Enumeration of Residual Leucocytes in Leucoreduced Blood Products on the New, Simple-to-Use BD FACSVia™ System: a Multicenter Study

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Summary

This application note discusses method comparison and inter-laboratory reproducibility studies performed at multiple centers for the new BD FACSVia™ System using the BD Leucocount™ assay. Our studies demonstrated that the BD FACSVia System has a simplified workflow and generated results for rWBC absolute counts equivalent to those for the BD FACSCalibur™ instrument. This new system provides an easy, reliable and rapid method for enumerating low levels of leucocytes in leucoreduced blood products.

BD FACSVia System

The BD FACSVia System is a small, easy-to-use flow cytometer equipped with a blue and a red laser, two light scatter detectors, and four fluorescence detectors. It features compact optical design, fixed alignment, and pre-optimized detector settings. Users do not have to adjust PMT voltages on the BD FACSVia System. BD FACSVia™ clinical software has an intuitive user interface (UI) to allow users to quickly compile information and generate data from the flow cytometer. Daily one-step instrument QC through the BD™ Cytometer Setup and Tracking (CS&T) procedure provides quality control for electronics, fluidics and optical performance as well as automatic optimization of instrument compensation. BD FACSVia clinical software tracks instrument QC results. The BD FACSVia System improves workflow efficiencies with automated processes and simplifies user experience for sample acquisition and analysis. A 24-tube sample loader is provided as a system option for automatic sample loading to enable high-throughput acquisition. The BD FACSVia System enables clinical laboratories to enumerate leucocytes in leucoreduced blood products using the BD Leucocount™ kit.

Clinical rationale and application

In past decades, performing leucoreduction for blood transfusion products has become standard practice in the developed world to improve clinical outcomes, and has also been gradually adopted globally by other countries in emerging markets.[1-3] Depleting leucocytes below a specific level for blood components helps to prevent or reduce short and long term adverse effects in transfusion, such as febrile reaction and HLA alloimmunization.[4, 5] Removal of greater than 99.9% of leucocytes (or a 3-log leucoreduction) in the red cell units and platelets can also significantly minimize potential transmission of viruses such as CMV and prevent transfusion-related bacterial sepsis.[6-9] European countries, the US and Canada have published guidelines to enforce universal leucoreduction in blood products aimed at improving the quality of blood components for transfusion. This requires establishment of reliable quality control processes, standards for leucoreduced blood products, as well as rapid and robust cell counting methods to assess residual leucocytes.

Flow cytometry has been the method of choice for centralized blood banks and clinical laboratories to enumerate leucocytes in peripheral blood samples because of its sensitivity, high sample throughput and robust inter-laboratory reproducibility. The flow cytometric method using the BD Leucocount assay on the BD FACSCalibur™ system provides good precision in low level leucocyte counting.[10] This approach uses BD Trucount™ tubes in conjunction with BD Leucocount reagent in one sample preparation step, resulting in lower pipetting variability. On the new BD FACSVia System, the BD Leucocount assay allows for the enumeration of low concentrations of leucocytes in leucoreduced blood products with a simplified, easy-to-use workflow.

Objective

The objective of this application note is to demonstrate performance equivalency between the BD FACSVia System and the BD FACSCalibur instrument using the BD Leucocount assay. Results of a multicenter method comparison study are presented to show low level leucocyte enumeration in leucoreduced platelets (PLTs) and red blood cells (RBCs) on the BD FACSVia System vs the predicate BD FACSCalibur instrument. An inter-laboratory reproducibility study is presented to show precision in residual white blood cell (rWBC) absolute counts on four BD FACSVia Systems across sites.

Methods

Study design

Method comparison

The institutional review board or ethics committee at four study centers reviewed and approved the study protocol before proceeding to site initiation of investigational activity. The leucoreduced PLT and RBC specimens (anticoagulants: CPD, CP2D, ACDA) were de-identified, enrolled and prepared using the BD Leucocount kit. Specimens then were measured in duplicate tubes on the BD FACSVia and BD FACSCalibur systems, respectively, at four study centers to determine rWBC absolute counts on each system. The study centers were Holland Lab, American Red Cross (ARC) located in Rockville, MD; New York Blood Center (NBC) in Long Island City, NY; BloodCenter of Wisconsin (BCW) in Milwaukee, WI; and MedLab, BD Life Sciences (MED) in San Jose, CA.

