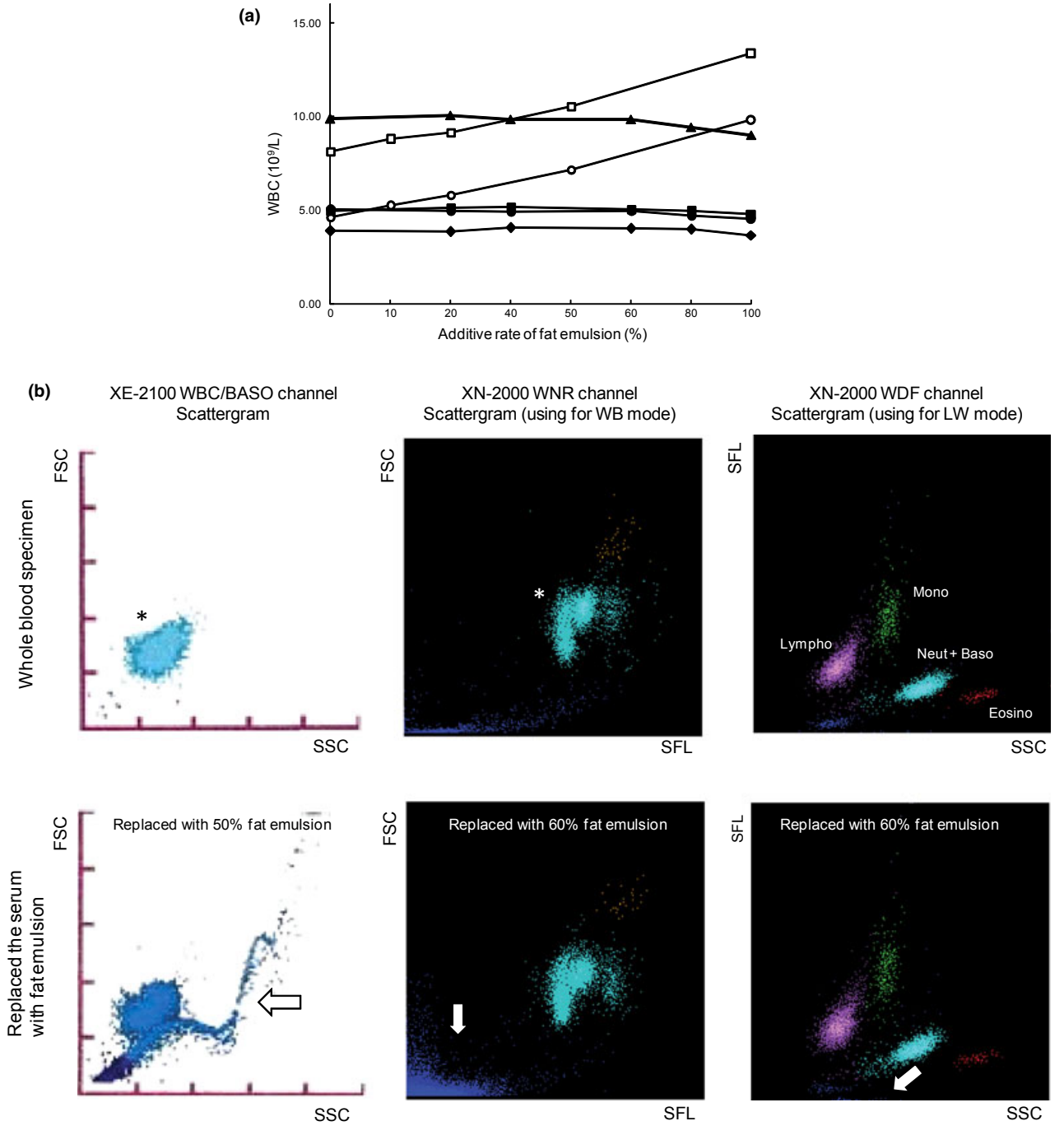
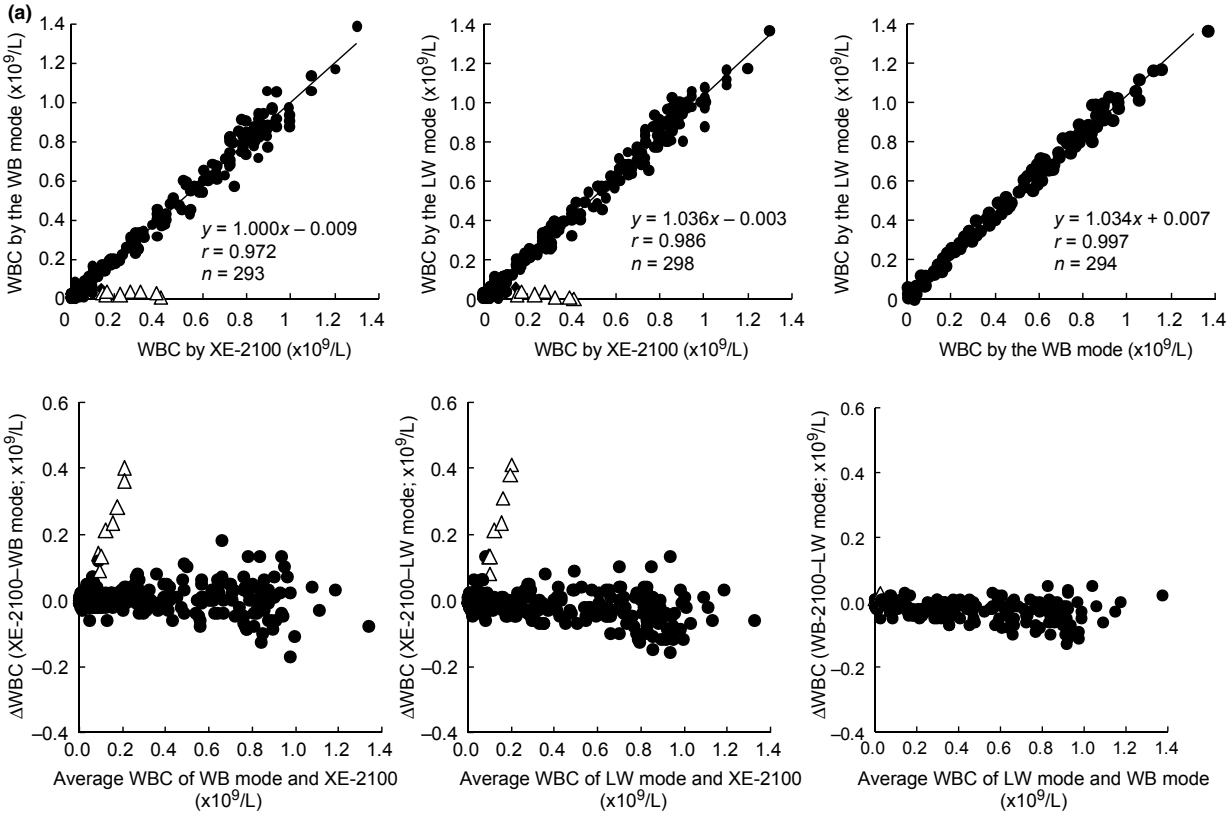


