Original Article

Evaluation of the Blood Bank mode Software of Sysmex XN-1000™ Hematology Analyzer for Counting Residual Red Blood Cells and Platelets in Platelet Concentrates, and Residual White Blood Cells in Leucocyte-Reduced Whole Blood

Fuminori ARISAWA[*1], Shinobu WAKAMOTO[*1], Mitsuaki AKINO[*1], Katsuya IKUTA[*2] and Shuichi KINO[*1]

[*1] *Japanese Red Cross Hokkaido Block Blood Center, 1-1-20, Nijyuyonken-nijo, Nishi-ku, Sapporo, 063-0802, Japan*
[*2] *Hokkaido Red Cross Blood Center*
Correspondence: Shinobu Wakamoto, Japanese Red Cross Hokkaido Block Blood Center, 1-1-20, Nijyuyonken-nijo, Nishi-ku, Sapporo, 063-0802, Japan
        Tel. +81-11-613-6640. E-mail: wakamoto@hokkaido.bc.jrc.or.jp

Background: *The Sysmex XN-1000 automated hematology analyzer (XN-1000) was equipped with Blood Bank mode (BB mode)* to count residual white blood cells (rWBC) and residual red blood cells (rRBC) in blood products. This mode is also intended for platelet enumeration in platelet concentrates (PCs). Presently, platelet, rRBC and rWBC counts are performed using several different methods. The BB mode has the potential to simultaneously perform these measurements using one sample. The aim of our study was to evaluate the utility of the BB mode on the XN-1000.*
* Blood Bank mode is a license software which can be equipped on the XN-10/20 hematology analyzer.

Method: *For RBC counting, we compared the BB mode and the manual counting method by measuring dilution series and routinely manufactured PCs. We also examined precision, accuracy and carryover of RBC counts with the BB mode. For platelet counting in PCs, we examined the correlation of platelet counts between the BB mode and the XS-1000i (Sysmex Corporation, Kobe, Japan). For WBC counting, we compared the BB mode with the flow cytometry (FCM) method by measuring dilution series and examining precision and accuracy of WBC counts with the BB mode.*

Result: *In the dilution study of RBC counts, the BB mode showed good linearity and correlation with manual counting in the 5–5,000 cells/μL range but did not meet linearity criteria in the low range of 5–50 cells/μL. In both the dilution study and with routinely manufactured PCs, the RBC counts obtained by the BB mode tended to be higher than those obtained by manual counting in the ≤ 50 cells/μL range. In the study to count platelets in PCs, there were no significant differences in the number of platelets obtained by the BB mode and the XS-1000i. The BB mode values correlated significantly with those of the XS-1000i. In the dilution study of WBC counts, the BB mode showed good linearity and correlation with the FCM method and found acceptable accuracy and precision results.*

Conclusion: *Our results suggest it is possible to perform simultaneous measurements of rRBCs, rWBCs, and platelet counts with one sample using the BB mode. Our findings suggest that further improvements of the BB mode RBC gating algorithm may lead to more accurate measurements of low-level RBCs in PCs.*

INTRODUCTION

It is important for quality control (QC) of manufactured blood components (in blood center) for transfusion to have accurate and reproducible counting methods for evaluating residual white blood cells (rWBCs) in each blood component, and residual red blood cells (rRBCs) and platelets content in platelet concentrates (PCs). For each of the blood products, cell counting is performed by different methods. Nageotte chamber counting and flow cytometry (FCM) are widely used methods for counting rWBCs. To detect rRBCs in blood components, the recommended method is either manual counting using a counting chamber[1] or visual inspection estimating redness of PCs.[2] For sensitive, reliable,

Notes: The specifications, performances and functions described here may be different depending on the regions or the countries due to the regulatory affairs, legal matters or local guidelines. For more details, please contact your regional affiliates or distributors.

and fast counting of rRBCs, FCM methods were developed which has not been universally accepted as a reference method for rRBC enumeration.[3-6] To measure platelet concentration in PCs, we, blood centers, use hematology analyzers which are designed for counting blood cells of healthy individuals or patients, although the platelet concentration in PCs is about fivefold higher than that of whole blood of healthy individuals.[7]

In Japanese regulation, the rWBCs are required to be $\leq 1 \times 10^6$ cells in leucocyte-reduced (LR) blood products and the FCM is used to quantify the rWBCs.[8] The rRBCs in PCs are required to be $\leq 1 \times 10^5$ /μL with the XS-1000i (Sysmex Corporation, Kobe, Japan) hematology analyzer. Japanese Red Cross standard operating procedure (JRC-SOP) requires that all PCs that show $\geq 1 \times 10^5$ /μL rRBCs with the XS-1000i be remeasured with manual counting. Our findings showed the remeasured rRBC counts were < 50 cells/μL. Platelet counts in PCs can be also measured with the XS-1000i. Measured values of $\geq 2,000 \times 10^3$/μL are considered unreliable due to equipment performance of the XS-1000i, therefore, JRC-SOP requires that these samples be diluted two-fold and then remeasured. It is important for manufacturing blood component to simplify the QC process and improve operational efficiency using an automated single-platform system.

Sysmex XN analyzer series with the Blood Bank mode (BB mode), which can measure blood cells in blood components including rWBCs and rRBCs, was successfully developed. It was reported that the BB mode may serve as an alternative to FCM for rWBC counting.[9] With RBC counts, the BB mode showed good correlation with FCM using expected and measured values with a quantification limit of 6 to 7 RBCs/μL.[10] According to the manufacturer's instructions for counting platelets, the difference between the expected and measured values of the BB mode should be within a 6% range of 1,001 to $5,000 \times 10^3$/μL, while that of the XS-1000i is within a 16% range of 2,001 to $5,000 \times 10^3$/μL. Therefore, it is expected that a high concentration of platelets in PCs could be measured accurately without dilution using the BB mode. However, evaluations have not been done with high platelets counts (concentration of $\geq 2,000 \times 10^3$/μL) using the BB mode or with rRBC measurements comparing the manual counting method to the BB mode.

