Received: 13 August 2020 | Accepted: 5 October 2020

DOI: 10.1111/trf.16196

BLOOD COMPONENTS

TRANSFUSION

Residual red cells in blood components: A multisite study of fully automated enumeration using a hematology analyzer

Chloe Cavagnetto[1] | Richard Alejo Blanco[1] | Hollie McKenna[2] | Laura Willmott[1] | Elif Aydogdu[3] | Nicola Akinyemi[3] | Helena Standring[3] | Simon Procter[3] | Johan W. Lagerberg[4] | Elin Johansson[5] | Harry Croxon[5] | Dirk de Korte[4] | Stephen F. Garner[1] | Atsushi Shirakami[6] | Jarob Saker[6] | Joachim Linssen[6] | Rebecca Cardigan[1,7]

[1]Component Development Laboratory, NHS Blood and Transplant, Cambridge, UK

[2]Clinical Services, NHS Blood and Transplant, UK

[3]Manufacturing and Product Development, NHS Blood and Transplant, UK

[4]Product and Process Development, Sanquin Blood Bank, Amsterdam, Netherlands

[5]Irish Blood Transfusion Service, Dublin, Ireland

[6]Scientific Affairs, Sysmex Corporation, Kobe, Japan

[7]Department of Haematology, University of Cambridge, Cambridge, UK

Correspondence
Richard Alejo Blanco, Component Development Laboratory, NHS Blood and Transplant, Long Road, Cambridge, CB2 0PT UK.
Email: richard.blanco@nhsbt.nhs.uk

Funding information
NHS Blood and Transplant; Sysmex Corporation

Abstract

Background: Manufacture of platelet concentrates (PCs) and plasma may fail to remove all residual red blood cells (rRBCs). Measuring rRBCs for compliance to guidelines has proven challenging, leading to an absence of a consensus methodology. Sysmex hematology analyzers with the Blood Bank mode (BB mode) analysis option offer the potential for automated rRBC counting. We therefore performed a two-site appraisal of the system.

Study Design and Methods: Performance characteristics were determined using platelet and plasma samples spiked with RBCs. Sample stability (n = 47) and the impact of sample type were also assessed. Components (platelets, n = 1474; plasma, n = 77) prepared using different routine manufacturing methods were tested to assess variation in rRBC concentration.

Abbreviations: BB mode, Blood Bank mode; BC, buffy coat; BFs, body fluids; CDL, Component Development Laboratory; CI, confidence interval; CSF, cerebrospinal fluid; CV, coefficient of variation; EDTA, ethylenediaminetetraacetic acid; hsA, high-sensitivity analysis; IBTS, Irish Blood Transfusion Service; LOD, limit of detection; LOQ, limit of quantitation; NHSBT, National Health Service Blood and Transplant; PAS, platelet additive solution; PCs, platelet concentrates; PPD, Department of Product and Process Development; QC, quality control; QM, quality monitoring; rRBCs, residual red blood cells; rWBCs, residual white blood cells; SBB, Sanquin Blood Bank; TACSI, Terumo Automated Centrifuge and Separator Integration; WB, whole blood.

Reprints will not be available

Results: Linearity studies up to 19 000 RBCs/μL demonstrated good correlation between expected and observed results ($R^2 \geq 0.9731$), and flow cytometric results also correlated well with BB mode ($R^2 = 0.9400$). Precision analysis gave a limit of quantitation of 6 to 7 RBCs/μL, and carryover was 0.03%. Ethylenediaminetetraacetic acid and plain tube results were not significantly different ($P \geq 0.10$), and samples were stable up to 24 hours. Apheresis PCs produced at two sites had lower rRBC concentrations (medians, 17 and 13 RBCs/μL) than those produced with the buffy coat method either manually (median, 681 RBCs/μL) or with the automated Terumo Automated Centrifuge and Separator Integration process (median, 81 RBCs/μL). All PCs failing visual inspection as having RBCs ≥4000 RBCs/μL were also detected by the BB mode.

Conclusion: The BB mode had acceptable performance characteristics and has the potential for integration into a fully automated process control system for rRBC enumeration in plasma and PCs.

KEYWORDS

blood components, haematology analyser, residual red cells

1 | INTRODUCTION

Blood component manufacturing processes for platelets and plasma may fail to remove all contaminating red blood cells (RBCs). These residual RBCs (rRBCs) can potentially lead to clinical complications such as reactions due to ABO blood group incompatibility or alloimmunization to Rh antigens.[1] ABO-related reactions may be minimized by hospitals having policies to maximize compatible transfusions, while Rh immunization may be avoided by similar policies combined with the appropriate use of anti-RhD prophylaxis.[2]

Although hospital practices reduce risks associated with transfusion of rRBCs, blood services are required to use manufacturing processes designed to minimize levels of rRBCs and to perform quality monitoring (QM) to ensure regulatory compliance. Guidelines for acceptable levels of rRBCs in platelet or plasma components vary by jurisdiction. For example, in accordance with European Union legislation, UK guidelines state that before freezing, fresh frozen plasma should contain less than 6000 RBCs/μL. However, there is no requirement to measure RBC contamination in platelet concentrates (PCs).[3,4]

For operational reasons, however, blood services may reduce rRBC levels below those stated in the guidelines. For example, use of pathogen inactivation technologies may require less than 4000 RBCs/μL before treatment.[5] Furthermore, although confounding factors make it difficult to define the critical RBC levels able to induce alloimmunization,[6] it may be below the 6000 RBCs/μL guideline, meaning that manufacturers may aim to reduce levels to as low as is practically possible. A recent study characterizing liquid plasma calculated that approximately 800 RBCs/μL may result in primary immunization to D.[7]