Figure 1. Evaluation of lipid interference for white blood cells (WBC) counting. (a) The six peripheral blood samples of which plasma were partially replaced (0%, 10%, 20%, 40%, 50%, 60%, 80%, or 100%) by intravenous fat emulsion (Intralipid® 20%, Fresenius Kabi Japan K.K.) were measured by WNR channel [for whole blood (WB) mode] (■, ●), WDF channel [for low WBC (LW) mode] (▲, ♦) of XN-2000 or XE-2100 (□, ○). (b) The pattern of scattergrams with or without addition of the fat emulsion. (*) and white arrow shows the WBC cluster and characteristic plots in an each specimen containing lipid particles.



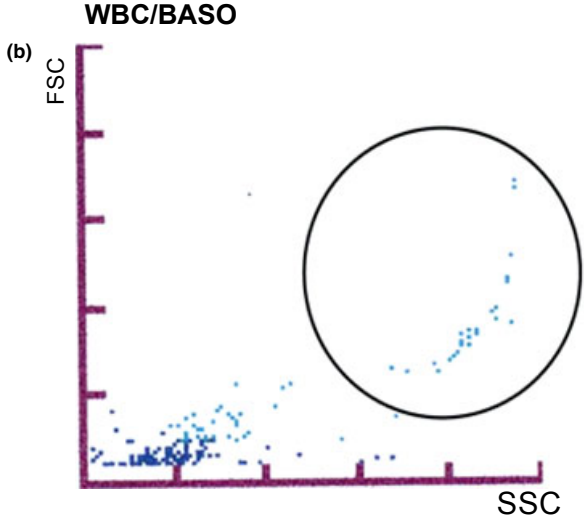


Figure 2. (a) The relationship between white blood cells (WBC) counts measured by XN-2000 and XE-2100. The relationships between WBC counts between the whole blood (WB) mode and XE-2100, the low WBC (LW) mode and XE-2100, and the LW and WB modes in XN-2000 are shown (upper graphs). The white triangles (△) represent a sample that has a discrepant WBC count between XN-2000 and XE-2100. And each Bland–Altman plots is shown in lower. (b) An example of a WBC/BASO scattergram evaluated by XE-2100. The abnormal distribution of a strand of dots appeared as 'Lissajous-like pattern' (○), generating a falsely high count, was recognized only in the XE-2100 analysis.

measured WBC counts by XN-2000 (the LW or WB modes) and XE-2100. All the 8 discrepant cases showed higher values of WBC counts up to 0.41×10^9/L in XE-2100 than those measured by XN-2000, which were all below 0.06×10^9/L actually. The WBC counts by XN-2000 agreed with those determined with the standard manual method by one person using Turk's solution and the Fuchs-Rosenthal chamber, which were from 0.003 to 0.069×10^9/L (0.003×10^9/L in 5 samples, 0.009, 0.031, and 0.069×10^9/L in each), suggesting spuriously high WBC counts in XE-2100. The WBC/BASO scattergram of XE-2100 generated abnormal distribution in all the discrepant samples, with the 'Lissajous-like pattern' (Figure 2b) [5–7]. The WBC/BASO channel of XE-2100 differentiates various cells according to size and morphological information obtained from the forward and side scatter light signals. The lipids generate particles large enough that are not correctly differentiated from blood cells because of their similarity in optical information. XE-2100 performs WBC classification using nucleic acid staining, so it could provide accurate WBC count from the differential channel. However, the result from that channel is for only service data in XE-2100.