In this study, we evaluated the performance of the BB mode to measure RBCs in a dilution series of artificially spiked PCs and with routinely manufactured PCs in our blood center. We compared the BB mode results to manual counting. We also compared results of the platelet count in PCs reported from the XN-1000/PLT-F and the XS-1000i. We then evaluated the possibility of using the BB mode as an alternative to FCM for WBC counting.

MATERIALS AND METHODS

Preparation of samples for dilution studies

To study linearity, precision, accuracy, and carryover, we prepared dilution series of RBCs and WBCs. Samples were produced from Apheresis PCs (Trima Accel®, TERUMO

BCT, Inc., Tokyo, Japan) and whole blood (WB) collected from voluntary blood donors. We used PCs, processed 3 days after collection and WBs, processed 2 days after collection. These samples did not meet blood product qualifications according to Japanese regulation. To prepare the dilution series, PCs (n = 3) were centrifuged to remove rRBC according to methods outlined in the JRC-SOP. The PCs did not contain measurable RBCs which was confirmed twice using a Neubauer hemocytometer as a diluting solution. WBs (n = 3) and diluting solutions were mixed to prepare the RBC suspension. The concentration of RBCs in the WBs was measured twice using the XS-1000i. Using the RBC concentration of 1×10^6 /μL, the suspensions were prepared and used as the initial sample. RBC concentrations in the initial samples were measured twice using the XS-1000i. These were further diluted with diluting solution so that the RBC concentration would be 5, 10, 20, 50, 100, 500, 1,000, 5,000 cells/μL (target values). In the dilution procedure, the exact amount of the sample and the diluted mixtures were added to the tube and weighed at the following times: (a) before the sample was added, (b) after the addition of the sample, (c) after the addition of the diluting solution. The expected values were calculated based upon these measurements and the concentration of the initial sample (d) using the formula below.

Expected value (cells/μL) = d × (b-a)/(c-a)

For WBC measurements, we prepared samples containing 0.1, 0.5, 1, 3, 5, 10, 20, 30 cells/μL (target values) as described previously with slight modifications.[11] A portion of the WB was transferred from the bag to a polypropylene tube to be used as the blood for spiking. The remaining blood was filtrated three times (Sepacell RZ-2000N, Asahi Kasei Medical Co., Ltd., Tokyo, Japan), which was proven to contain no measurable leucocytes by FCM (BD Leucocount™ Kit, BD Biosciences, San Jose, CA, USA) with a BD FACSCalibur™ (BD Biosciences, San Jose, CA, USA), and used as a diluting solution. The concentration of WBCs in the nonfiltered WB was measured twice by the XS-1000i. Based on the WBC concentration, 100 cells/μL of leucocyte suspension was prepared and used for the initial samples. The WBC concentrations in the initial samples were measured twice using the XS-1000i. These were further diluted with LR-WB so the WBC concentration would meet the criteria described above. The expected values were calculated using the same procedure previously outlined for the RBC series.

Measurement of RBCs or WBCs in the samples for dilution studies

Plain tubes without anticoagulant (Asia Kizai Co., Ltd., Tokyo, Japan) or elastomer caps (VENOJECT II, TERUMO CORPORATION, Tokyo, Japan) were used in this study to measure RBCs and WBCs using the BB mode (software version 22.15). RBC counts in the dilution series were measured by the BB mode (PLT pack) and by manual counting with the improved Neubauer hemocytometer. The BB mode (PLT pack) procedure was as follows: 1 mL of sample was added to the plain tubes and measured using the auto-sampler mode. The manual counting procedure was as follows: the samples were thoroughly mixed and loaded into

each chamber of the improved Neubauer hemocytometer. The samples were left to sediment before RBC were counted using a microscope at 200 × magnification. WBC counts in the dilution series were measured by the BB mode (PLT pack) using the same procedures as previously outlined for the RBC dilution series and by FCM. Assessing precision and accuracy of the BB mode procedure were performed at 5, 10, 20, 50 cells/μL for RBCs and at 0.5, 1, 3, 10 cells/μL for WBCs in three independent experiments. Ten aliquots from the RBC and WBC concentrations were measured once per tube. Precision was evaluated using the coefficient of variation (CV) which was calculated from 10 repeated measurements at each concentration. We defined a CV of < 20% as good precision.[12] The accuracy was considered acceptable when at least 80% of observed values fell within 20% of the expected values.[13]

Carryover, using RBC suspensions in PCs, was evaluated according to appropriate guidelines.[14] The highest concentration in the dilution series, 5,000 cells/mL (H), was measured 3 times, and then the non-spiked sample (L) was measured 3 times. The percentage carryover was calculated using the formula (L1-L3)/(H3-L3) × 100.

Measurement of rRBCs in routinely manufactured PCs

To measure the number of rRBCs in PCs, samples were collected from apheresis PCs (Trima Accel) that were routinely manufactured in our blood center (n = 40). Counting rRBC was performed using the BB mode and the improved Neubauer hemocytometer.