Measuring rRBC levels to ensure compliance has been a challenge as the concentrations are too low for enumeration by routine hematology analyzers. Consequently, several alternative approaches have been described. Manual visual inspection of components is used, with some blood services developing visual assessment guides,[8] which reflect that rRBC levels give an observable redness in PC color. For example, both National Health Service Blood and Transplant (NHSBT) in England and Sanquin Blood Bank (SBB) in the Netherlands use a visual guide to identify PCs and plasma needing to be discarded due to rRBC levels exceeding their guidelines. However, these methods based on the use of color charts are subjective and cannot discriminate low levels of rRBCs. More quantitative but still manual techniques using counting chambers are performed by other organizations.[9] Limitations of both visual inspection and manual counting led to the development of flow cytometric approaches for measuring rRBCs, but there is still no consensus or reference methodology for rRBC enumeration.[1,10–12]

Interest in the use of hematology analyzers to count rRBCs was renewed by the latest generation of instruments being able to count low levels of cells in body fluids (BFs). Assessment of the cerebrospinal fluid (CSF) analysis mode on an ADVIA 120 hematology analyzer (Siemens, Berlin, Germany) found that although sample

predilution was required for counting of rRBCs, the assay met the required performance characteristics.[13]

The Sysmex XN series of analysers (Sysmex Corp., Kobe, Japan) also includes an option for low-level cell counting in BFs that has been reported as giving comparable results with microscopy, although limitations were noted with levels of RBCs in BFs less than 1000 cells/μL.[14] This analysis mode has also been described as being suitable for rRBC enumeration in leukoreduced plasma, with satisfactory performance at 6000 RBCs/μL, although it was unsuitable for counting residual white blood cells (rWBCs).[15]

Further development of BF analysis on the Sysmex analyzer led to a new research mode, called high-sensitivity analysis (hsA) in which sample analysis volume was increased, and erythrocytic cells were measured on a dedicated flow cytometric RBC channel. This gave a lower limit of quantitation (LOQ) for RBC in CSF of 10 cells/μL.[16]

It was possible that the high concentration of platelets and plasma in blood components when compared with BF would impact on the detection levels for residual cells using the hsA mode. Thus, Sysmex developed the Blood Bank mode (BB mode), which maintained the hsA mode features but modified the gating strategy for optimal detection of residual cells in blood components and introduced automated mixing and sampling before analysis. This BB mode has been described as a potential alternative to flow cytometry for rWBC enumeration[17] but also offers the ability to simultaneously count rRBCs in both plasma and PCs.

The aims of our multisite study in three countries were to characterize fully for the first time the performance of the BB mode for rRBC enumeration, and to subsequently assess the variation in rRBC levels in PCs and plasma generated by different routine manufacturing processes.

2 | MATERIALS AND METHODS

The Sysmex BB mode uses flow cytometric measurements of light scatter to count rRBCs in blood components. Initial assessment of performance characteristics was performed by both NHSBT and SBB, with specifically prepared samples spiked with calculated concentrations of RBCs. A selection of samples were also tested in parallel by flow cytometry at SBB. Subsequently, rRBC levels were measured in PC and plasma components, manufactured in routine production environments (Figure 1).

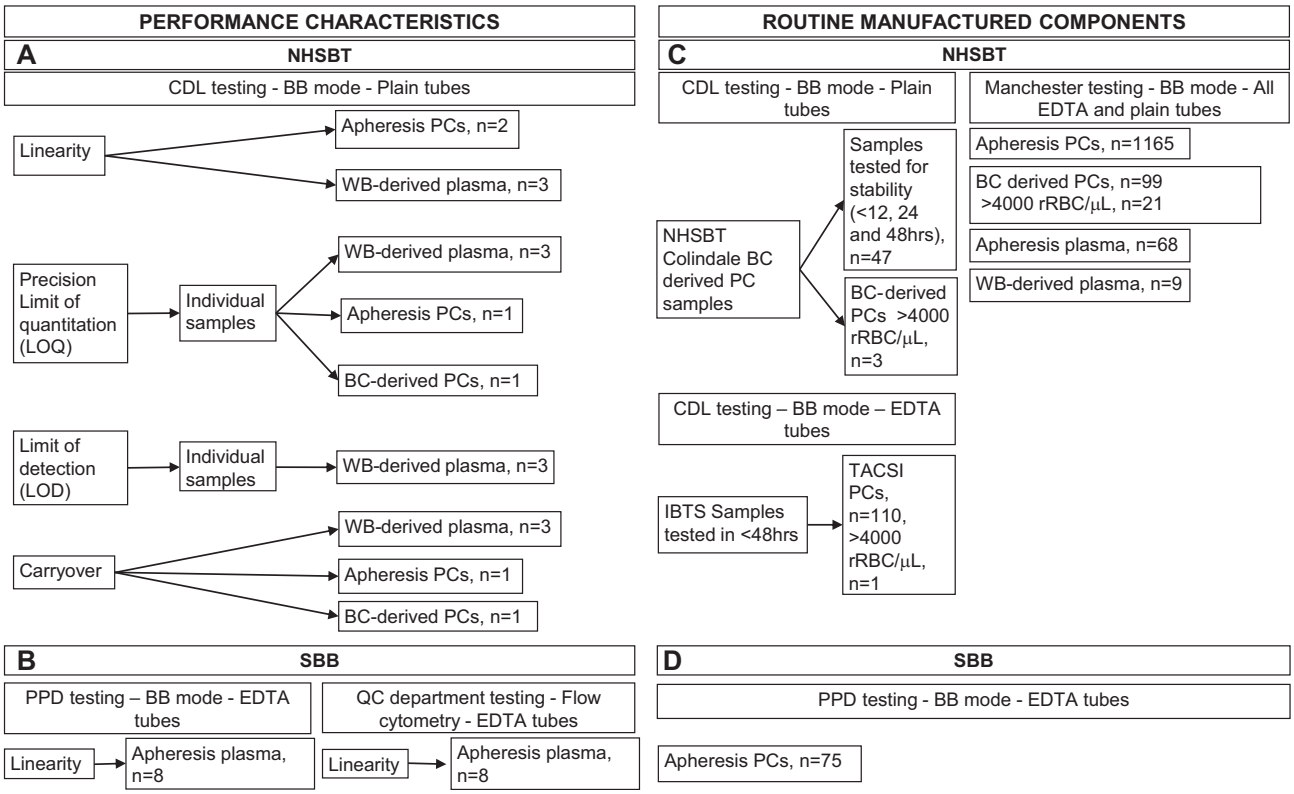

FIGURE 1 Schematic of assessment protocol. Performance characteristic testing performed at (A) NHSBT CDL with use of the BB mode; and (B) Sanquin with use of the BB mode and flow cytometry. Routine manufactured components tested at (C) NHSBT CDL and Manchester with the BB mode; and (D) Sanquin with the BB mode. n = numbers of samples collected and tested

2.1 | Blood components

Components from NHSBT were manufactured according to UK guidelines.[3] Both plasma and pooled buffy coat (BC)-derived platelets suspended in platelet additive solution (PAS) were obtained from manual processing of whole blood (WB). Apheresis machines (Trima Accel, Terumo BCT, Surrey, UK) were used to generate both leukoreduced PCs suspended in plasma and plasma alone.