The spuriously high WBC counts by automated hematology analyzers are caused by the contamination of cellular and noncellular particles originating from the specimen [3]. In the current study, it was found that the abnormal clusters due to noncellular particles such as lipids have been cleared from both their WNR and WDF channels for WBC analysis. This improvement is confirmatory of the principle for fractionating WBC in XN-Series, where a fluorescent dye with a high affinity to nucleic acids has been introduced, theoretically enabling the discrimination of WBC from non-WBC particles such as lipids [4]. Therefore, we considered lipemia specimens no longer interfere with WBC counting using XN analyzer.

The method comparison study using 25 samples with an extremely LW range $<0.10 \times 10^9$/L revealed that WBC counts by the LW and the WB modes had moderate correlation with the manual method, with a regression line being $y = 0.5828x + 0.015$ ($r = 0.7829$) and $y = 0.6424x + 0.0156$ ($r = 0.7358$), respectively. A combination of the low slope and the positive interception suggests more influences of falsely high values on the lower range of WBC. In all the samples, the WNR scattergram of XN-2000 displayed no apparent abnormal clusters. In such a case, influences by the carryovers from the previous analysis might be also considered.

In contrast to the WB mode, the LW mode dose not discard the differential data even in the extremely LW range ($0.01–0.1 \times 10^9$/L), and the absolute numbers as well as the proportion of each cell fraction are displayed. As the reference method for comparison, differential WBC counts were determined with the manual method by one person, where 10–100 cells were counted in May-Grunwald-Giemsa-stained smears of samples having 0.003×10^9/L or more WBC counts. The method comparison study using 29 samples with WBC $<0.10 \times 10^9$/L revealed that data for differentials displayed only by the LW mode had moderate correlation with the manual method for neutrophil and lymphocyte counting with a regression line being $y = 1.1039x + 0.0123$ ($r = 0.8730$) and $y = 0.668x + 0.0037$ ($r = 0.7903$), respectively. These suggest that the absolute numbers of neutrophil or lymphocyte counts generated by the LW mode could be used as immediate and reliable data, as supported by improvement in precision and accuracy in terms of elimination of the interference by noncellular particles.

In conclusion, evaluation of the analytical performance of the newly developed automated hematology analyzer XN-2000 for the WBC counting in a low range revealed that a spuriously high count due to the interference by the contamination of lipids had been eliminated. Furthermore, the LW mode, which was newly equipped in the analyzer, had better performance than the WB mode in precision as evaluated by within-run reproducibility for a low range of WBC counts down to 0.10×10^9/L, and the WBC differentials for neutrophil and lymphocyte were reportable with moderate correlation with the manual method for an extremely low range of WBC counts $<0.10 \times 10^9$/L. The LW mode of XN-2000 can be used as reference for the manual method or in place of it, in a selective manner, depending on the range of WBC counts in leucopenia.

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References

1. Passweg J, Halter J, Bucher C, Gerull S, Heim D, Rovó A, Buser A, Stern M, Tichelli A. Hematopoietic stem cell transplantation: a review and recommendations for follow-up care for the general practitioner. Swiss Med Wkly 2012;142:w13696.
2. Schmidt M, Koelbl H. Adjuvant chemotherapy in early breast cancer. Minerva Ginecol 2012;64:53–65.
3. Zandecki M, Genevieve F, Gerard J, Godon A. Spurious counts and spurious results on haematology analysers: a review. Part II: white blood cells, red blood cells, hemoglobin, red cell indices and reticulocytes. Int J Lab Hematol 2007;29:21–41.
4. Briggs C, Longair I, Kumar P, Singh D, Machin SJ. Performance evaluation of the Sysmex haematology XN modular system. J Clin Pathol 2012;65:1024–30.
5. Doornbos RM, Hoekstra AG, Deurloo KE, De Grooth BG, Sloot PM, Greve J. Lissajous-like patterns in scatter plots of calibration beads. Cytometry 1994;16:236–42.
6. Hoekstra AG, Doornbos RM, Deurloo KE, Noordmans HJ, Grooth BG, Sloot PM. Another face of Lorenz-Mie scattering: monodisperse distributions of spheres produce Lissajous-like patterns. Appl Opt 1994;33:494–500.
7. Mori Y, Mizukami T, Hamaguchi Y, Tsuruda K, Yamada Y, Kamihira S. Automation of bone marrow aspirate examination using the XE-2100 automated hematology analyzer. Cytometry B Clin Cytom 2004;58:25–31.