Measurement of platelet count in routinely manufactured PCs

Plain tubes without anticoagulant or rubber caps (Erma Inc., Saitama, Japan) were used to measure platelet counts using the XS-1000*i* and the BB mode (software version 22.15). The samples were collected from apheresis PCs (Trima Accel, TERUMO BCT and Component Collection System (CCS), Haemonetics Corporation, Braintree, MA, USA) (n = 101). In order to compare the measured values of platelet counts from the XS-1000*i* and the BB mode, the remaining samples (measured using the XS-1000*i* for QC on platelet content in PCs) were remeasured using the BB mode. In the XS-1000*i* measurement, the samples showing platelet concentration of ≥ 2,000 × 10³/μL were diluted two-fold and then remeasured.

Statistical analysis

The data was analyzed using Microsoft Excel (Microsoft Corporation) computer software. Linearity was assessed by regression analysis and determined by a coefficient of determination (r^2). We defined a r^2 of ≥ 0.98 as having adequate linearity. Correlation was assessed by Spearman's rank correlation coefficient. The significance of differences between these two procedures was determined by paired t-test. A p-value of ≤ 0.05 was considered a significant difference.

RESULTS

Dilution studies of the RBC

First off we examined the linearity of RBC counts using the BB mode. The observed values were plotted against the expected values with the regression line (***Fig. 1***). The BB mode showed good linearity in the range of 5–5,000 cells/μL (R^2 = 0.9996, y = 0.9434x + 8.4661). Since the concentration of rRBC in apheresis PCs tended to be < 50 cells/μL, based upon our QC testing, we focused on the 5–50 cells/μL range. In this range, the BB mode did not meet linearity criteria (R^2 = 0.9366, y = 0.9456x + 6.3397). The observed values of the BB mode significantly correlated with those of manual counting in the 5–5,000 cells/μL (R = 0.9869, P < 0.01) range and the low range of 5–50 cells/μL (R = 0.9272, P < 0.01) (***Fig. 2***).

CVs and accuracy are shown in ***Table 1***. CVs were ≤ 20% in the 5–50 cells/μL range for experiments 2 and 3, while in experiment (Exp.) 1, the CV did not meet the < 20% at 5 and 10 cells/μL. The accuracy did not reach 80% in the 5–20 cells/μL range in Exp. 1, or at 5 and 10 cells/μL in Exp. 3. The carryover assessment of RBCs in PCs showed a percentage carryover within the 0.00–0.13% (***Table 2***) range suggesting a low percentage of carryover.

In the dilution series, the measured values of the BB mode tended to be higher than the expected and the measured values using manual counting in the 5–50 cells/μL range (***Table 3***). The RBC counts in the diluting solution measured with manual counting was 0 cells/μL as described in "Materials and Methods", however the RBC count with the BB mode was not 0 cells/μL (range, 1–7 cells/μL). This finding may indicate that the BB mode has a tendency to overestimate RBC counts.

The representative scattergrams of the RBC-spiked PC samples (Exp. 1–3), and the Blank-PC samples (Exp. 1–3) are shown in ***Fig. 3*** and ***Fig. 4***.

Measurement of the number of rRBC in routinely manufactured PCs

40 donor PC samples routinely produced in our blood center following JRC-SOP, were used to compare rRBC measurements from manufactured PCs using the BB mode and the manual counting method. All measured rRBC values from the BB mode and the manual counting method met the Japanese criteria of ≤ 1×10⁵ /μL in PCs, which is displayed by XS-1000*i*, and the highest value was 5 cells/μL (1.5 cells per bag) measured by the BB mode (P < 0.0001) (***Table 4***). However, the BB mode values were higher than those reported from manual counting which supports the findings from the RBC dilution study.

Measurement of platelet counts in routinely manufactured PCs

We also compared platelet count results from the BB mode (PLT-F) and the XS-1000*i*. There were no significant differences in the results obtained from the two systems. The BB mode values correlated significantly with those of the XS-1000*i* in the 954–2,416 × 10^3/μL range (R = 0.9837, P < 0.01) and the ≥ 1,500 × 10^4/μL range (R = 0.9564, P < 0.01) (***Fig. 5***). The representative PLT-F scattergram of routinely manufactured PC samples is shown in ***Fig. 6***.
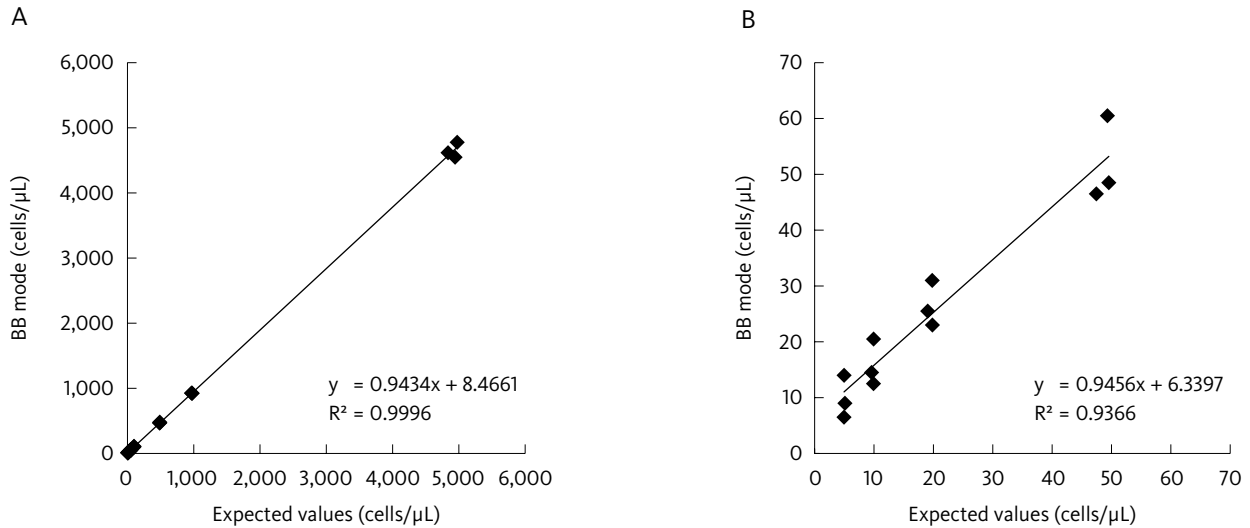

