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XN-1000 Hematology Analyzer as an Alternative to Flow Cytometry for Measuring Residual Cells in Blood Components

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Background: Measuring residual cells in blood products is legally required for monitoring the manufacturing process and ensuring recipient safety. We compared the accuracy and performance of the two methodologies.

Methods: Residual white blood cells (rWBCs), red blood cells (rRBCs), and platelets (rPLTs) were measured in RBC concentrates (rWBCs), fresh frozen plasma (rWBCs, rRBCs, and rPLTs), and PLT concentrates (rWBCs and rRBCs) using the Sysmex XN-1000 hematology analyzer (Sysmex, Kobe, Japan) equipped with Blood Bank mode and standard flow cytometry (fluorescence-activated cell sorting; FACS).

Results: rWBC counts in RBC concentrates and plasma were similar between XN-1000 and FACS. In pooled pathogen-inactivated PLT concentrates, XN-1000 yielded higher rWBC counts. Correlations between XN-1000 and FACS were moderate for rWBCs (0.42, 95% confidence interval: 0.15–0.69) in RBC inline-filtrated WBC-depleted RBC concentrates. In plasma, correlations were high for rWBC, rRBC, and rPLT counts, with Spearman correlation coefficients of 0.82–0.97. In pathogen-inactivated PLT concentrates, correlations were moderate for rWBCs (0.58, 0.33–0.84) and rRBCs (0.61, 0.35–0.87) in pooled samples but not significant in apheresis-derived samples. Median differences between FACS and XN-1000 were generally low, but XN-1000 overestimated residual cell counts in a subset of measurements. Residual cell cut-off values were surpassed in >90% of RBC concentrates, plasma, and apheresis pathogen-inactivated PLT concentrates using both methods. In pooled pathogen-inactivated PLT concentrates, 91.2% and 70.6% surpassed the cut-off using FACS and XN-1000, respectively.

Conclusions: Sysmex XN-1000 is suitable for residual cell measurements in RBC concentrates and plasma, with some limitations for PLT concentrates.

Key Words: Blood bank mode, Blood components, Flow cytometry, Residual cells, Sysmex XN-1000

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INTRODUCTION

White blood cell (WBC) depletion to a minimal content is crucial in blood component manufacturing, particularly to reduce the risk of transmitting intracellular pathogens such as cytomegalovirus, Epstein–Barr virus, or human T-cell lymphotropic virus [1–3]. Furthermore, allogenic donor WBCs can cause febrile nonhemolytic transfusion reactions and WBC antigen alloimmunization and transfusion-associated acute lung injury [2]. The risk of acquiring transfusion-associated graft-versus-host disease diminishes after WBC depletion [4]. Residual red blood cells (rRBCs) in fresh frozen plasma (FFP) or platelet (PLT) concentrates can cause adverse reactions due to ABO blood group incompatibility or alloimmunization to rhesus antigens [5, 6]. Residual PLTs (rPLTs) in FFP should also remain below a certain cut-off because they can lead to human WBC and PLT antigen alloimmunization, and their freezing-induced activation may result in the release of vasoactive substances [7].

Residual cell numbers in blood components need to be routinely evaluated as part of QC in blood establishments to monitor the manufacturing performance and identify products that do not meet the required standards. According to the guidelines on the preparation, use, and quality assurance of blood components by the European Directorate for the Quality of Medicines and Healthcare of the Council of Europe (EDQM blood guide) [8], 90% of the WBC-depleted products (RBC concentrates, FFP, and PLT concentrates) tested should not exceed 1×10^6 residual white blood cells (rWBCs)/unit. For non-WBC-depleted FFP, rWBCs should not exceed 0.1×10^9 cells/L in 90% of the units tested [8]. Typically, for FFP, rRBCs should not exceed 6×10^9 cells/L, and rPLTs should not exceed 50×10^9 cells/L in 90% of the units tested [8]. Various methods are available to measure residual cells [9], such as manual microscopy using a Nageotte hemocytometer [10] and microvolume fluorimetry. Flow cytometry with corresponding reagents has become the most widely used method for counting residual cells in blood products [6, 9, 11–13].

The hematology analyzer Sysmex XN-1000 (Sysmex Austria GmbH, Vienna, Austria) equipped with Blood Bank mode (BBM) software (Sysmex, Kobe, Japan) has recently become available as a potential alternative to flow cytometry for counting residual cells in blood components [6, 11]. This method has several advantages over flow cytometry, including reduced time and fewer handling steps for personnel. Additionally, it provides an automated, standardized method, and hematology analyzers are already required for other purposes in blood establishments. The Sysmex XN-1000 equipped with BBM is not yet widely used in blood establishments for measuring residual cells in routine blood product QC because its accuracy and ability to meet required standards for routine practice remain uncertain.

We measured residual cells in RBC concentrates, FFP, and PLT concentrates using the Sysmex XN-1000 with BBM and flow cytometry to directly compare the two methods and clarify whether they perform equally well.

MATERIALS AND METHODS

Blood products

All blood components were routinely manufactured and subjected to routine QC at the Central Institute for Blood Transfusion and Immunology of the University Hospital in Innsbruck, Austria. In addition to routine QC measurements, including the measurement of residual cells with flow cytometry (defined as the standard method), measurements were performed using the Sysmex XN-1000 equipped with BBM. In total, 101 RBC concentrates, 115 FFP units, 67 non-pathogen-inactivated PLT concentrates, and 56 pathogen-inactivated PLT concentrates were analyzed. We compared rWBC counts in RBC concentrates, rWBC, rRBC, and rPLT counts in FFP, and rWBC and rRBC counts in PLT concentrates.

Blood components (RBC concentrates, FFP, and pooled PLT concentrates) were collected from whole blood donors at mobile blood donation events across the Federal State of Tyrol, Austria. These events were organized by the blood donation service of the Tyrolean Red Cross in collaboration with the Central Institute for Blood Transfusion and Immunology of the University Hospital in Innsbruck, Austria, according to standard operating procedures. Whole blood was collected using two collection bag systems. To produce WBC-depleted RBC concentrates and FFP, the whole-blood inline filtration system CompoSelect 4F (REF PQ31555; Fresenius Kabi, Bad Homburg, Germany) containing citrate-phosphate-dextrose as an anticoagulant and saline, adenine, glucose, and mannitol (SAGM) as an additive solution for RBCs was used (hereafter referred to as “Fresenius”). To produce WBC-depleted RBC concentrates, non-WBC-depleted FFP, and buffy coats, the RBC inline filtration system quadruple top-bottom LCRD2 450 mL CPD/SAGM system (reference LQT610U; Macopharma, Tourcoing, France) was used (hereafter referred to as “Macopharma”).

