



Performance Evaluation of the BD Leucocount™ Assay on the BD FACSLyric™ Flow Cytometer and BD FACSCalibur™ System using RBC and PLT samples

Poster #143

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Abstract

Introduction: Leukoreduction of blood products reduces risks of adverse reactions associated with blood transfusion, such as febrile reactions, cytomegalovirus transmission and HLA alloimmunization. Quality control procedures for residual white blood cells (rWBCs) in leukoreduced blood products are critical to ensure effectiveness and safety of blood transfusion. The BD Leucocount™ Kit measures rWBCs in leukoreduced platelet (PLT) and red blood cell (RBC) products. The BD Leucocount™ Reagent contains propidium iodide (a fluorescent nucleic acid dye) and RNase for the enzymatic digestion of RNA in the specimen. BD Trucount™ Tubes are included in the kit to determine absolute rWBC count using flow cytometry.

Methods: 1) The BD Leucocount™ Kit was evaluated on the BD FACSLyric™ Flow Cytometer in comparison to the BD FACSCalibur™ System using leukoreduced RBC (N=68) or PLT (N=61) samples. 2) The Limit of Quantitation (LOQ) of the BD Leucocount™ Assay was evaluated on the BD FACSLyric™ Flow Cytometer following the Clinical & Laboratory Standards Institute (CLSI) guideline EP17-A2. Target rWBC absolute counts of 1, 2, 3 and 4 cells/μL were used to prepare four PLT sample pools. Twenty replicates of each target low concentration pool were prepared for one lot of the BD Leucocount™ Reagent. Ten of the stained PLT samples were acquired on the BD FACSLyric™ Flow Cytometer (test system) and the other ten were acquired on the BD FACSVia™ System (predicate system). The LOQ was measured three times on three testing days using leukoreduced PLT from three donors.

Results: 1) Comparison of the BD FACSLyric™ vs BD FACSCalibur™ Systems: Deming regression analysis of rWBC absolute counts showed slope of 0.954 (95% CI 0.917, 0.991), intercept of 0.100 (95% CI 0.012, 0.188), R² of 1.00 for PLT; slope of 1.078 (95% CI 1.012, 1.144), intercept of -0.006 (95% CI -0.136, 0.123), R² of 0.997 for RBC. The mean relative bias of rWBC absolute count with 95% Limits of Agreement was 1.94% (LoA -51.53%, 55.41%) for PLT and 7.13% (LoA -40.51%, 54.77%) for RBC. 2) LOQ of the BD Leucocount™ Assay on the BD FACSLyric™ Flow Cytometer was 1.06, 1.03 and 1.02 cells/μL in three repetitive tests.

Conclusion: Comparison of absolute rWBC counts of leukoreduced PLT and RBC samples between the BD FACSLyric™ System and the BD FACSCalibur™ System demonstrated agreeable results. LOQ of the BD Leucocount™ Assay on the BD FACSLyric™ System was measured as 1.06 cells/μL.

Methods

Residual WBC Count Method Comparison Study

- Instruments: BD FACSLyric™ System and BD FACSCalibur™ System
- Reagent: BD Leucocount™ Kit
- Samples: Leukoreduced RBCs and leukoreduced PLTs
- Range of rWBC concentrations: 0-350 cells/μL
- Single replicate per rWBC concentration acquired on BD FACSLyric™ System and on the BD FACSCalibur™ System

Limit of Quantitation (LOQ) Study

- Instruments: BD FACSLyric™ System and BD FACSVia™ System
- Reagent: BD Leucocount™ Kit
- Samples: Leukoreduced PLTs
- Target rWBC Concentrations: 1, 2, 3, and 4 cells/μL
- 20 replicates per target rWBC concentration; 10 replicates each acquired on the BD FACSLyric™ System and the BD FACSVia™ System

Instrument Setup

- BD FACSLyric™ System
 - Performed Performance QC with BD™ CS&T beads
 - Performed BD Leucocount™ Assay/Tube Settings Setup with BD CS&T beads
- BD FACSCalibur™ System
 - Performed Cytometer Setup with BD Calibrite™ 3-color kit with optimization for BD Leucocount™ Assay
- BD FACSVia™ System
 - Performed Performance QC with BD™ CS&T beads