Fig. 1 *Linearity of RBC counts by the BB mode*
rRBC counts in dilution series prepared from PCs were performed using the BB mode. All individual values (5–5,000 cells/μL) (A) and the values from 5 to 50 cells/μL (B) obtained from three independent experiments were plotted against expected values. The regression lines are shown.
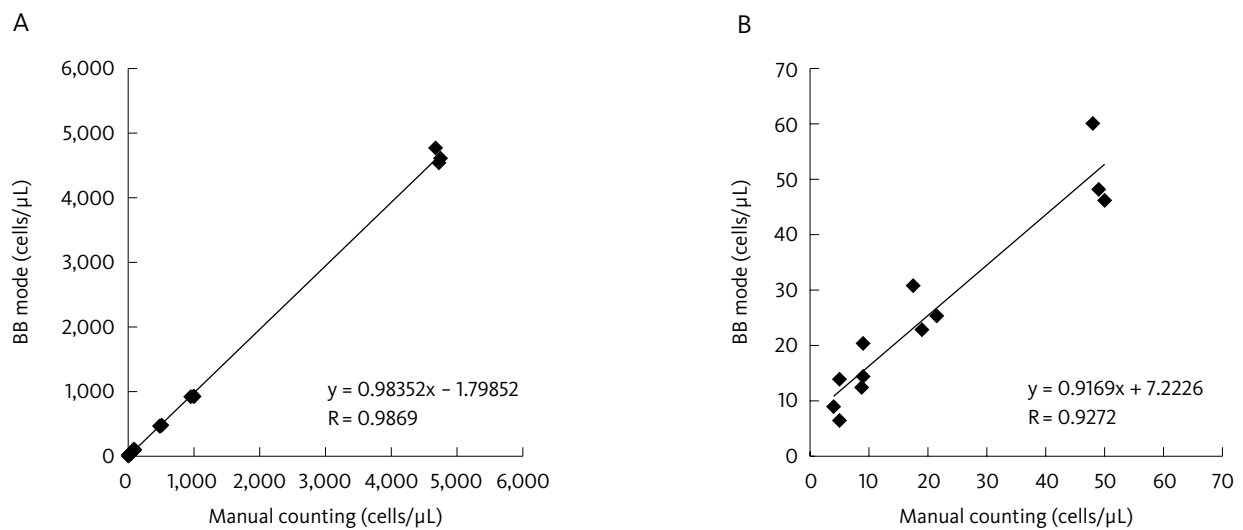

Fig. 2 *Correlation of RBC counts between the BB mode and improved Neubauer hemocytometer*
rRBC counts in dilution series prepared from PCs were performed - using the BB mode and by the manual counting method. All independent values (5–5,000 cells/μL) (A) and the values from 5 to 50 cells/μL (B) obtained from three independent experiments were plotted. The regression lines are shown.

Table 1 *Accuracy and precision of the BB mode for RBCs*

Target values (cells/µL)	Exp. 1				Exp. 2				Exp. 3			
	Expected values (cells/µL)	Observed values (cells/µL)	CV (%)	Accuracy (%)	Expected values (cells/µL)	Observed values (cells/µL)	CV (%)	Accuracy (%)	Expected values (cells/µL)	Observed values (cells/µL)	CV (%)	Accuracy (%)
50	49	49 ± 5	11	90	50	50 ± 3	6	100	47	49 ± 3	6	100
20	20	21 ± 3	15	70	20	21 ± 2	8	100	19	21 ± 1	6	100
10	10	20 ± 4	20	0	10	10 ± 1	14	100	10	13 ± 2	15	40
5	5	19 ± 4	20	0	5	5 ± 1	16	90	5	9 ± 2	17	0

Values are expressed as mean ± SD of ten samples.
Accuracy is expressed as percentage of observed values that fall within ± 20% of the expected values.
Coefficient variation (CV) is calculated as the SD of the date, divided by the mean and multiplied by 100 to give a percentage score.

Table 2 *Carryover examination of the BB mode*

Target values (cells/µL)		Exp. 1			Exp. 2			Exp. 3		
		Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3
	H1	4,629	4,602	4,620	4,547	4,649	4,577	4,753	4,746	4,753
5,000	H2	4,572	4,609	4,523	4,544	4,504	4,600	4,739	4,709	4,714
	H3	4,515	4,630	4,574	4,561	4,617	4,554	4,739	4,796	4,754
	L1	17	11	15	1	1	1	6	3	4
0	L2	14	13	16	1	1	1	3	3	2
	L3	14	17	14	1	0	1	4	3	3
carryover (%)		0.067	– 0.130	0.022	0.000	0.022	0.000	0.042	0.000	0.021

Table 3 *rRBC count in dilution study*

Target values (cells/µL)	Expected values (cells/µL)	Observed values (cells/µL)	
		BB mode	Manual counting
50	48.7 ± 1.5	52.3 ± 7.6	49.0 ± 1.0
20	19.7 ± 0.6	26.7 ± 4.0	19.7 ± 2.1
10	10.0 ± 0.0	16.3 ± 4.2	9.0 ± 0.0
5	5.0 ± 0.0	10.0 ± 3.6	4.7 ± 0.6

Values are expressed as mean ± SD (n = 3).