After whole-blood inline filtration (Fresenius), the whole blood was centrifuged in a Cryofuge 6000i (Thermo Fisher Scientific, Dreieich, Germany) at $4,068\times g$, 22°C for 10 mins or a Sigma

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8-KBS (Sigma Laborzentrifugen GmbH, Osterode, Germany) at 4,068×*g*, 22°C for 13 mins. Plasma and RBCs were separated using the automated blood component extractor Macopress Smarter (Macopharma). For the RBC inline filtration system (Macopharma), whole blood was centrifuged using the Cryofuge 6000i at 3,507×*g*, 22°C for 14 mins or the Sigma 8-KBS at 4,068×*g*, 22°C for 13 mins. Plasma, RBCs, and buffy coats were separated using the Macopress Smarter.

Pooled PLTs were manufactured from six buffy coats using the CompoStop flex Triple, T&B set (reference FT52600; Fresenius Kabi). Pools were centrifuged using the Sigma 8-KBS at 560×*g*, 20°C for 9 mins and 10 secs and then automatically separated by the Macopress Smarter through a WBC filter into the storage container of the pooling set (hereafter referred to as "pooled").

Apheresis PLT donors donated at the Central Institute for Blood Transfusion and Immunology of the University Hospital in Innsbruck, according to standard operating procedures. Apheresis PLTs were collected using the Trima Accel cell separator (Terumo BCT, Zaventem, Belgium) or the Amicus cell separation platform (Fresenius Kabi) (hereafter referred to as "apheresis-derived").

All PLT components were WBC-depleted and suspended in 35% plasma and 65% SSP+ PLT additive solution (PAS-E) (reference SSP2130U; Macopharma) or Intersol PLT additive solution (PAS-C) (reference RGR8109B; Fresenius Kabi) (hereafter referred to as "non-pathogen-inactivated"). All PLT products were further pathogen-inactivated using the INTERCEPT blood system (amotosalen/UVA) per the manufacturer's instructions, using large-volume or dual-storage processing sets (Cerus Europe, Amersfoort, The Netherlands) (hereafter referred to as "pathogen-inactivated").

Residual cells were measured across various blood components, including Fresenius and Macopharma RBC concentrates (rWBCs/μL), Fresenius and Macopharma plasma (rWBCs/μL, rRBCs/μL, and rPLTs/μL), and pooled and apheresis PLT concentrates before and after pathogen inactivation (rWBCs/μL and rRBCs/μL).

Analysis with the hematology analyzer

Samples were collected into 2-mL plain tubes without anticoagulant (Greiner Bio-One, Kremsmünster, Austria) or EDTA tubes (when stated explicitly) and manually inverted 10 times immediately before analysis on the Sysmex XN-1000, using the BBM per the manufacturer's instructions. QC measurements of the analyzer were performed daily prior to sample analysis with three levels of XN-Check material, PLT check material, and additional XN-Check body fluid (BF) control material (Sysmex) to monitor analyzer performance. To our knowledge, controls for residual cell measurements with BBM are lacking; therefore, we measured rWBCs in XN-Check BF levels 1 and 2 using the BBM. XN-Check BF level 1 contains 70–80 WBCs/μL (depending on the batch) and is, therefore, in a relatively low range (comparable to that of rWBCs/μL in blood products). No controls were available for rRBCs and rPLTs. RBC concentrates were measured using the RBC-Pack+residual cell profile. FFP and PLT concentrates were measured using the PLT-Pack+residual cell profile, as no specific profile is currently available for plasma products. All measurements of residual cells in plasma were performed before freezing, but we refer to "FFP" throughout the manuscript because this is the final product.

Supplemental Data Table S1 shows the results of precision analysis (interday and intraday precision) using two controls (BF levels 1 and 2) measured with the Sysmex XN-1000. For BF levels 1 and 2, the CVs were 5.6% and 2.8% for interday precision and 4.0% and 1.5% for intraday precision, respectively. Residual cell measurements with the Sysmex XN-1000 equipped with BBM are hereafter referred to as "XN-1000."

Flow cytometry

Samples were collected into 2-mL plain tubes without anticoagulant (Greiner Bio-One). To count residual cells in FFP, we used the BD Plasma Count Kit and BD Trucount tubes (both from Becton Dickinson BD] Biosciences, San Jose, CA, USA) per the manufacturer's instructions. To count rWBCs in RBC and PLT concentrates, we used the BD Leucocount Kit (BD Biosciences) and BD Trucount tubes per the manufacturer's instructions. rRBC counts in PLT concentrates were determined using a CD235a-PE (Glycophorin A)-conjugated antibody (reference A07792; Beckman Coulter, Vienna, Austria) in a separate tube. In detail, we mixed 50 μL of BD Cell Wash (Becton Dickinson Biosciences), 5 μL of conjugated antibody, and 10 μL of sample in BD Trucount tubes, followed by a 15-min incubation at room temperature, then added 500 μL of BD Cell Wash, and mixed the samples before analysis in the flow cytometer. For fluorescence-activated cell sorting (FACS) analysis, BD FACSVia and BD FACSLyric flow cytometers (BD Biosciences) were used. Residual cell measurements using flow cytometry were hereafter referred to as "FACS."

Linearity studies

Blood components were WBC-depleted and double-filtered using an additional WBC reduction filter of the whole blood inline

filtration system CompoSelect 4F (reference FT52600; Fresenius Kabi) for whole blood and the CompoStop Flex2F PLT processing and WBC reduction system (reference FD51600; Fresenius Kabi) for PLT concentrates. ABO-compatible whole blood with a known WBC concentration was used for spiking to generate samples with calculated concentrations of 35.2, 17.6, 8.8, 4.4, 2.2, and 1.1 WBCs/μL.