Inter-laboratory reproducibility

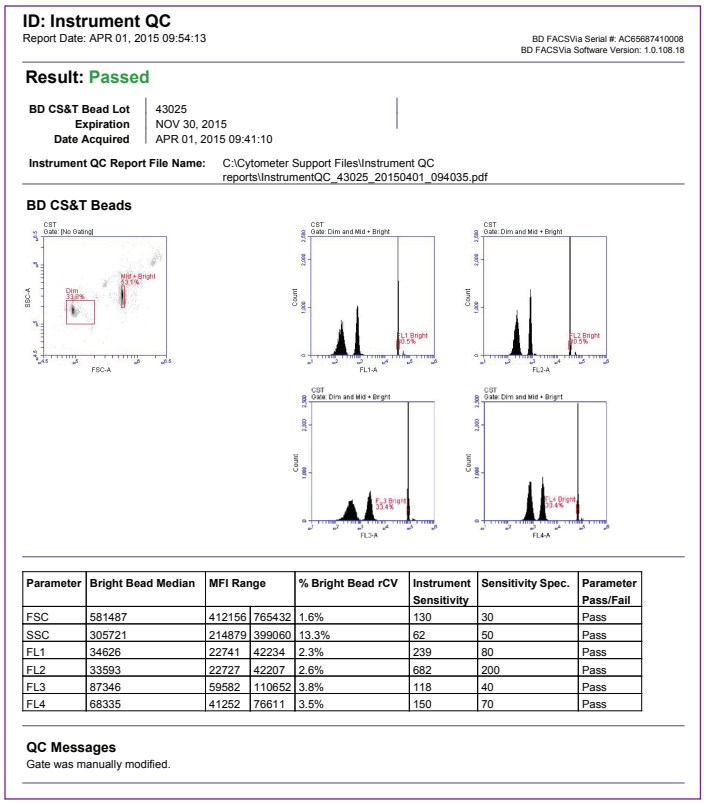
To evaluate the reproducibility of the rWBC absolute count on the BD FACSVia instrument, BD Leucocount control cells with high and low rWBC concentrations were prepared in BD Trucount tubes with the BD Leucocount kit. Samples were run once per day on four BD FACSVia Systems at three study centers (ARC, BCW and MED) for 20 operational days. At least two operators per site prepared samples and performed the reproducibility study.

Daily instrument setup and QC

The daily instrument setup and QC consists of a simple single step on the BD FACSVia System using BD™ CS&T beads and BD FACSVia clinical software. The process is performed in less than five minutes. BD CS&T beads are fluorochrome dyed polystyrene beads consisting of equal quantities of 3-µm bright, 3-µm mid and 2-µm dim fluorescent microspheres. The CS&T beads were prepared by adding 2 drops of beads into 500 µL of deionized water. They were run on the BD FACSVia instrument to generate an Instrument QC report in less than five minutes, as shown in Figure 1.

During instrument QC, BD FACSVia clinical software verified that the CS&T bright beads were placed within the target fluorescence channels on the instrument. Fluorescence compensation was optimized based on the daily bright bead MFI values. Bright bead robust coefficients of variation (rCVs) were also measured during setup for each fluorescence channel and compared with preset QC criteria. Instrument sensitivity was calculated automatically during the CS&T setup process and subject to predefined specifications. To achieve quality control of the optics, electronics and fluidics of the system, a "Pass" on the Instrument QC report had to be obtained on the study day before sample acquisition on the BD FACSVia System. User adjustment and optimization of PMT settings are not part of the instrument setup because the BD FACSVia flow cytometer uses fixed detector voltages with auto-optimized fluorescence compensation. BD FACSVia clinical software automatically tracks daily instrument QC results.