Components from SBB were manufactured according to Council of Europe guidelines.[4] Apheresis machines (Trima Accel, Terumo BCT; and MCS+, Haemonetics Corp., Braintree, Massachusetts), were used to manufacture leukoreduced PCs suspended in PAS and plasma alone.

The Irish Blood Transfusion Service (IBTS) produced pooled BC-derived PCs in PAS, according to Council of Europe guidelines,[4] with use of the Terumo Automated Centrifuge and Separator Integration (TACSI) system, as previously described.[18]

2.2 | Hematology analyzers

Cell counts on ethylenediaminetetraacetic acid (EDTA) WB samples and BCs used for spiking experiments were obtained with an XN-1000 analyzer (XN-20 model, software version 22.11, Sysmex UK) based at the Component Development Laboratory (CDL) at NHSBT Cambridge, and on a XT2000i analyzer (Sysmex Netherlands) in the Department of Product and Process Development (PPD) at SBB, Amsterdam.

Testing of spiked samples to determine the performance characteristics of rRBC BB mode analysis, such as linearity, precision, and carryover, were performed with use of the BB mode on the XN-1000 analyzer at the CDL. Samples were collected into plain tubes without anticoagulant (Greiner Bio-One, Monroe, North Carolina), tested within 10 hours of preparation, and mixed by manual inversion only, a maximum of eight times immediately before testing (Figure 1A). PPD performed linearity studies with samples in EDTA tubes (Becton Dickinson, Utrecht, Netherlands), tested within 5 hours, and mixed first by manual inversion and then automatically on an XN-1000 (XN-10 model, software version 22.13) using the BB mode (Figure 1B).

Validation using routine manufactured components utilised the BB mode on the XN-1000 in CDL, XN-2000 (dual XN-10 format, software version 22.10) within the manufacturing department at NHSBT Manchester (Figure 1C) and the XN-1000 at PPD, SBB (Figure 1D).

Analyzer performance was monitored using the manufacturer's quality control (QC) material.

2.3 | Flow cytometric enumeration of RBCs

Additional rRBC analysis was performed by the SBB QC department with a flow cytometer (FACSCalibur, Becton Dickinson) and the BD Plasma Count kit,[12] which according to the instructions for use is suitable for counting up to 3000 RBCs/μL in plasma (Figure 1B). FACSCalibur performance was monitored with polymethylmethacrylate beads (Calibrite beads, Becton Dickinson).

2.4 | Linearity studies

Two units of apheresis PCs and three WB-derived plasma units were tested at NHSBT CDL (Figure 1A). These components were spiked with ABO and D-compatible WB and subsequently serially diluted to generate calculated concentrations of 6000 to 6 RBCs/μL for platelet samples, and 4000 to 1 RBCs/μL for plasma samples. One dilution series per unit was produced. Inherent rRBCs in components were measured with the BB mode, and the values were taken into account when determining the expected rRBC value in spiked components.

Eight units of apheresis plasma were tested by PPD, SBB (Figure 1B). These components were centrifuged for 30 minutes at 2800 *g* to produce cell-free plasma and spiked with aliquots of BCs and subsequently serially diluted to generate calculated concentrations ranging from 19 000 to 0 RBCs/μL. Two dilution series per unit were tested and the results averaged. Aliquots of samples were also transported at ambient temperature to the QC department for testing by flow cytometry, coordinated to occur at the same time.

2.5 | Precision, limit of quantitation, and carryover

At CDL, samples were prepared from WB-derived plasma (n = 3) at 50, 25, 13, and 6 RBCs/μL, from BC-derived PCs in PAS (n = 1) with a calculated concentration of 20 RBCs/μL, and apheresis PCs in plasma (n = 1) at 50, 25, 15, and 7 RBCs/μL. Ten aliquots were then made from each sample and every aliquot tested once. Results were used to determine within-run precision, with the

mean concentration and coefficient of variation (CV) being calculated. Results from the three WB-derived plasma and apheresis PC samples were also used to define the LOQ (Figure 1A), regarded as the lowest concentration at which the CV was 20% or less.[16,19]

Carryover, using three WB-derived plasma and two platelet samples (one BC derived and one apheresis), was assessed according to the appropriate guidelines (Figure 1A).[20] Briefly, a high sample of 4000 RBCs/μL (H) was tested three times followed by aliquots of the corresponding nonspiked components (L) and the percentage carryover calculated with the formula (L1-L3) / (H3-L3) × 100.