Statement of ethical approval

No ethical approval was required, as only blood products that were routinely manufactured at the Central Institute for Blood Transfusion and Immunology in Innsbruck, Austria were analyzed, and no donor data were used. The donors signed informed consent that residual material of the donation can be used for research purposes.

Statistical analysis

The data were not normally distributed, as verified by plotting histograms (Supplemental Data Figs. S1 and S2). Continuous variables were reported as median and interquartile range (IQR). Differences between residual cell counts measured with XN-1000 and FACS were tested using the sign test of matched pairs. In a sensitivity analysis, we compared residual cells measured with FACS and XN-1000 sampled in plain or EDTA tubes.

Correlations were assessed using Spearman correlation coefficients. Point estimates of correlation coefficients <0.4, 0.4 to <0.7, and ≥ 0.7 were interpreted as low, moderate, and strong, respectively. Agreements were depicted in Bland–Altman plots accompanied by median differences and limits of agreement using the 2.5th and 97.5th percentiles [14, 15].

Pass rates were calculated according to cut-off values defined in the EDQM blood guide [8] per blood product (unit) or per volume (L) and are reported with Agresti–Coull 95% confidence intervals (CIs).

All statistical tests were two-sided, and statistical significance was set to $P \leq 0.05$. The data were analyzed using Stata version 15.1 and R version 4.1.0.

RESULTS

Residual cells in various blood components measured by FACS and XN-1000

Table 1 summarizes the measurement results of the various blood components assessed with FACS and XN-1000. In RBC inline-filtrated RBC concentrates (Macopharma; N = 40), the median rWBC count was 0.1 cells/μL (IQR, 0.0–0.2 cells/μL) using FACS and 0.1 cells/μL (0.1–0.3 cells/μL) using XN-1000 (*P* for difference = 0.856). In whole-blood inline-filtrated RBC concentrates (Fresenius; N = 61), the median rWBC count was 0.4 cells/μL (IQR 0.2–0.7 cells/μL) using FACS and 0.4 cells/μL (0.2–0.9 cells/μL) using XN-1000 (*P* for difference = 0.427). In plasma products derived from the RBC inline filtration system (Macopharma; non-WBC-depleted; N = 60), the median rWBC count was 2.0 cells/μL (1.0–5.0 cells/μL) using FACS and 2.5 cells/μL (0.9–6.6 cells/μL) using XN-1000 (*P* for difference >0.999), and the median rRBC count was 49.5 cells/μL (29.5–133.5 cells/μL) using FACS and 47.0 cells/μL (32.5–139.5 cells/μL) using XN-1000 (*P* for difference = 0.193). rPLT counts were significantly higher for FACS than for XN-1000 (5,332.0 [3,779.5–7,325.0] cells/μL vs. 4,000.0 [3,000.0–6,000.0] cells/μL; *P* for difference <0.001). In plasma products derived from the whole-blood inline filtration system (Fresenius; WBC-depleted; N = 55), the median rWBC count was 0.0 cells/μL (0.0–0.0 cells/μL) using FACS and 0.0 cells/μL (0.0–0.1 cells/μL) using XN-1000 (*P* for difference <0.001), and the median rRBC count was 200.0 cells/μL (148.0–270.0 cells/μL) using FACS and 196.0 cells/μL (143.0–263.0 cells/μL) using XN-1000 (*P* for difference = 0.684). rPLT counts were significantly higher for FACS than for XN-1000 (165.0 [79.0–2,007.0] cells/μL vs. 0.0 [0.0–1,000.0] cells/μL; *P* for difference <0.001). rPLT counts in WBC-depleted plasma (Fresenius) were prominently lower than those in non-WBC-depleted plasma (Macopharma), as PLTs were also removed during WBC reduction. For pathogen-inactivated pooled PLT concentrates (N = 34), rWBC counts were significantly lower for FACS than for XN-1000 (1.1 [0.4–1.7] cells/μL vs. 2.3 [1.4–3.0] cells/μL; *P* for difference <0.001). Similarly, for pathogen-inactivated apheresis PLT concentrates (N = 22), rWBC counts were significantly lower for FACS than for XN-1000 (0.0 [0.0–0.1] cells/μL vs. 0.8 [0.6–1.1] cells/μL; *P* for difference <0.001).

Supplemental Data Table S2 provides data on residual cells measured with FACS and XN-1000 sampled in plain or EDTA tubes. For most blood components, we did not detect differences between EDTA and plain tubes. However, for RBC concentrates derived using the Macopharma system, rWBC counts were significantly higher in plain tubes than in EDTA tubes.

Figs. 1 and 2 depict scatterplots and Spearman correlation coefficients comparing residual cell counts measured with FACS and XN-1000 in routine samples. In RBC concentrates derived using the Macopharma system, we observed a moderate correlation between the two methodologies for rWBC counts, with a correlation coefficient of 0.42 (95% CI, 0.15–0.69). In plasma,

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Table 1. Distribution of residual cells in various blood components

Blood component	Subgroup	N	Method	Residual cell type, median (IQR)		
				rWBCs/μL	rRBCs/μL	rPLTs/μL
RBC concentrate	Fresenius	61	FACS	0.4 (0.2–0.7)	-	-
		61	XN-1000	0.4 (0.2–0.9)	-	-
				P=0.427	-	-
	Macopharma	40	FACS	0.1 (0.0–0.2)	-	-
		40	XN-1000	0.1 (0.1–0.3)	-	-
				P=0.856	-	-
FFP	Fresenius	55	FACS	0.0 (0.0–0.0)	200.0 (148.0–270.0)	165.0 (79.0–2,007.0)
		55	XN-1000	0.0 (0.0–0.1)	196.0 (143.0–263.0)	0.0 (0.0–1,000.0)
				P<0.001	*P*=0.684	*P*<0.001
	Macopharma	60	FACS	2.0 (1.0–5.0)	49.5 (29.5–133.5)	5,332.0 (3,779.5–7,325.0)
		60	XN-1000	2.5 (0.9–6.6)	47.0 (32.5–139.5)	4,000.0 (3,000.0–6,000.0)
				P>0.999	*P*=0.193	*P*<0.001
PLT concentrate before pathogen inactivation	Pooled	45	FACS	1.0 (0.6–2.0)	202.0 (166.4–240.1)	-
		45	XN-1000	1.0 (0.4–2.1)	320.0 (160.0–539.0)	-
				P=0.755	*P*<0.001	-
	Apheresis- derived	22	FACS	0.2 (0.1–0.3)	80.5 (54.0–125.5)	-
		22	XN-1000	0.6 (0.2–4.6)	4.0 (1.0–7.0)	-
				P=0.167	*P*<0.001	-
PLT concentrate after pathogen inactivation	Pooled	34	FACS	1.1 (0.4–1.7)	188.1 (138.8–234.6)	-
		34	XN-1000	2.3 (1.4–3.0)	284.0 (159.0–438.0)	-
				P<0.001	*P*=0.003	-
	Apheresis-derived	22	FACS	0.0 (0.0–0.1)	84.5 (49.9–116.8)	-
		22	XN-1000	0.8 (0.6–1.1)	5.0 (2.0–6.0)	-
				P<0.001	*P*<0.001	-