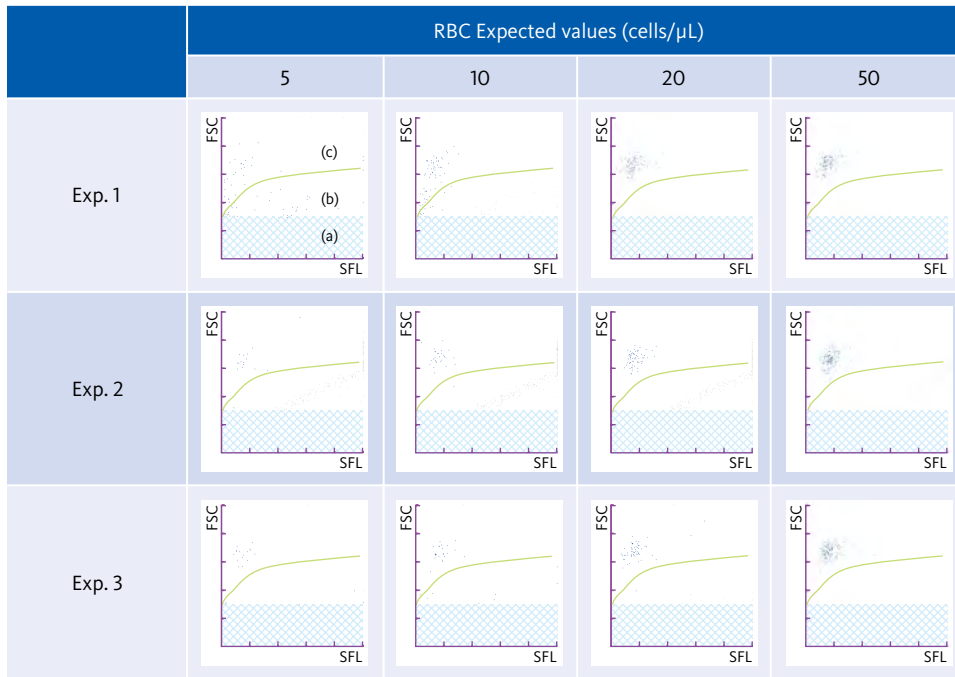|	RBC Expected values (cells/µL)			
	5	10	20	50
Exp. 1				
Exp. 2				
Exp. 3				

Fig. 3 *Representative Scattergrams of RBC-spiked PC samples (Exp. 1–3)*
All scattergrams were obtained from the Ret channel with schematic gatings shown as: (a) below-threshold zone, (b) interference zone, (c) RBCs.
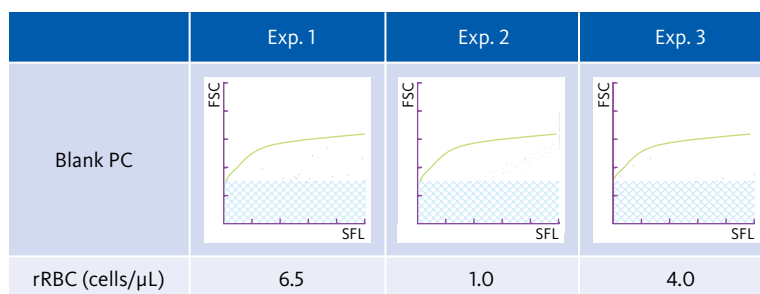*Note: The gatings / thresholds are not seen in the actual Ret scattergram.*

	Exp. 1	Exp. 2	Exp. 3
Blank PC			
rRBC (cells/µL)	6.5	1.0	4.0

Fig. 4 *Representative Scattergrams of Blank-PC samples (Exp. 1–3)*
The data represents the mean of 2 consecutive measurement on the XN-1000.

Table 4 *rRBC counts in routinely manufactured PCs measured with the BB mode and using the manual counting method (n = 40)*

Method	(cells/μL)		(×10⁶ cells/bag)	
	mean ± SD	Range	mean ± SD	Range
BB mode	2.2 ± 1.2*	0.5–5.0	0.5 ± 0.3*	0.1–1.2
manual counting	0.8 ± 0.9	0.0–0.3	0.2 ± 0.2	0.0–0.7

* P < 0.05 vs. manual counting

A

B

y = 1.0437x − 59.3605
R = 0.9837
(n = 101)