2.6 | Limit of detection

Limit of detection (LOD) was assessed by testing three WB-derived plasma samples (Figure 1A) spiked to give calculated RBC concentrations of 25, 13, and 6 RBCs/μL and tested to give 30 values at each cell concentration. The mean and 95% confidence intervals (CIs) were calculated

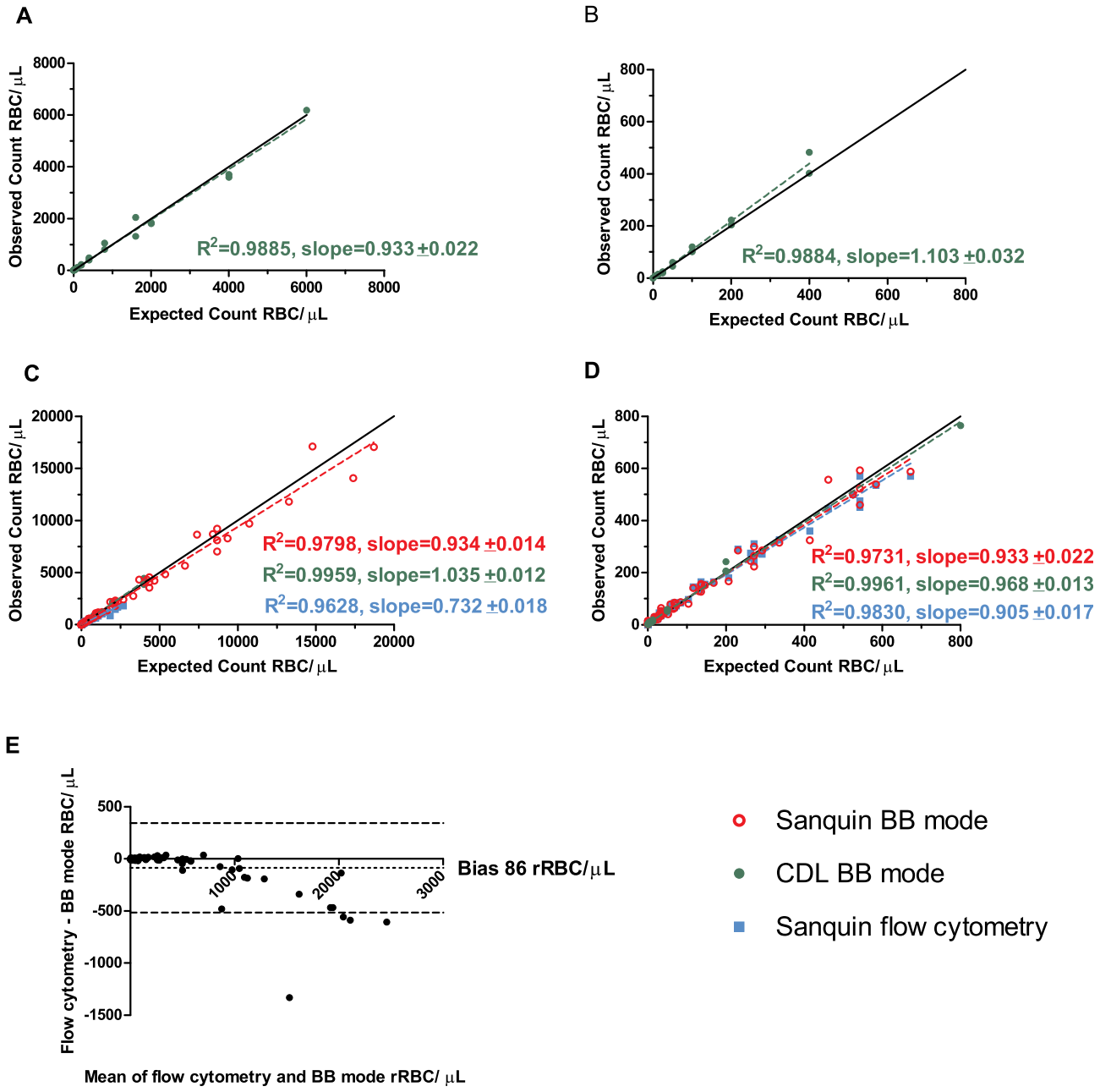

FIGURE 2 Linearity studies. Linear regression of observed vs expected rRBC results. (A) Platelets, by the Sysmex XN-1000 BB mode; and (C) plasma, by the Sysmex XN-1000 BB mode and flow cytometry. Graphs were expanded to show greater clarity for lower cell numbers for (B) platelets, and (D) plasma. For all linearity graphs; the black solid line is the line of equivalence. (E) Results from Bland-Altman analysis comparing PPD BB mode and flow cytometry data for plasma

TABLE 1 Precision analysis results of Sysmex BB mode using plasma and PC samples

	Plasma[a]		Platelets[a]			
			Pooled BC		Apheresis	
Expected RBCs/μL	Observed RBCs/μL[b]	CV[c]	Observed RBCs/μL	CV	Observed RBCs/μL	CV
50	54	8	–[d]	–	52	8
20-25	30	10	22	12	24	12
13-15	15	15	–	–	17	16
6-7	8	21	–	–	7	16

[a]For plasma n = 3, while for platelets n = 1 for each method of manufacture. Observed counts were corrected for inherent RBCs in nonspiked samples.
[b]RBCs/μL (average for plasma).
[c]CV (average coefficient of variation for plasma).
[d]Not tested.
Abbreviation: PC, platelet concentrate.

and compared with the corresponding values for nonspiked plasma samples (n = 30). The LOD was defined as the lowest concentration at which the 95% CI results did not overlap with that of the nonspiked samples.[21]

2.7 | Validation with standard components prepared in a routine manufacturing environment

Within NHSBT Manchester, components were sampled into EDTA and plain tubes (Greiner Bio-One) as previously described.[17] Having been processed for routine QM, with appropriate mixing, tubes with sufficient remaining volume were manually inverted twice and placed on the XN-2000 analyzer, for automatic mixing and rRBC enumeration in the BB mode.

Paired EDTA and plain tube samples tested were apheresis PCs (n = 1165), BC PCs (n = 99), apheresis plasma (n = 68), and WB-derived plasma (n = 9). These paired samples enabled potential issues associated with sample type to be identified. In addition, 21 EDTA-only samples from BC PCs that had been identified by visual inspection as production failures due to high rRBC levels were also tested. All testing was completed within 10 hours of component sampling (Figure 1C).

Plain tube BC PC samples (n = 50) that had undergone standard QM at the NHSBT Colindale site, North London, were transported to CDL for testing. Forty-seven of these were used to assess sample stability over time by storing at ambient temperature and testing at approximately less than 12, 24, and 48 hours after component sampling, the remaining three were found to have rRBC levels greater than 4000 rRBCs/μL (Figure 1C).

Samples from IBTS PC components manufactured with the TACSI process were collected into EDTA tubes.