In this study, the BD FACSCalibur system was set up according to the *BD FACSComp Software Reference Manual* and the manufacturer's instructions included with the BD Leucocount kit using BD FACSComp™ software. BD FACSComp software generated PMT and compensation values specific for the BD Leucocount assay based on the measurement of BD Calibrite™ beads.



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Report Date: APR 01, 2015 09:54:13
BD FACSVia Serial #: AC65687410008
BD FACSVia Software Version: 1.0.108.18

Result: Passed

BD CS&T Bead Lot 43025
Expiration NOV 30, 2015
Date Acquired APR 01, 2015 09:41:10

Instrument QC Report File Name: C:\Cytometer Support Files\Instrument QC reports\InstrumentQC_43025_20150401_094035.pdf

BD CS&T Beads

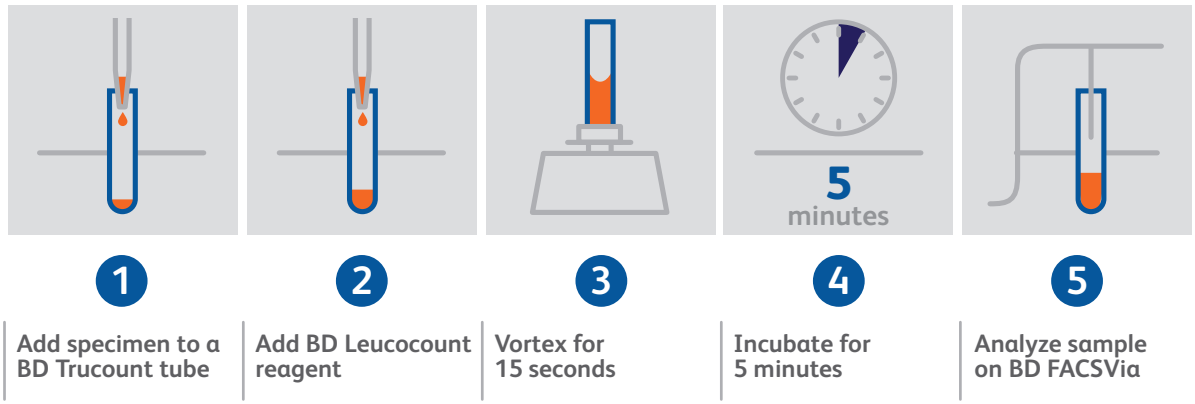
Parameter	Bright Bead Median	MFI Range		% Bright Bead rCV	Instrument Sensitivity	Sensitivity Spec.	Parameter Pass/Fail
FSC	581487	412156	765432	1.6%	130	30	Pass
SSC	305721	214879	399060	13.3%	62	50	Pass
FL1	34626	22741	42234	2.3%	239	80	Pass
FL2	33593	22727	42207	2.6%	682	200	Pass
FL3	87346	59582	110652	3.8%	118	40	Pass
FL4	68335	41252	76611	3.5%	150	70	Pass

QC Messages
Gate was manually modified.

Figure 1. Instrument QC report on the BD FACSVia System using BD CS&T beads

Sample staining and preparation

In the method comparison study, four samples per donor were stained and prepared using instructions included with the BD Leucocount kit. Four hundred microliters of BD Leucocount reagent was mixed with 100 µL of leucoreduced PLTs or RBCs in a BD Trucount tube. Fluorescence-labeled samples were vortexed and incubated in the dark for five minutes. If prepared samples were not analyzed on cytometers immediately, they were kept at room temperature protected from light. Samples subsequently were analyzed on cytometers within 24 hours if PLTs and RBCs were prepared within 24 hours of leucoreduction, or analyzed within 60 minutes if they were stained within 48 hours of leucoreduction. In the inter-laboratory reproducibility evaluation, PLT and RBC control cells containing two concentration levels of rWBCs were fluorescence labeled with BD Leucocount reagent in duplicated BD Trucount tubes per level. The stained and prepared BD Leucocount control samples were analyzed on the BD FACSVia flow cytometer within 60 minutes after preparation.