y = 1.0828x − 137.7932
R = 0.9564
(n = 72)
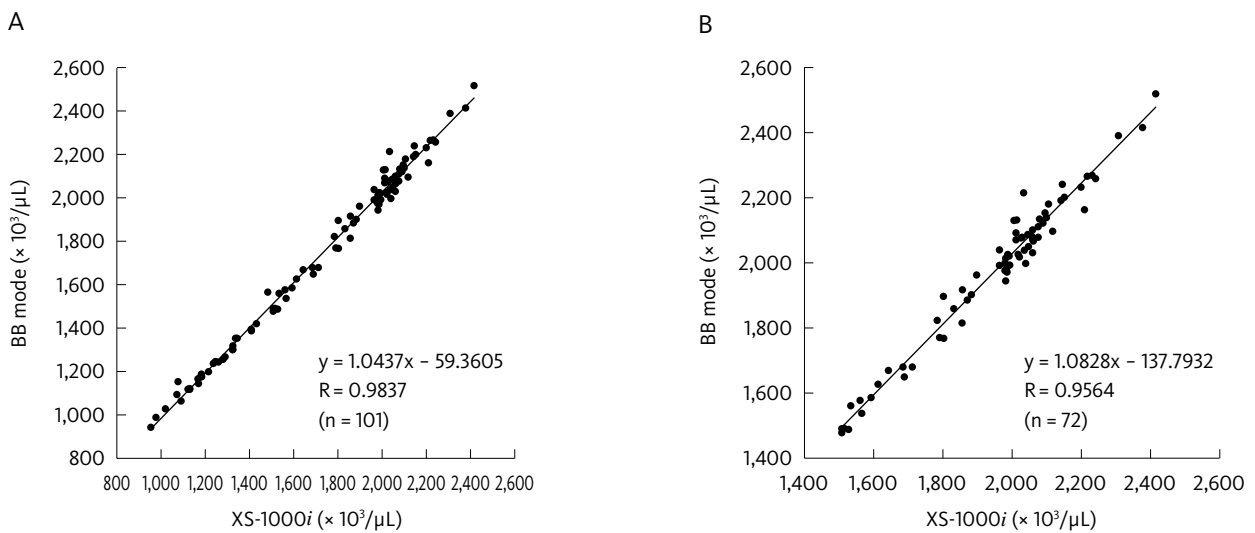
Fig. 5 *Correlation of platelet counts between XS-1000i and the BB mode in routinely manufactured PCs*
The remaining samples, measured with the XS-1000i for QC on PC platelet content, were remeasured with the BB mode.
The sample values using the XS-1000i and the BB mode were plotted. The dotted lines represent 95% CI.
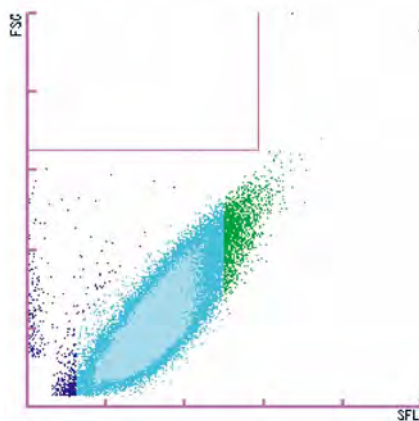
Fig. 6 *Representative PLT-F scattergram of Platelet Concentrate*

Dilution studies of the WBC

We examined the linearity of WBC counts from the BB mode using the same procedure as the RBC dilution study. The BB mode showed good linearity in the 0.1–30 cells/μL range ($R^2 = 0.9919$, $y = 0.9872x + 0.1246$) and in the 0.1–5.0 cells/μL range ($R^2 = 0.9807$, $y = 0.9550x + 0.1776$) (**Fig. 7**). The observed values of the BB mode significantly correlated with those of FCM in the 0.1–30 cells/μL range ($R = 0.9858$, $P < 0.01$) and in the 0.1–0.5 cells/μL low range ($R = 0.9740$, $P < 0.01$) (**Fig. 8**). CVs were ≤ 20% and the WBC accuracy was ≥ 80% in all experiments at ≥ 3 cells/μL (date not shown).
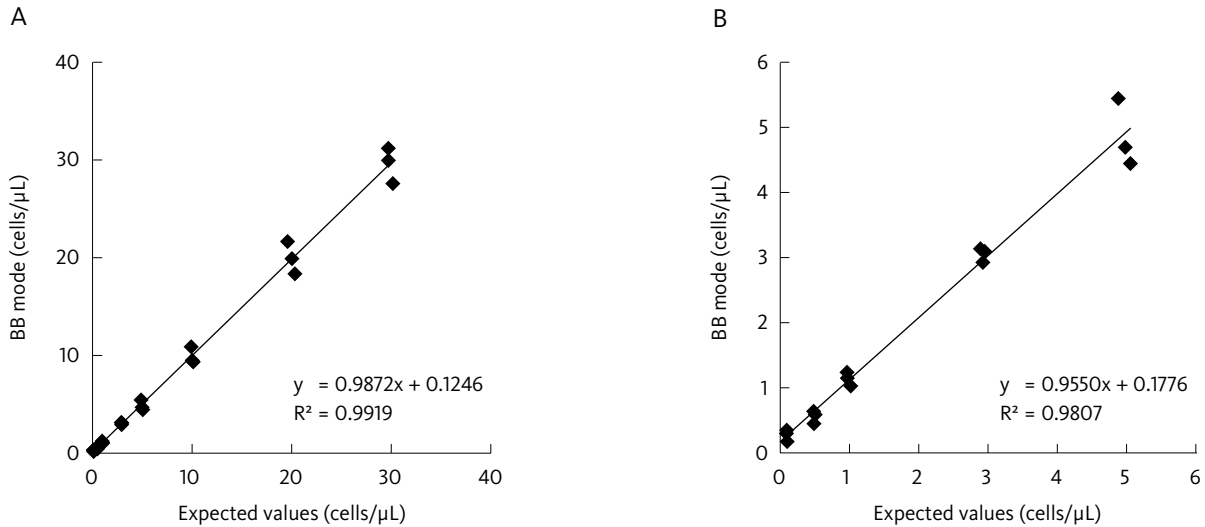