TABLE 2 LOD analysis results for the BB mode with use of samples from plasma (n = 3)

	Observed RBCs in plasma	
Expected RBCs/μL	Mean RBCs/μL	95% CI
0	1.8	1.4-2.1
6	8.4	7.7-9.1
13	14.5	13.7-15.4
25	29.5	28.2-30.8

Abbreviations: CI, confidence interval; LOD, limit of detection.

Having undergone QM, 111 samples, one of which had been identified by visual inspection as a production failure, were transported to CDL at ambient temperature and tested in the BB mode within 48 hours of component sampling (Figure 1C).

Apheresis PCs from Sanquin were routinely sampled into EDTA tubes. Having been processed for QM with appropriate mixing, samples (n = 75) were tested on the XN-1000 using the BB mode within 8 hours of component sampling (Figure 1D). Overall, samples underwent similar mixing sequences to those at NHSBT Manchester.

2.8 | Statistical analysis

Data were analyzed with computer software (PRISM version 5; GraphPad Software Inc, San Diego, California). The BB mode results from plain and EDTA tubes were analyzed with a two-tailed, paired Wilcoxon signed-rank test at 95% CI. The stability of sample BB mode results over time were analyzed with use of a Friedman test with Dunn's Multiple Comparison posttests. The correlation between expected and measured results and flow cytometry and BB mode

results was analyzed with linear regression; *P* values less than 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Linearity

With use of the BB mode for apheresis PCs and WB-derived or apheresis plasma, there was both excellent linear correlation and good agreement between the expected and observed RBC counts with R^2 of 0.9798 or greater and best-fit linear regression slope values of 0.933 or greater for all cases over the full range of concentrations tested at CDL and PPD (Figure 2A,C). As a concentration of 800 RBCs/μL has been postulated as a limit for alloimmunization, the correlation between expected and observed results at 800 RBCs/μL or less was examined and found to be excellent, with R^2 values of 0.9731 or greater and slope values of 0.933 or greater for all cases (Figure 2B,D).

With flow cytometry, there was a reasonable correlation between expected and observed results with $R^2 = 0.9628$, slope = 0.732 over the full range of RBC concentrations tested, 3000 RBCs/μL or less (Figure 2C), which improved when the range was limited to 800 RBC/μL or less, $R^2 = 0.9830$, slope = 0.905 (Figure 2D). Thus,