Differences between residual cell counts measured with Sysmex XN-1000 and FACS in plain tubes were tested using the sign test of matched pairs.
Abbreviations: IQR, interquartile range; FACS, fluorescence-activated cell sorting; (r)PLTs, (residual) platelets; (r)RBCs, residual red blood cells; rWBCs, residual white blood cells; FFP, fresh frozen plasma.

we observed high correlations for rWBC, rRBC, and rPLT counts, with correlation coefficients of 0.82–0.97. In pathogen-inactivated pooled PLT concentrates, correlations were moderate for rWBC and rRBC counts, with correlation coefficients of 0.58 (0.33–0.84) and 0.61 (0.35–0.87), respectively (Fig. 2). For pathogen-inactivated apheresis-derived PLTs, the correlation coefficients were not significantly different from zero for both rWBC and rRBC counts. We further performed spiking experiments with known rWBC counts for RBC concentrates, plasma, and PLT concentrates, revealing a high linear correlation between expected and observed WBC counts using FACS and XN-1000, with $R^2 \geq 0.993$ (Supplemental Data Fig. S3). Bland–Altman plots showed lower biases with FACS for RBC concentrates (–1.13 rWBCs/μL) and PLT concentrates (–0.20 rWBCs/μL), but a higher bias for FFP (0.30 rWBCs/μL) (Supplemental Data Fig. S3). Values obtained with FACS were lower than expected (–9.1% to –24.2%), except for the highest value in FFP (Supplemental Data Table S3). Values obtained using XN-1000 were higher than the expected values for RBC concentrates (4.1%–12.1%), except for one value, which was lower than the expected value (Supplemental Data Table S3). For FFP and PLT concentrates, values were lower than the expected values when using XN-1000 (–6.4% to –28.8%), except for one value that was the same as the expected value (Supplemental Data Table S3).

Bland–Altman plots comparing FACS and XN-1000 results are provided in Figs. 3 and 4. For Fresenius and Macopharma RBC