Worklist setup

The workspace of BD FACSVia clinical software provides functionalities for sample acquisition, analysis and information management. It can be easily set up, saved and reopened before or after sample acquisition for future analysis or further acquisition as needed. The workspace file is also portable.

Creating a worklist for process controls and samples can be easily completed inside the workspace of BD FACSVia clinical software. An example of the BD FACSVia worklist setup is shown in Figure 2, in which the Leucocount test definition was selected as the clinical assay application for our study.

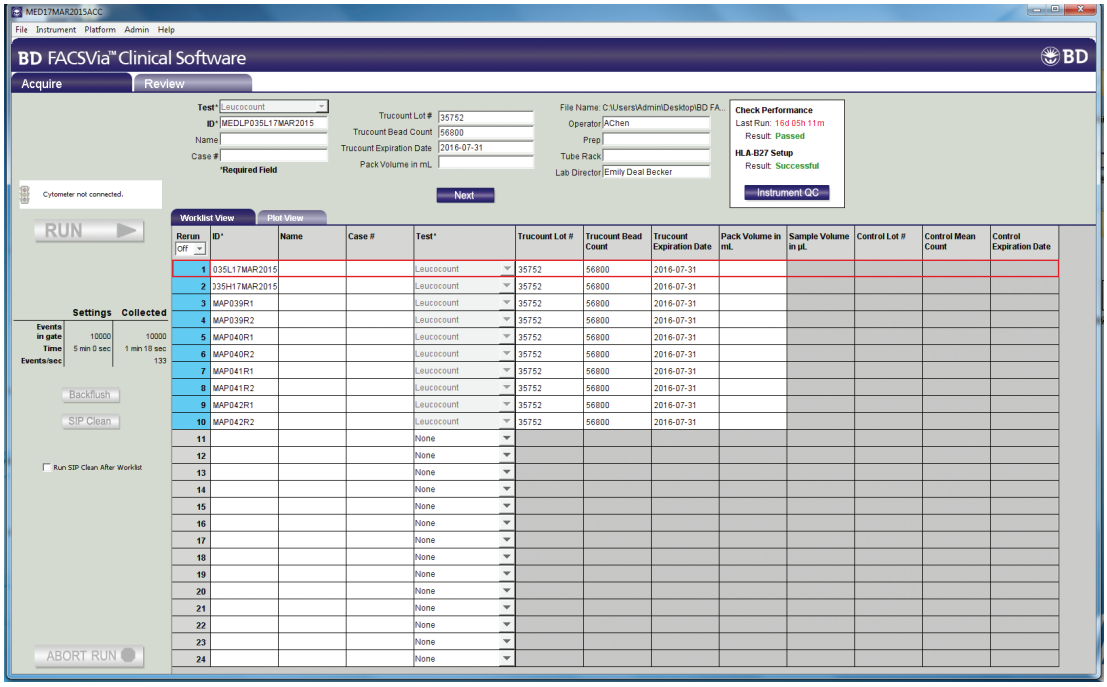


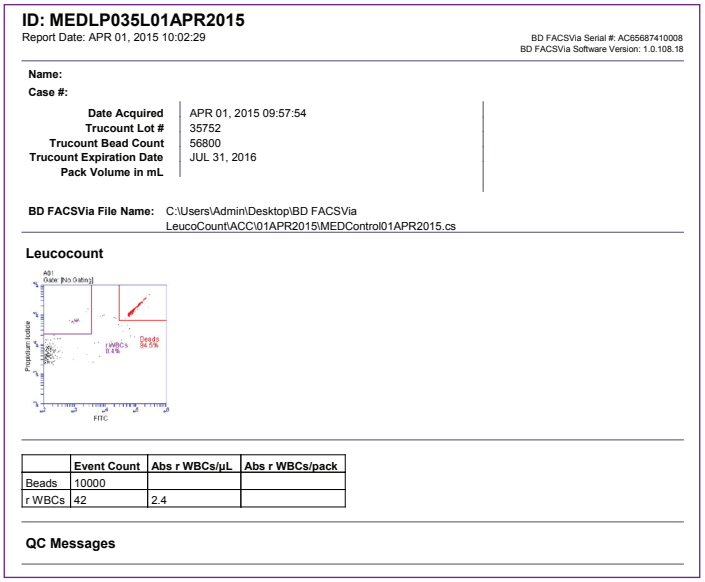
Figure 2. Example of worklist setup on the BD FACSVia System