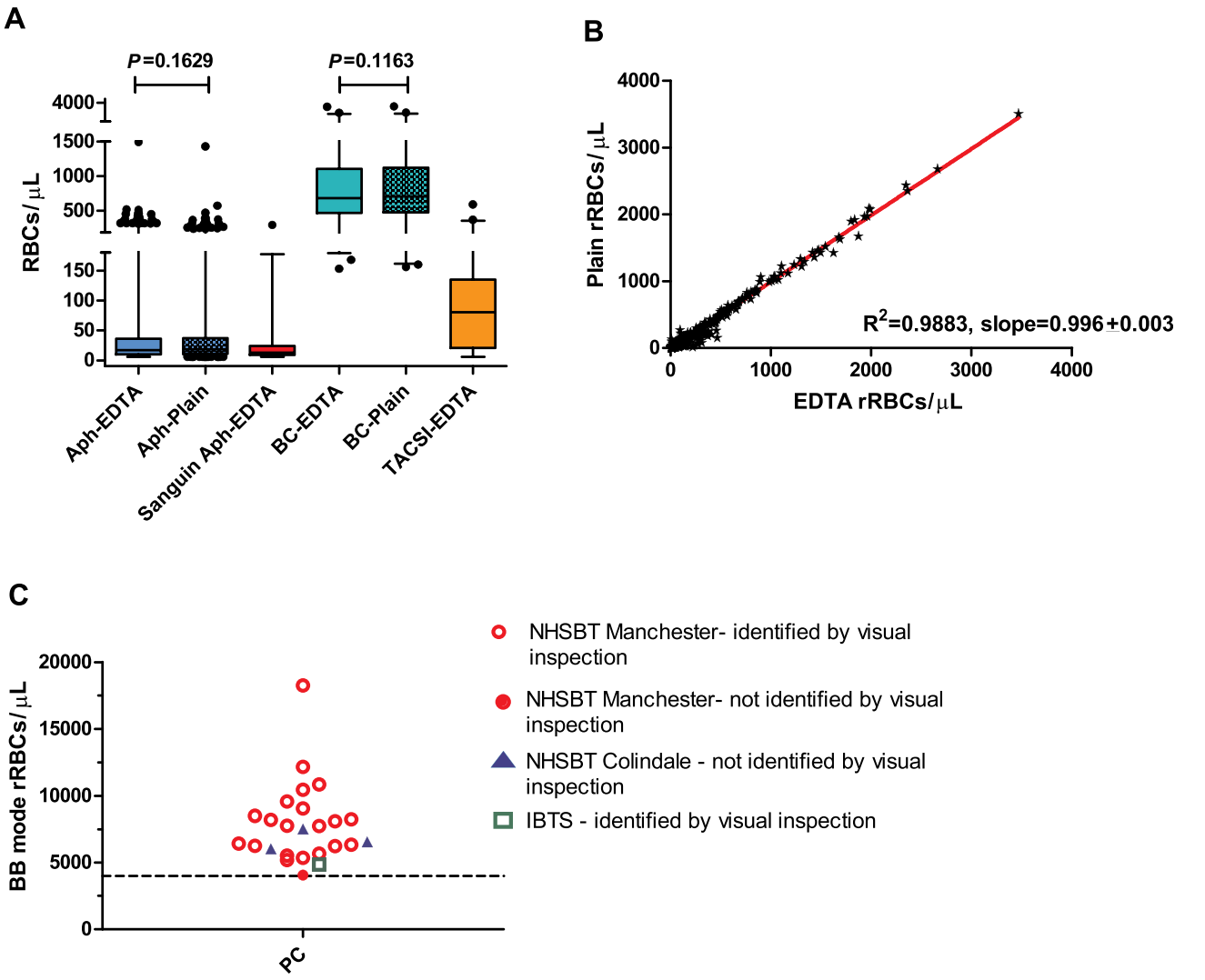

FIGURE 3 PC BB mode results from plain and EDTA tubes, and rRBC failures. (A) The range of BB mode results from EDTA (clear boxes) and plain tubes (spotted boxes), with values ≥6 and < 4000RBC/μL, taken from NHSBT Manchester paired apheresis PCs (blue box; n = 998), Sanquin PPD apheresis PCs (red box; n = 71), NHSBT Manchester paired BC-PCs (green box; n = 99), and IBTS TACSI PCs (orange box; n = 110). Boxes represent the 25 and 75 percentile values with the horizontal bar corresponding to the median value, the whiskers the 2.5 to 97.5 percentile ranges and black full circles the outliers. (B) Regression analysis (red line) of paired EDTA and plain tube data above the LOQ from NHSBT Manchester apheresis and BC PCs (n = 1097). (C) BC derived PCs (n = 25) identified by BB mode as containing ≥4000 RBCs/μL

there was an underestimation in measured flow cytometry results, which increased as RBC concentration increased. The manufacturer's instructions for use for the Plasma Count kit states this phenomenon can be caused by agglutination of RBCs and is observed at concentrations above 3000 RBCs/μL. Our results showed that this occurred at lower RBC concentration than suggested, as when expected results were greater than 300 RBCs/μL there was an average underestimation of approximately 17%.

The flow cytometry and BB mode results correlated well ($R^2 = 0.9400$); however, Bland-Altman analysis up to 3000 RBCs/μL reiterated the pattern identified in the linearity analysis, with flow cytometry showing a bias of underestimation that increased at higher RBC concentrations (Figure 2E).

3.2 | Precision, LOQ, carryover, and LOD

Precision testing showed CVs for apheresis PCs and WB-derived plasma of 8% at 50 RBCs/μL, which increased to approximately 12% with BC and apheresis PCs at 20 to 25 RBCs/μL and a maximum of 21% at 6 to 7 RBCs/μL (Table 1). The average CV% for combined plasma and PCs at this concentration was, however, less than 20%, therefore giving an overall average LOQ of 6 to 7 RBCs/μL (Table 1).

Carryover assessment for PCs and WB-derived plasma showed an average value of 0.03% (range, 0%-0.15%).

Using three WB-derived plasma components, the nonspiked samples gave an average count of 1.8 RBCs/μL. The 95% CI values, however, did not overlap with the values for 6 RBCs/μL, nor did any of the 95% CI values for samples containing concentrations of 13 and 25 RBCs/μL overlap with each other, indicating an LOD of at least 6 RBCs/μL (Table 2).

3.3 | Validation with standard components produced in a routine manufacturing department

Blood components were analyzed to compare the degree of rRBC contamination occurring in components derived from different manufacturing processes, to determine potential variation in results according to the sample tube type, and to assess sample stability over time.

3.3.1 | Variation in rRBC concentration and impact of sample type

In total, 1598 components were tested (Figure 1), of which 223 gave results below the previously identified LOQ of 6 RBCs/μL and were therefore not included in any further analysis.

Acquisition of PC data into EDTA tubes enabled comparison of the rRBC values across all three manufacturing sites. The degree of rRBC contamination in the apheresis PCs was similar between sites (NHSBT median, 17 RBC/μL vs SBB median, 13 RBC/μL) and was less than observed in NHSBT BC PCs (median, 681 RBC/μL). Notably, rRBC contamination in BC PCs produced with use of the TACSI process was reduced to a median of 81 RBCs/μL (Figure 3A).

From the 1325 apheresis and BC PCs that were less than 4000 RBCs/μL, 1097 had paired residual counts from both EDTA and plain tubes, which showed no significant difference between their rRBC counts ($P \geq 0.1163$; Figure 3A).

Although results from both tube types correlated well, it is worth noting that a small number of individual samples below approximately 200 RBCs/μL showed a bias toward higher rRBC counts in EDTA tubes (analysis of best-fit linear regression values obtained from Figure 3B shows that the rRBC count in EDTA tubes was 3 when the plain tube count was 0).

The BB mode identified 25 BC PCs with RBC counts of 4000 RBCs/μL or greater. Interestingly, 4 (16.0%) of these had not been initially identified as failures by visual inspection but had counts between 4056 and 7516 rRBCs/μL (Figure 3C).

Of the 77 plasma units, 25 had paired quantitated EDTA and plain tube samples (apheresis, n = 24; WB, n = 1), these units had rRBC counts above the LOQ and all met

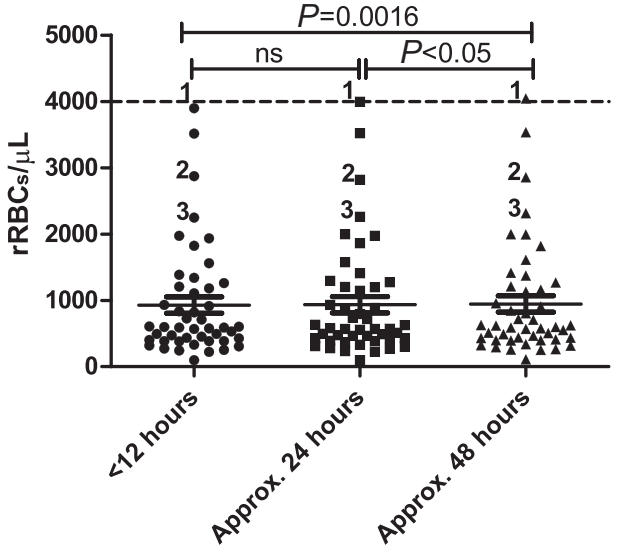

FIGURE 4 BB mode sample stability study. rRBC BB mode results from BC PC component samples stored and tested on multiple occasions over 48 hours (plain; n = 47). For illustration purposes, numbers 1, 2 and 3 track individual samples at each time point. ns = not significant, $P > 0.05$

the NHSBT guidelines of less than 4000 RBCs/μL based on their BB mode results. These plasma units demonstrated similar rRBC counts irrespective of tube type (EDTA: median, 10; range, 6-326; plain: median, 7; range, 6-567), with no significant difference between them ($P = 0.57$).