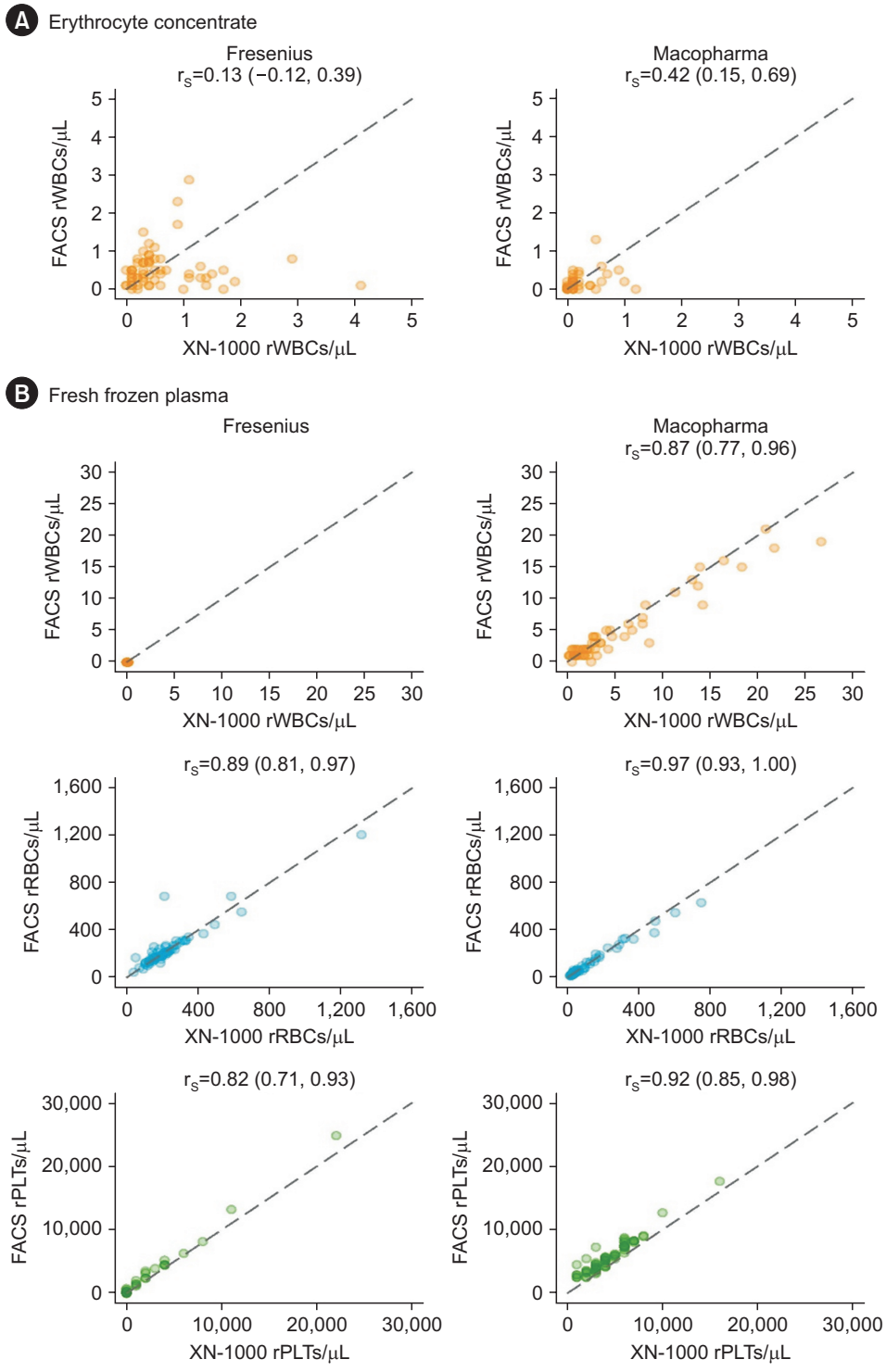

Fig. 1. Scatterplots of residual cell counts measured with XN-1000 and FACS in (A) RBC concentrates and (B) FFP. r_S, Spearman correlation coefficient.
Abbreviations: FACS, fluorescence-activated cell sorting; rPLTs, residual platelets; rRBCs, residual red blood cells; rWBCs, residual white blood cells; FFP, fresh frozen plasma.

concentrates, median differences were close to zero and increased with higher averages of rWBC counts. Similarly, median differences for Fresenius and Macopharma plasma were zero. However, in Macopharma plasma, XN-1000 tended to overestimate rWBC counts. Median differences in rPLT counts were 164 (limits of agreement: 19, 2,188) in Fresenius plasma and 1,246 (140, 3,509) in Macopharma plasma. In pathogen-inactivated PLT concentrates, XN-1000 overestimated rWBC and rRBC counts, and this overestimation tended to increase with higher averages.

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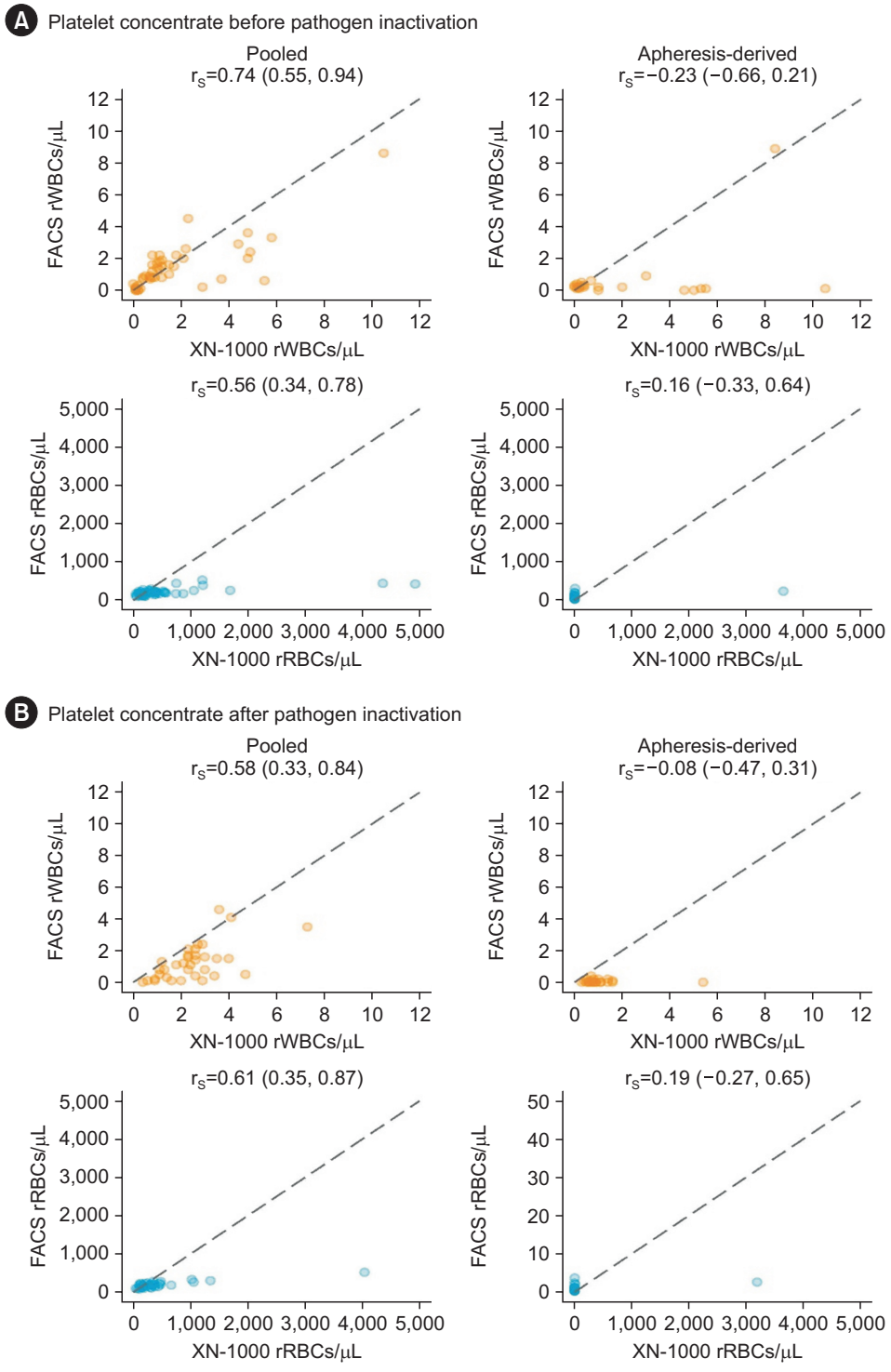

Fig. 2. Scatterplots of residual cell counts measured with XN-1000 and FACS in PLT concentrates (A) before and (B) after pathogen inactivation. r_S, Spearman correlation coefficient.
Abbreviations: FACS, fluorescence-activated cell sorting; PLT, platelet; rRBCs, residual red blood cells; rWBCs, residual white blood cells.