Enumeration of residual white blood cells

The BD FACSVia System acquires samples based on the worklist setup in BD FACSVia clinical software. The BD Leucocount test definition contains optimized instrument settings for BD FACSVia clinical software, with which labeled cells and beads can be readily distinguished. The threshold of the BD Leucocount assay was set at 2,500 for FL2-H. For each sample, a fixed number of 10,000 bead events was counted (<3 minutes of acquisition time) as stopping criteria. The BD Leucocount test definition provides a locked BD Leucocount template with gates in place to view labeled cells and beads during acquisition and analysis. A FITC (FL1) vs propidium iodide (FL2) plot was used in the template, where the BD Trucount beads region was labeled for beads percentage and the rWBCs region was labeled for the rWBC% in total events, as shown in Figure 3. Gating regions can be adjusted manually when necessary for optimal identification of rWBCs and BD Trucount beads. Absolute counts of rWBCs were determined automatically by BD FACSVia clinical software, and a report was generated after optimal gating was determined for sample analysis (Figure 3). In our study, the BD FACSVia™ Loader was used at each of the four study sites for high-throughput operation.

Sample acquisition and analysis on the predicate BD FACSCalibur instruments was performed using the BD CellQuest™ software template with an FL1 vs FL2 dot plot. A threshold of 300 was set on the FL2 fluorescence channel. A threshold of 332 was set on the FL3 fluorescence channel. Regions were set around the population of beads (R1) and the population of rWBCs (R2), and they were adjusted manually as needed. For each sample, a fixed number of 10,000 bead events were counted (<3 minutes of acquisition time) as stopping criteria. The number of rWBCs per microliter was calculated with the following equation using the expression editor:

$$\frac{\text{n events in R2} * \text{n beads in a Trucount tube}}{\text{n events in R1} * \text{volume of blood component added}}$$



ID: MEDLP035L01APR2015
Report Date: APR 01, 2015 10:02:29

BD FACSVia Serial #: AC65687410008
BD FACSVia Software Version: 1.0.108.18

Name:
Case #:

Date Acquired	APR 01, 2015 09:57:54
Trucount Lot #	35752
Trucount Bead Count	56800
Trucount Expiration Date	JUL 31, 2016
Pack Volume in mL	

BD FACSVia File Name: C:\Users\Admin\Desktop\BD FACSVia LeucoCount\ACC\01APR2015\MEDControl01APR2015.cs