3.3.2 | Sample stability over time

In 47 BC PCs with less than 4000 RBCs/μL, there was no significant increase in rRBC count between the initial test at less than 12 hours and the next test at 24 hours ($P > 0.05$); however, the counts did increase significantly when measured at 48 hours ($P = 0.0016$; Figure 4). The magnitude of the increase, however, was relatively small with the mean result of 929 RBCs/μL at less than 12 hours, only increasing to 946 RBCs/μL at 48 hours, representing a less than 2% increase over that time.

4 | DISCUSSION

Blood services ensure that the levels of rRBC in non-RBC components are kept to a minimum to maintain quality and reduce transfusion-associated risks. Guidelines exist for acceptable levels of rRBCs, but ensuring compliance requires rRBC enumeration as part of QM process control.

Although manual RBC counting by microscopy is sometimes considered as a "gold standard," its inherent errors and limitations as a reference method, at least in diagnostic work, have been known for many years.[22,23] Flow cytometric methods may be an alternative, but the requirement for specialist equipment and the problem of RBC agglutination[12] mean that the technology may not be applicable to routine component manufacturing environments. Thus, for many organizations, this lack of appropriate technology means that levels of rRBCs are monitored solely by visual inspection, ensuring that the redness of a component is below a subjective limit.

Recent developments in hematology analyzers have highlighted their potential for counting rRBCs in blood components. Early studies, however, suggested that manual sample processing steps could inhibit introduction into routine automated QM processes.[13] A study that used the BF mode on the Sysmex XN-10 analyzer concluded that it was suitable for rRBC counting in plasma at a level of 6000 RBCs/μL, but manual mixing was required and performance with PCs was not assessed.[15]

More recently, the Sysmex BB analysis mode on XN-1000 analyzers has been described as a potential alternative to flow cytometry for rWBC enumeration in blood components.[17] This software also offers the potential for fully automated rRBC enumeration in both plasma and PCs, without any sample preparation. We therefore carried out the first evaluation of this facility for rRBC counting with components spiked with known concentrations of RBCs to assess performance and then measured the levels of rRBCs in components generated via different manufacturing processes.

The assessment of linearity with use of spiked components was performed independently by two blood services, with spiked plasma tested by both NHSBT and SBB and spiked PCs, both in PAS and plasma, tested by NHSBT. Results demonstrated good concordance between the outcomes from the individual site assessments, with overall excellent linear correlation ($R^2 \geq 0.9798$) and agreement between the expected and observed RBC counts.

The BB mode also demonstrated good LOD/LOQ values of 6 to 7 rRBCs/μL or less, which compares favorably with those previously observed for flow cytometry (<40 rRBCs/μL, CV 11.8%),[1] and by the Advia 120 hematology analyzer (CV <15% at <200 rRBCs/μL)[13] and is comparable to the 10 RBCs/μL value previously ascribed to the Sysmex hsA mode for CSF analysis.[16] It is possible that use of components with confirmed absence of RBCs and spiked concentrations less than 7 rRBCs/μL may reveal lower LOD and LOQ values for the BB mode.

Although there is not a recognized reference method for comparison with the BB mode, we used the Plasma Count kit as an additional method for linearity assessment, and the methods generated reproducible and comparable data at RBC levels less than 3000/μL ($R^2 = 0.9400$). The previously described problem of flow cytometry underestimating RBC concentrations as values increase[12] was also observed in our study but was not seen with the BB mode. This emphasizes the suitability of the BB mode as an option to quantitate rRBC levels in plasma and PCs up to and above the levels required for pathogen inactivation (<4000 RBCs/μL)[5] and those mentioned in international guidelines (absence of red color, <6000 RBCs/μL).[3,4]

Our study of manufactured components using the BB mode showed that levels of rRBC in apheresis PC were similar to those previously described[1,9,13] and about 45-fold lower than in manually prepared BC PCs. This reflects other studies that used different technologies, which found that apheresis PCs had between 8- and 21-fold lower levels of rRBCs than BC PCs (Table 3). Our data also showed for the first time that moving from manually processed BC PCs to automated TACSI processing further reduced rRBC levels, going from a median value of 681 to 81 RBCs/μL. Our observation of very low levels of rRBCs in plasma is also similar to other studies (Table 3).

TABLE 3 Previous studies with rRBC data

Study reference	rRBC Method	Component	n=	rRBCs/μL		
				Median	Mean	Range
Santana and Dumont[1]	FC	PC: Aph	193	17.4		
Culibrk et al[13]	HA	PC: BC	166	605		80-15 040
		PC: Aph	58	70		5-1380
Reckhaus et al[9]	Microscopy	PC: BC	120[a]		304	152-1662
		PC: Aph	120[a]		14	3-80
Lambrecht et al[12]	FC	Plasma	2666	623		0-959
Backholer et al[7]	FC	Plasma	119	<100		
Petersson and Ekblom[15]	HA	Plasma	25			All <6000

[a]n = based on 10 per month for a year. Actual number not given in paper.
Abbreviations: Aph, apheresis; BC, buffy coat; FC, flow cytometry; HA, hematology analyzer; PC, platelet concentrate.

BB mode analysis identified all components with rRBCs of 4000/μL or greater that had been detected by visual inspection, and notably detected others that had not initially been observed visually. Overall, data presented here on rRBCs in routine manufactured PCs is the largest published to date, owing to the limitations of using other methods at large scale.

In contrast to our previous observations with rWBC measurements with the BB mode,[17] the sample tube type did not significantly alter the measurements of rRBC in either platelet or plasma components. At levels less than 200 RBCs/μL, however, there was an indication of some anomalous readings. Furthermore, in initial precision studies at less than 50 RBCs/μL, where tubes were repeat sampled up to 10 times, higher CVs were obtained due to counts increasing as the sampling increased. These observations may reflect artefacts induced by excessive mixing or residual particles from rubber caps entering the tube. Overall, further studies may identify and confirm potential preanalytical variables able to affect results, particularly at levels of less than 200 RBCs/μL. Such testing will also enable users to further assess performance characteristics with use of their own reagents and components.