Classification of residual cells in different blood components measured by FACS and XN-1000 according to cut-off values

Table 2 summarizes pass rates based on the cut-off values specified in the EDQM blood guide [8]. In RBC concentrates, FFP, and pathogen-inactivated apheresis-derived PLT concentrates, >90% of units/volumes analyzed passed the cut-off values for both FACS and XN-1000. In pathogen-inactivated pooled PLT concentrates, 91.2% (95% CI, 76.3%–97.7%) and 70.6% (53.7%–83.3%) of blood products passed the cut-off for rWBC

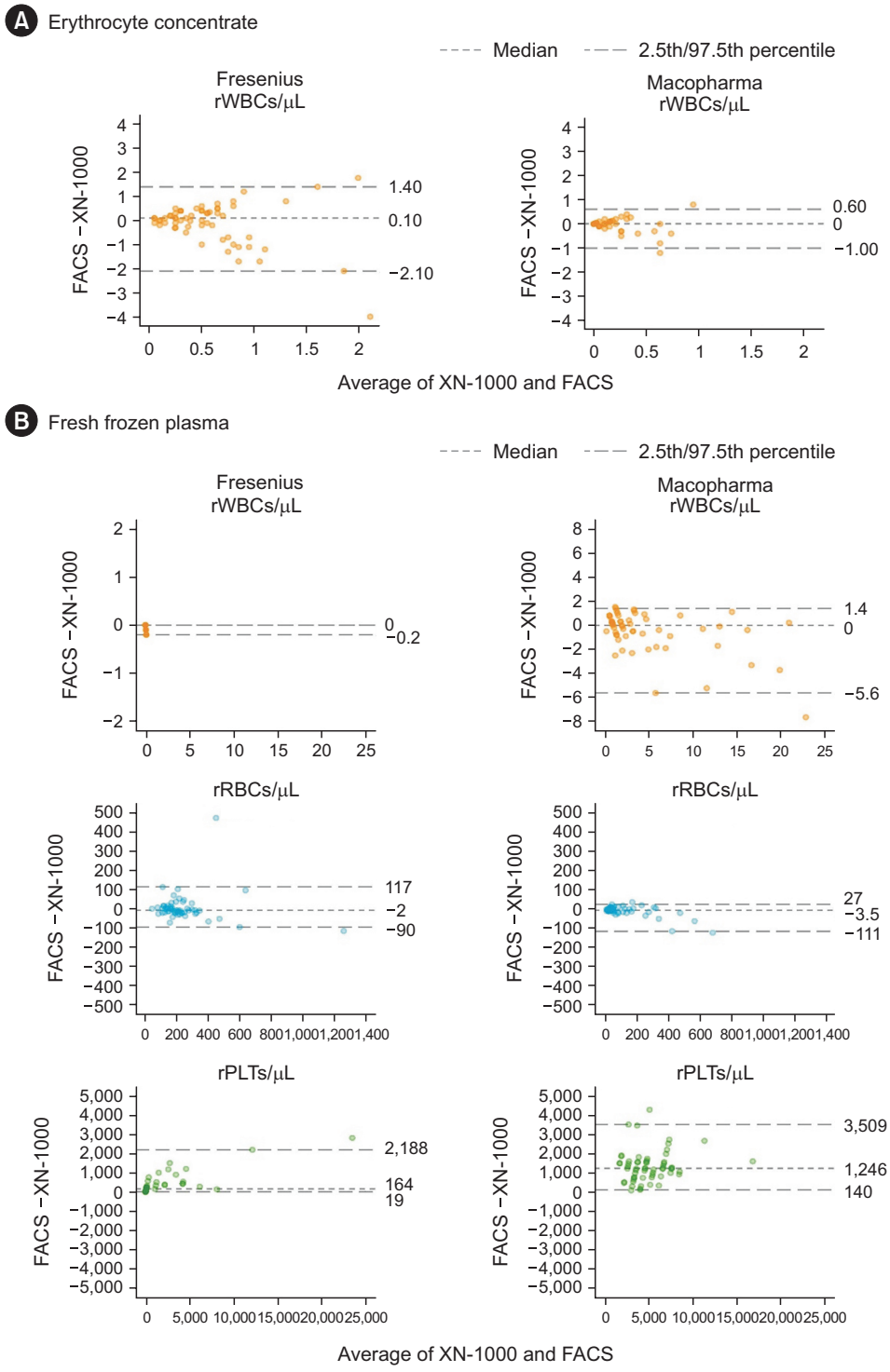

Fig. 3. Bland–Altman plots of residual cell counts measured with XN-1000 and FACS in (A) RBC concentrates and (B) FFP. The limits of agreement are estimated using the 2.5th and 97.5th percentiles, and the average bias is estimated as the median of the differences.

Abbreviations: FACS, fluorescence-activated cell sorting; rPLTs, residual platelets; rRBCs, residual red blood cells; rWBCs, residual white blood cells; FFP, fresh frozen plasma.

counts for FACS and XN-1000, respectively. For rRBC counts in PLT concentrates, no cut-off values are specified in the EDQM blood guide; therefore, we could not calculate pass rates.

rWBC counts by FACS and XN-1000 in PLT concentrates at different time points

Supplemental Data Fig. S4 depicts mean rWBC counts measured on different days after donation in apheresis-derived and pooled PLT concentrates using XN-1000 and FACS, showing ex-