Leucocount

	Event Count	Abs r WBCs/µL	Abs r WBCs/pack
Beads	10000		
r WBCs	42	2.4	

QC Messages

Figure 3. BD Leucocount assay report on the BD FACSVia System

Results

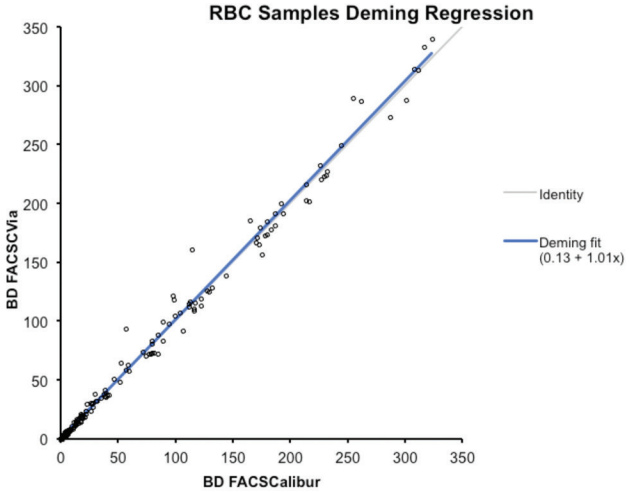
Method comparison

Two hundred fifty-two (252) PLT and 278 RBC evaluable specimens were analyzed for the absolute count of rWBCs measured on the BD FACSVia and BD FACSCalibur systems. Concentration levels of rWBCs in these specimens spanned four rWBC bins, as shown in Table 1. Statistical analysis was performed separately for each specimen type (PLT and RBC). Deming regression analysis recommended by the CLSI Guideline EP09-A3 was applied to the rWBC absolute count, with results from the BD FACSVia System compared to the BD FACSCalibur system. Deming regression slope, intercept, and R-squared were calculated for PLTs and RBCs and are shown next to the Deming regression plots in Figure 4.

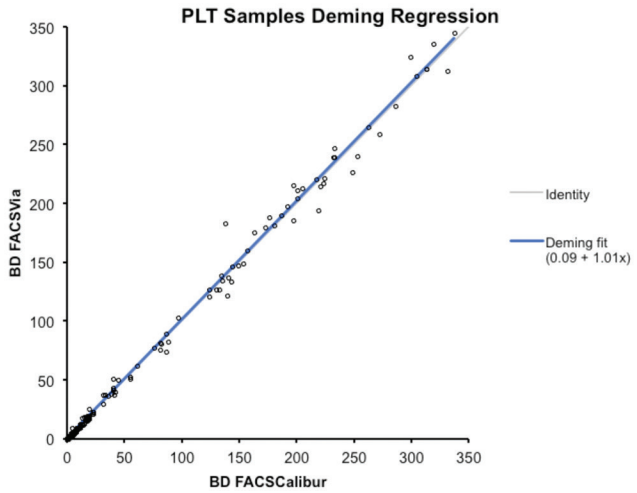
Table 1: rWBC concentration level in PLTs and RBCs

Bin (cells/µL)	PLT (n)	RBC (n)
0 ≤ rWBC < 1	56	59
1 ≤ rWBC < 5	61	72
5 ≤ rWBC <25	67	62
25 ≤ rWBC <350	68	85

Figure 4. Regression plots



Absolute counts of rWBCs in RBCs
Statistics summary
Slope with 95% CI: 1.01 (0.99, 1.03)
Intercept with 95% CI: 0.13 (-0.36, 0.61)
N: 278
R^2: 0.99



Absolute counts of rWBCs in PLTs
Statistics summary
Slope with 95% CI: 1.01 (0.99, 1.03)
Intercept with 95% CI: 0.09 (-0.28, 0.45)
N: 252
R^2: 1.00

Inter-laboratory reproducibility

In the inter-laboratory reproducibility study, 40 data points per control level were repeatedly collected on the BD FACSVia System for each sample type (PLT and RBC) at each center over 20 operational days. Total variance was the combination of the following factors: site/instrument, day and within run repeatability variance. The standard deviation (SD) and percent coefficient of variation (%CV) with upper 95% confidence limit were calculated for the total inter-laboratory reproducibility and within-run repeatability for the absolute count of rWBCs, as shown in Tables 2 and 3.

Table 2: Inter-laboratory reproducibility on the BD FACSVia system

Control cells	rWBC Mean (cells/µL)	SD (Upper 95% SD)	%CV (Upper 95% %CV)
PLT High	16.49	1.07 (1.19)	6.46 (7.16)
PLT Low	7.30	0.69 (0.78)	9.49 (10.52)
RBC High	17.10	1.28 (1.44)	7.51 (8.32)
RBC Low	6.76	0.73 (0.81)	10.76 (11.92)

Table 3: Within-run repeatability on the BD FACSVia system

Control cells	rWBC Mean (cells/µL)	SD (Upper 95% SD)	%CV (Upper 95% %CV)
PLT High	16.49	0.81 (0.95)	4.89 (5.63)
PLT Low	7.30	0.60 (0.71)	8.24 (9.50)
RBC High	17.10	0.90 (1.06)	5.24 (6.04)
RBC Low	6.76	0.69 (0.82)	10.25 (11.82)

Discussion

In our method comparison study, we evaluated BD Leucocount assay results on the new BD FACSVia System in comparison with the BD FACSCalibur system at four study centers. The rWBC concentration levels assessed spanned from 0 to 350 cells/µL. Our results demonstrated that BD Leucocount reagent with BD Trucount beads gave equivalent rWBC absolute counts for the PLT and RBC samples run on the BD FACSVia System. The BD FACSVia System closely agrees with the BD FACSCalibur instrument as analyzed using the Deming regression method.