This is the first report on the use of the BB mode software for the enumeration of rRBCs in both artificially spiked and manufactured blood components. This two-site assessment that used multiple component types demonstrated that the BB mode on the XN-Series analyzer can provide acceptable performance characteristics and is able to identify components that should be discarded due to their high levels of RBC contamination. In addition, its ability to measure RBCs at levels far below current guidelines mean that it is also suitable for monitoring component production processes designed to reduce rRBCs as far as possible, perhaps even to a level that may reduce the risk of causing RBC alloimmunization.

In summary, residual cell analyses for many blood manufacturers is a mandatory QM test for components. The BB software appears to have the potential to provide a single-platform solution, with one sample for the simultaneous assessment of both residual WBCs and RBCs, at the same time as measuring routine QM parameters such as platelet count. Results from this initial work support continued studies into the role of hematology analyzers to further evaluate and validate their potential in optimizing both component manufacturing and subsequent QM testing processes.

ACKNOWLEDGMENTS

We thank the various donor clinics and manufacturing facilities for the supply of blood samples and components. We acknowledge lab staff at the CDL, Sanquin, and IBTS and the manufacturing teams for their support during this study.

CONFLICT OF INTEREST

The validation of the Blood Bank mode software described within the document was partly funded by Sysmex, the manufacturers of the software and the hematology analyzers used in this study. A. Shirakami, J. Saker, and J. Linssen are employees of Sysmex. All the other authors declare no conflicts of interest.

ORCID

Richard Alejo Blanco https://orcid.org/0000-0001-5162-7696

REFERENCES

1. 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):966–972.

2. Estcourt LJ, Birchall J, Allard S, et al. Guidelines for the use of platelet transfusions. Br J Haematol. 2017;176(3):365–394.
3. MacLennan S. Guidelines for the blood transfusion services in the United Kingdom. 8th ed. London: TSO, 2013.
4. European Directorate for the Quality of Medicines and Healthcare (EDQM). Guide to the preparation, use and quality assurance of blood components. 20th ed. Strasbourg: EDQM of the Council of Europe; 2020.
5. Picker SM, Speer R, Gathof BS. Evaluation of processing characteristics of photochemically treated pooled platelets: Target requirements for the INTERCEPT blood system comply with routine use after process optimization. Transfus Med Oxf Engl. 2004;14(3):217–223.
6. Hendrickson JE, Tormey CA. Understanding red blood cell alloimmunization triggers. Hematol Am Soc Hematol Educ Program. 2016;2016(1):446–451.
7. Backholer L, Green L, Huish S, et al. A paired comparison of thawed and liquid plasma. Transfusion. 2017;57(4):881–889.
8. Canadian Blood Services. Canadian Blood Services Visual Assessment Guide [Internet]. 2009. Available from: https://professionsleducation.blood.ca/en/transfusion/best-practices/visual-assessment-guide
9. Reckhaus J, Jutzi M, Fontana S, et al. Platelet transfusion induces Alloimmunization to D and non-D rhesus antigens. Transfus Med Hemotherapy Off Organ Dtsch Ges Transfusionsmedizin Immunhamatologie. 2018;45(3):167–172.
10. Pichler J, Printz D, Scharner D, Trbojevic D, Siekmann J, Fritsch G. Improved flow cytometric method to enumerate residual cells: Minimal linear detection limits for platelets, erythrocytes, and leukocytes. Cytometry. 2002;50(4):231–237.
11. Schmidt M, Spengler H-P, Lambrecht B, Hourfar MK, Seifried E, Tonn T. A new one-platform flow cytometric method for residual cell counting in platelet concentrates. Transfusion. 2009;49(12):2604–2611.
12. Lambrecht B, Spengler H-P, Nauwelaers F, Bauerfeind U, Mohr H, Müller TH. 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):1195–1204.
13. Culibrk B, Stone E, Levin E, Weiss S, Serrano K, Devine DV. Application of the ADVIA cerebrospinal fluid assay to count residual red blood cells in blood components. Vox Sang. 2012;103(3):186–193.
14. Fleming C, Brouwer R, Lindemans J, et al. Validation of the body fluid module on the new Sysmex XN-1000 for counting blood cells in cerebrospinal fluid and other body fluids. Clin Chem Lab Med. 2012;50(10):1791–1798.
15. Petersson A, Ekblom K. Evaluation of a routine hematology analyzer for quality control of leukoreduced plasma. Transfusion. 2019;59(10):3214–3218.
16. Fleming C, Russcher H, Brouwer R, Lindemans J, de Jonge R. Evaluation of Sysmex XN-1000 high-sensitive analysis (hsA) research mode for counting and differentiating cells in cerebrospinal fluid. Am J Clin Pathol. 2016;145(3):299–307.
17. 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):155–164.
18. Sandgren P, Hild M, Sjödin A, Gulliksson H. Storage of Buffy-coat-derived platelets in additive solutions: in vitro effects on platelets prepared by the novel TACSI system and stored in plastic containers with different gas permeability: Platelets prepared from BC, processed on the novel TACSI system. Vox Sang. 2010;99(4):341–347.
19. Tholen D. Protocols for determination of limits of detection and limits of quantitation. Approved Guidelines (Internet). 2004; https://ci.nii.ac.jp/naid/10030014457/en
20. 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):613–627.
21. Dzik WH. Leukocyte counting during process control of leukoreduced blood components. Vox Sang. 2000;78(suppl 2):223–226.
22. Biggs R, Macmillan RL. The error of the red cell count. J Clin Pathol. 1948;1(5):288–291.
23. Reference method for the enumeration of erythrocytes and leucocytes. International Council for Standardization in Haematology; prepared by the expert panel on cytometry. Clin Lab Haematol. 1994;16(2):131–138.

How to cite this article: Cavagnetto C, Alejo Blanco R, McKenna H, et al. Residual red cells in blood components: A multisite study of fully automated enumeration using a hematology analyzer. *Transfusion*. 2021;61:568–578. https://doi.org/10.1111/trf.16196