Fig. 7 *Linearity of WBC counts by the BB mode*
rWBC counts in dilution series prepared from WBs were performed with the BB mode. All individual values (0.1–30 cells/μL) (A) and the values from 0.1 to 5 cells/μL (B) obtained from three independent experiments against expected values were plotted. The regression lines are shown.
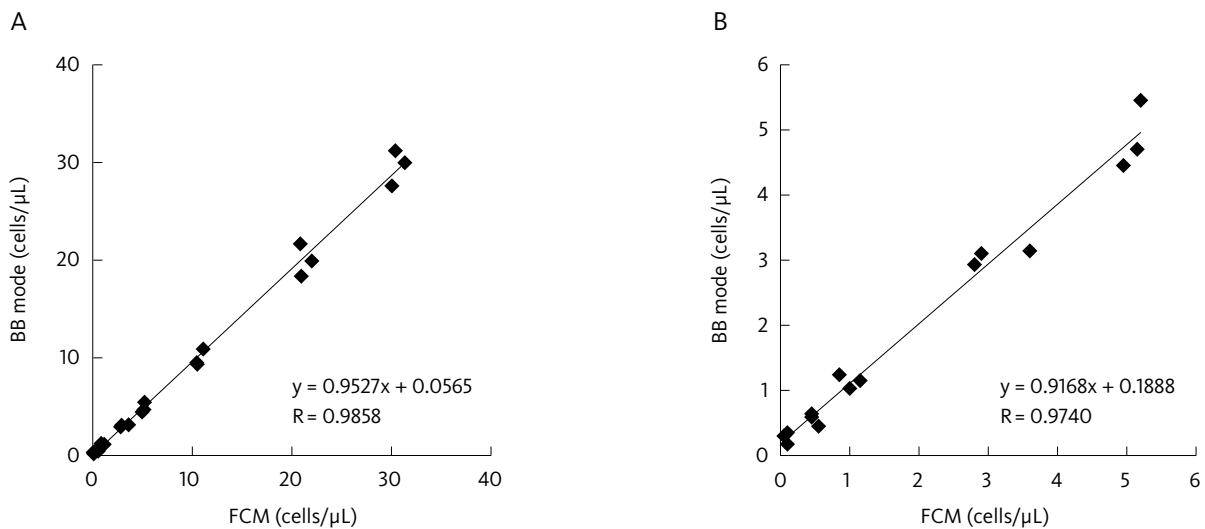

Fig. 8 *Correlation between the BB mode and FCM in WBC counting*
rWBC counts of dilution series prepared from WBs were performed using the BB mode and FCM. All independent values (0.1–30 cells/μL) (A) and the values from 0.1 to 5 cells/μL (B) obtained by plotting from three independent experiments were plotted. The regression lines are shown.

DISCUSSION

rWBCs and rRBCs in blood components must be ≤ specified levels. These levels are markedly lower than whole blood of healthy individuals in order to reduce alloimmunization to HLA or RBC antigens and other rWBC-related adverse transfusion effects.[15-19] On the other hand, the platelet content in PCs is about fivefold higher than levels in whole blood of healthy individuals. It is not possible to simultaneously count these cells in different ranges using a conventional hematology analyzer. At present, the Sysmex XN analyzer series is now equipped with the BB mode which allows simultaneous testing of cell counts. In the current study, we evaluated the utility of the BB mode which is available on the XN-1000.

In RBC counting experiments, the BB mode showed good linearity in the 5–5,000 cells/µL range (***Fig. 1***). This finding is comparable with the previous report by Cavagnetto *et al.*[10] They noted that the BB mode showed good linearity in the 6–6,000 cells/µL range ($r^2 = 0.9885$) based upon experiments with apheresis PCs. In addition, our results showed significant correlation between observed values of the BB mode and those of manual counting in the 5–5,000 cells/µL range. However, in the 5–50 cells/µL range, the BB mode did not meet linearity criteria (***Fig. 2***). In our study, the dilution series experiments with artificially spiked PCs and routinely manufactured PCs, showed a tendency for the BB mode to overestimate RBC concentrations of ≤ 50 cells/µL compared to expected values and observed values with manual counting (***Table 3***, ***4***). Therefore, it is suggested that the overestimation of RBCs observed in the BB mode contributed to the inappropriate linearity. In the study of precision and accuracy of the BB mode in the 5–50 cells/µL range, results obtained from the three independent experiments (***Table 1***, Exp. 1, 2, 3), each performed with PCs from three different blood donors were inconsistent. The CV did not meet the < 20% goal at 5 and 10 cells/µL (Exp. 1) in one of the three independent experiments (Exp. 1). Accuracy did not reach 80% in the 5–20 cells/µL range with Exp. 1, or at 5 and 10 cells/µL in Exp. 3. Cavagnetto *et al.* showed that CVs were < 20% in the range of 6–50 cells/µL range in one experiment using apheresis PC. It is considered that the CV discrepancy between our results and their report is due to the difference in the number of PCs used in the experiments. At present, it is speculated that the overestimation of RBCs observed in the BB mode and the different results observed with the accuracy and precision study (depending on PCs) may be due to the BB mode gating algorithm to detect RBCs. In the current algorithm, the gating for rRBC becomes nonfunctional if the total dot counts (detected in area (b) and (c); see ***Fig. 3***) are < setting value. The BB mode did not meet criteria for linearity and reproducibility in counting < 50 cells/µL of RBCs during this study. However, it is thought that the BB mode would not yield false-negative results with counting RBCs in PCs due to underestimation of RBCs. Therefore, this occurrence will not presumably affect feasibility of the BB mode to test QC of blood components. Nevertheless, it is expected that adjustments to the BB mode gating algorithm may be needed to detect low concentration of RBCs in PCs making it possible to count RBCs more accurately and reliably.

Our evaluation results of the BB mode's ability to count platelets in PCs indicated there were no significant differences in measured values between the BB mode and the XS-1000*i* (***Fig. 5***). Our findings showed that the BB mode significantly correlated with the XS-1000*i*. The samples of > 2,000 × 10³/µL were diluted two-fold and measured using the XS-1000*i* while the samples measured by the BB mode were not diluted. These results indicate that the BB mode has the ability to accurately measure samples of > 2,000 × 10³/µL without a two-fold dilution and may therefore serve as an alternative to XS-1000*i* for platelet counting with routinely manufactured PCs.