Additional bias analysis was performed to compare the rWBC absolute count within the two low bins (Table 1) on the BD FACSVia vs BD FACSCalibur systems. In the first low bin, less than 1 cell/µL of rWBCs was measured side by side on the BD FACSVia and BD FACSCalibur flow cytometers. The mean absolute bias was 0.07 (95% CI 0.02, 0.12) cells/µL for PLTs (n = 56), and -0.03 (-0.08, 0.02) for RBCs (n = 59). In the next low leucocyte bin, in which residual leucocytes were more than 1 cell/µL, but less than 5 cells/µL, the mean absolute bias was -0.05 cells/µL (-0.21, 0.11), and the relative bias 0.61% (-4.67%, 5.88%) for PLTs (n = 61). In this bin, for RBC samples (n = 72), the mean absolute bias was 0.11 cells/µL (-0.08, 0.31), and the mean relative bias 5.73% (-1.75%, 13.20%). These results demonstrate that for low to extremely low levels of leucocytes, the BD FACSVia System can provide results as accurate as the BD FACSCalibur system for the leucoreduced PLT and RBC samples. To meet standards proposed by the Council of Europe that the residual leucocytes number be $<1 \times 10^6$ per unit in leucoreduced blood components,[11] equating to <3.3 cells/µL, a sensitive leucocyte enumeration method is critical to accurately count low levels of leucocytes. In this regard, the BD FACSVia flow cytometer provides a tool that meets sensitivity requirements for blood centers and clinical laboratories that deliver leucocyte QC services for blood transfusion units on a routine basis.

The BD FACSVia System demonstrated good reproducibility in the evaluation of the BD Leucocount assay across three centers. At least two operators at each site performed the study for 20 operational days. BD Leucocount control cells that contained two concentration levels (high and low) of rWBCs in PLTs and RBCs were used for the study. The same PLT and RBC control cells were measured for the absolute count of rWBCs on four BD FACSVia instruments at three centers. Our inter-laboratory reproducibility study showed that, using BD Leucocount reagent with BD Trucount beads, the BD FACSVia System met study acceptance criteria for leucoreduced blood products across multiple sites.

Moreover, the BD FACSVia System utilizes a simplified workflow to effectively integrate daily instrument QC, Levey-Jennings tracking, sample information management, sample acquisition and analysis, report generation and review, as well as laboratory information system (LIS) data output. This new system allows users to complete the clinical cytometric workflow using significantly fewer clicks with BD FACSVia clinical software. For a sample batch of 24 tubes running on the BD FACSVia loader, the average operational time to complete the overall workflow for the BD Leucocount assay is about one hour. This workflow includes instrument QC, sample acquisition, analysis and report approval. Compared with the BD FACSCalibur system, the overall operational time can be reduced by ~20% for running the BD Leucocount assay in 24 tubes on the BD FACSVia System when performing data analysis simultaneously with sample acquisition.

Conclusion

In this application note, we demonstrated that the performance of the BD FACSVia System is equivalent to the BD FACSCalibur instrument for low level leucocyte enumeration, as well as offering ease-of-use features. We validated inter-laboratory reproducibility on the BD FACSVia System and demonstrated an overall increase in productivity on this new system through a simplified workflow.

The BD FACSVia System generates reliable and consistent results, improves work flow efficiencies, helps to minimize cost of operation and supports lab productivity by providing the following features:

- Automatic instrument startup and shutdown
- Easy one-step instrument setup and QC
- Easy, intuitive, clinical software integrated into a single application for setup, acquisition, and analysis
- Portable, reusable workspace file
- Easy LIS connectivity
- Minimized service needs and instrument maintenance
- Small footprint and light weight

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