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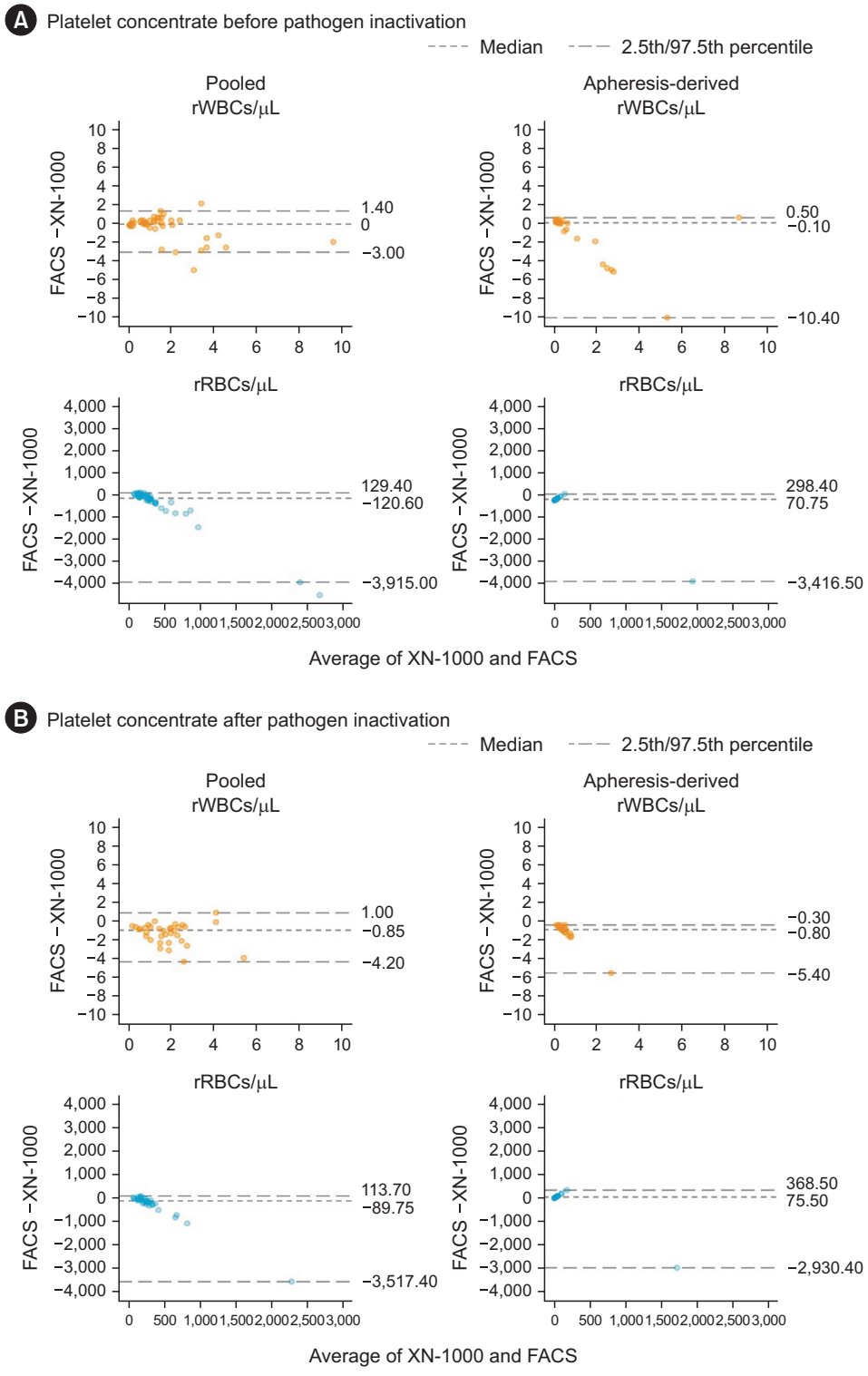

Fig. 4. Bland–Altman plots of residual cell counts measured with XN-1000 and FACS in PLT concentrates (A) before and (B) after pathogen inactivation. The limits of agreement are estimated using the 2.5th and 97.5th percentiles, and the average bias is estimated as the median of the differences.
Abbreviations: FACS, fluorescence-activated cell sorting; PLT, platelet; rRBCs, residual red blood cells; rWBCs, residual white blood cells.

ceptionally high values for products measured on day 2 after pathogen inactivation when using XN-1000, but not when using FACS.

DISCUSSION

We directly compared Sysmex XN-1000 equipped with BBM with FACS, a standard method for quantifying residual cells in blood

Table 2. Residual cells in different blood components classified according to cut-off values

Blood component	Subgroup	Method	Pass rate (95% CI) (number below cut-off/above cut-off)		
			rWBC	rRBC	rPLT
RBC concentrate	Fresenius	Cut-off	$<1\times10^6$ rWBCs/unit	-	-
		FACS	100.0% (92.9, 100.0%) (61/0)		
		XN-1000	98.4% (90.4, 100.0%) (60/1)		
	Macopharma	Cut-off	$<1\times10^6$ rWBCs/unit	-	-
		FACS	100.0% (89.6, 100.0%) (40/0)		
		XN-1000	100.0% (89.6, 100.0%) (40/0)		
FFP	Fresenius	Cut-off	$<1\times10^6$ rWBCs/unit	$<6\times10^9$ rRBCs/L	$<50\times10^9$ rPLTs/L
		FACS	100.0% (92.2, 100.0%) (55/0)	100.0% (92.2, 100.0%) (55/0)	100.0% (92.2, 100.0%) (55/0)
		XN-1000	100.0% (92.2, 100.0%) (55/0)	100.0% (92.2, 100.0%) (55/0)	100.0% (92.2, 100.0%) (55/0)
	Macopharma	Cut-off	$<0.1\times10^9$ rWBCs/L	$<6\times10^9$ rRBCs/L	$<50\times10^9$ rPLTs/L
		FACS	100.0% (92.8, 100.0%) (60/0)	100.0% (92.8, 100.0%) (60/0)	100.0% (92.8, 100.0%) (60/0)
		XN-1000	100.0% (92.8, 100.0%) (60/0)	100.0% (92.8, 100.0%) (60/0)	100.0% (92.8, 100.0%) (60/0)
PLT concentrate before pathogen inactivation	Pooled	Cut-off	$<1\times10^6$ rWBCs/unit	-	-
		FACS	88.9% (76.0, 95.6%) (40/5)		
		XN-1000	80.0% (66.0, 89.3%) (36/9)		
	Apheresis-derived	Cut-off	$<1\times10^6$ rWBCs/unit	-	-
		FACS	95.5% (76.5, 100.0%) (21/1)		
		XN-1000	68.2% (47.1, 83.8%) (15/7)		
PLT concentrate after pathogen inactivation	Pooled	Cut-off	$<1\times10^6$ rWBCs/unit	-	-
		FACS	91.2% (76.3, 97.7%) (31/3)		
		XN-1000	70.6% (53.7, 83.3%) (24/10)		
	Apheresis-derived	Cut-off	$<1\times10^6$ rWBCs/unit	-	-
		FACS	100.0% (82.5, 100.0%) (22/0)		
		XN-1000	95.5% (76.5, 100.0%) (21/1)		

Residual cells per unit refer to residual cells per blood product.
Abbreviations: CI, confidence interval; FACS, fluorescence-activated cell sorting; (r)PLTs, (residual) platelets; (r)RBCs, (residual) red blood cells; rWBCs, residual white blood cells, FFP, fresh frozen plasma.