The BB mode also showed good linearity and correlation with FCM in counting WBCs in both the 0.1–30 cells/µL range and the low range of 0.1–5.0 cells/µL (***Fig. 7***, ***8***). The BB mode showed acceptable accuracy and precision at > 3 cells/µL. In Japan, the rWBC are required to be < 1 × 10⁶ cells per blood product with cut-off values of approximately > 3 cells/µL for LR-WBs at a volume of 502 mL (the upper volume limit). Therefore, our results indicate that the BB mode has the ability to measure low levels of rWBC in blood products and may be a suitable alternative to FCM. To our knowledge, this was the first time the utility of the BB mode for counting low concentrations of WBCs in LR-WBs was demonstrated. Our results are consistent with previous reports by Blanco *et al.* who looked at PCs and plasma and Lagerberg and Korte who studied red blood cell components (RCCs).[9, 20] Blanco *et al* determined that the BB mode had good linearity in the 0.3–32 cells/µL range for PCs and plasma. Lagerberg and Korte showed that the BB mode had good linearity in the ≤ 16 cells/µL range with RCCs.

In conclusion, our results suggest that the BB mode will make it possible to simultaneously measure rWBCs in each blood component, and rRBCs and platelets in PCs for QC using one sample. This automated method is expected to improve operating efficiency, simplify the manufacturing process and reduce costs.

ACKNOWLEDGEMENTS

References

1) *Reckhaus J, Jutzi M, Fontana S, et al. Platelet Transfusion Induces Alloimmunization to D and Non-D Rhesus Antigens. Transfus Med Hemother. 2018; 45(3): 167–72.*

2) *Canadian Blood Services. Canadian Blood Services Visual Assessment Guide. 2009. URL: https://profedu.blood.ca/sites/msi/files/VAG_en.pdf*

3) *Santana JM, Dumont LJ. A flow cytometric method for detection and enumeration of low-level, residual red blood cells in platelets and mononuclear cell products. Transfusion. 2006; 46(6): 966–72.*

4) *Pichler J, Printz D, Scharner D, et al. Improved flow cytometric method to enumerate residual cells: Minimal linear detection limits for platelets, erythrocytes, and leukocytes. Cytometry. 2002; 50(4): 231–7.*

5) *Schmidt M, Spengler H-P, Lambrecht B, et al. A new one-platform flow cytometric*

method for residual cell counting in platelet concentrates. *Cytometry*. 2009; 49(12): 2604–11.

6) Lambrecht B, Spengler H-P, Nauwelaers F, et al. Flow cytometric assay for the simultaneous determination of residual white blood cells, red blood cells, and platelets in fresh-frozen plasma: validation and two years' experience. *Transfusion*. 2009; 49(6): 1195–204.

7) van der Meer PF, Dijkstra-Tiekstra MJ, Mahon A, et al. Counting platelets in platelet concentrates on hematology analyzers: a multicenter comparative study. *Transfusion*. 2009; 49(1): 81–90.

8) Ministry of Health, Labour and Welfare, URL: https://www.mhlw.go.jp/shingi/2003/06/s0604-3k.html

9) Blanco RA, Cavagnetto C, Willmott L, et al. The use of a hematology analyzer with a new generation of software as an alternative to flow cytometry for enumerating residual white blood cells in blood components. *Transfusion*. 2020; 60(1): 155–64.

10) Cavagnetto C, Blanco RA, McKenna H, et al. Residual red cells in blood components: A multisite study of fully automated enumeration using a hematology analyzer. *Transfusion*. 2020; 61(2): 568–78.

11) Wakamoto S, Fujihara M, Akino M, et al. Evaluation of ADAM-rWBC for counting residual leucocytes in leucocyte-reduced whole blood and apheresis platelet concentrates. *Transfus Med*. 2016; 26(3): 231–5.

12) van der Meer PF, Gratama JW, van Delden CJ, et al. Comparison of five platforms for enumeration of residual leucocytes in leucoreduced blood components. *Br J Haematol*. 2001; 115(4): 953–62.

13) Dzik WH. Leukocyte counting during process control of leukoreduced blood components. *Vox Sang*. 2000; 78(suppl 2): 223–6.

14) International Council for Standardization in Haematology, Writing Group; Briggs C, Culp N, Davis B, et al. ICSH guidelines for the evaluation of blood cell analysers including those used for differential leucocyte and reticulocyte counting. *Int J Lab Hematol*. 2014; 36(6): 613–27.

15) Dzik S, Aubuchon J, Jeffries L, et al. Leukocyte reduction of blood components: Public policy and new technology. *Transfus Med Rev*. 2000; 14(1): 34–52.

16) Vamvakas EC, Blajchman MA. Universal WBC reduction: the case for and against. *Transfusion*. 2001; 41(5): 691–712.

17) Beckman N, Sher G, Masse M, et al. Review of the Quality Monitoring Methods Used by Countries Using or Implementing Universal Leukoreduction. *Transfus Med Rev*. 2004; 18(1): 25–35.

18) Vamvakas EC. Universal White Blood Cell Reduction in Europe: Has Transmission of Variant Creutzfeldt-Jakob Disease Been Prevented? *Transfus Med Rev*. 2011; 25(2): 133–44.

19) Santana JM, Dumont LJ. A flow cytometric method for detection and enumeration of low-level, residual red blood cells in platelets and mononuclear cell products. *Transfusion*. 2006; 46(6): 966–72.

20) Lagerberg JW, Korte D. Improved accuracy in counting residual white blood cells in red cell concentrates using new blood bank mode software of SYSMEX XN-1000 hematology analyzer. *Transfusion*. 2020; 60(10): 2456–7.