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products. Overall, although absolute values differed between the two methods for some measurements, Sysmex XN-1000 performed well in accurately determining whether a product passed the defined cut-off values for RBC concentrates and FFP; however, it had difficulty in accurate determination in pooled PLT concentrates.

Our findings suggest that the Sysmex XN-1000 is a suitable alternative method for quantifying residual cells in blood products, as previously validated for rWBCs [11, 16–19], rRBCs [6, 17], and rPLTs [17]. Consistent with previous findings [11], we found strong correlations over a broad concentration range between expected and observed rWBC counts using FACS and Sysmex XN-1000 in double-WBC-reduced products spiked with defined numbers of WBCs. Our study is unique in comparing measurements of relevant residual cells in RBC concentrates and FFP produced using two blood collection systems (Fresenius and Macopharma), demonstrating robust pass rates for the XN-1000, independent of the collection system used. Additionally, we analyzed PLT concentrates from pooled buffy coats and apheresis before and after pathogen inactivation, identifying challenges in measuring pathogen-inactivated pooled PLT concentrates with the XN-1000, as the pass rate was only 70.6% for these products. We considered evaluating the performance of the XN-1000 on pathogen-inactivated products essential, as routine quality control measurements should utilize the final product, specifically post-pathogen inactivation, when such procedures are implemented.

Although discrepancies existed between both methods for some blood products, Sysmex XN-1000 accurately discriminated whether RBC concentrates or FFP met the cut-off criteria defined in the EDQM blood guide. Absolute rWBC counts were significantly higher in Macopharma RBC concentrates measured in plain tubes than in those measured in EDTA tubes, potentially reducing aggregate formation during sampling. However, since the pass rate for the cut-off criteria reached 100% with plain tubes, this difference lacks practical relevance. For all other categories, no significant differences were observed for the Sysmex XN-1000 when using EDTA tubes instead of plain tubes. Given the unclear cause of lower absolute rWBC/μL counts in Macopharma RBC concentrates with EDTA tubes, we advocate for plain tube usage to avoid underestimating residual cell counts. However, the XN-1000 inaccurately measured pathogen-inactivated pooled PLT concentrates, overestimating absolute values. Specifically, rWBC counts were significantly higher with XN-1000 (2.3 cells/μL; IQR, 1.4–3.0 cells/μL) than when using FACS (1.1 cells/μL; IQR, 0.4–1.7 cells/μL). This overestimation significantly impacts compliance with the predefined cut-off ($<1\times10^6$ rWBCs/unit), achieved in 91.2% (95% CI, 76.3%–97.7%) of units tested with FACS but only 70.6% (53.7%–83.3%) of those tested with Sysmex XN-1000. Given identical pathogen inactivation processes for pooled and apheresis-derived products and overestimation observed only in pooled products, we assume that pathogen inactivation does not play a role. Furthermore, similar mean rWBC counts in pooled and apheresis-derived products when measured with Sysmex XN-1000 and FACS on day 1 post-donation imply the pooling process is not the cause. Instead we assume that time points of measurement influenced overestimation, with higher values on day 2 using XN-1000, but not with FACS. In routine practice, this issue is non-relevant for apheresis-derived products, which are routinely measured on day 1 after pathogen inactivation (donation on day 0, pathogen inactivation completed on day 1) but is crucial for pooled products, typically measured on day 2 (donation on day 0, pooling on day 1, pathogen inactivation completed on day 2). Consequently, we recommend using the Sysmex XN-1000 for measuring rWBC counts in PLT concentrates no later than day 1 post-donation. These limitations of the Sysmex XN-1000 system require careful consideration for full-scale implementation.

In addition to performance, method feasibility merits consideration. Hematology analyzers such as the Sysmex XN-1000 system simplify operation compared to flow cytometry as they eliminate manual handling steps beyond sample mixing and autosampler insertion. The Sysmex XN-1000 achieves measurements in approximately 3 minutes per sample without preparatory steps, in contrast to flow cytometry, which requires approximately 20 mins for sample preparation, followed by measurement. Flow cytometry involves additional steps, including reagent addition and incubation before analysis, which prolongs the overall processing time. Furthermore, flow cytometry requires trained biomedical personnel for sample preparation, whereas hematology analyzers function automatically.

Furthermore, measurements with the Sysmex XN-1000 are not only less time-consuming, thereby reducing personnel costs, but are also more cost-effective. Additionally, the higher degree of automation might lead to more standardized results. Although the advantages of simplicity, cost-efficiency, and time effectiveness have been highlighted for the Sysmex XN-1000 [3], blood establishments involved in manufacturing pooled platelet (PLT) products and performing pathogen inactivation may still require a flow cytometer for accurate residual cell measurements.

Our study has several strengths, including the comprehensive evaluation of the Sysmex XN-1000 against FACS in measuring

residual cells in RBC concentrates, FFP, and PLT concentrates, with head-to-head comparisons aligned with the EDQM blood guide. We also assessed the impact of different blood collection systems, timing, pathogen inactivation, production methods, and sampling tube selection on the Sysmex XN-1000 measurements. One limitation of our study is that, although all available QC measurements were performed for the Sysmex XN-1000 analyzer, we still lack specific controls for the BBM with residual cells in the expected low concentration range. Another limitation is that rPLT counts using XN-1000 were only obtained rounded to $\times 10^3/\mu L$, whereas measurements with FACS were obtained accurately. This less precise measurement using XN-1000 may, in theory, have biased the revealed differences.

In conclusion, Sysmex XN-1000 is a suitable alternative method for measuring residual cells in RBC concentrates and FFP but has some limitations for PLT concentrates.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.3343/alm.2024.0448.

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AUTHOR CONTRIBUTIONS

Conceptualization: Siller A and Amato M; Investigation: Siller A, Schmidt D, and Amato M; Data curation: Siller A and Seekircher L; Formal analysis: Seekircher L and Tschiderer L; Project administration: Siller A and Amato M; Supervision: Siller A, Schennach H, Willeit P, and Amato M. Writing original draft: Siller A; Writing—review and editing: Seekircher L, Schmidt D, Tschiderer L, Schennach H, Willeit P, and Amato M. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

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