Process Controls

- BD Leucocount™ PLT Control, High and Low
- BD Leucocount™ RBC Control, High and Low

Sample Staining Procedure using BD Leucocount™ Kit

- Add 100 μL of well-mixed control or sample to a BD Trucount™ tube
- Add 400 μL of BD Leucocount™ reagent per tube and gently vortex
- Incubate the tubes for 5min at room temperature in the dark

Acquisition Stopping Criteria

- 10,000 events for BD Trucount™ beads

Results (1): Method Comparison Study

Note: Single-donor platelet (SPLT) and platelet (PLT) are used interchangeably.

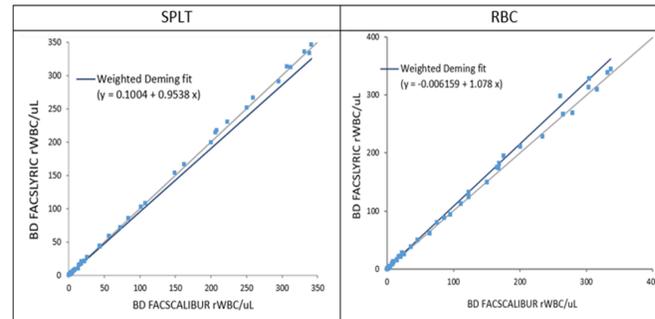


Figure 1. Deming regression plots for single-donor platelet (SPLT or PLT) and RBC (BD FACSLyric™ vs. BD FACSCalibur™)

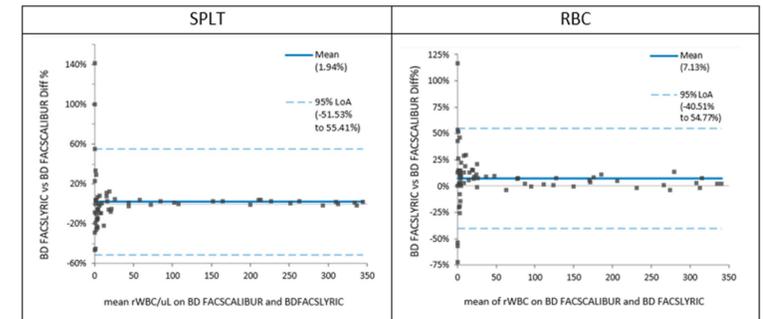


Figure 2. Bland-Altman analysis results for SPLT (or PLT) and RBC (BD FACSLyric™ vs. BD FACSCalibur™)

Table 1. Deming regression results for SPLT (or PLT) and RBC (BD FACSLyric™ vs. BD FACSCalibur™)

Parameter	SPLT (or PLT)			RBC		
	Estimate	95% CI		Estimate	95% CI	
Intercept	0.100	0.012	0.188	-0.006	-0.136	0.123
Slope	0.954	0.917	0.991	1.078	1.012	1.144
R ²	1.000			0.997		

- Deming regression analysis results were 0.954 (slope), 0.100 (intercept), 1.000 (R²) for Single-donor platelet (SPLT or PLT) and 1.078 (slope), -0.006 (intercept), 0.997 (R²) for RBC.
- Bland-Altman analysis was performed to determine the %bias in rWBC absolute counts on the BD FACSLyric™ System vs. the BD FACSCalibur™ system. Results of Bland-Altman analysis showed that mean bias with 95% limits of agreement were 1.94% (-51.53%, 55.41%) for SPLT (or PLT), and 7.13% (-40.51%, 54.77%) for RBC.

Results (2): Limit of Quantitation (LOQ) Study

Criteria of 30% CV for imprecision for both test (BD FACSLyric System) and predicate (BD FACSVia System) and absolute bias of 1 cell/μL between test and predicate systems were used for the evaluation. Absolute total error criteria are defined for each low concentration pool in Table 2. LOQ measurement results for the BD Leucocount assay is shown in Table 3 and 4.

Table 2. Absolute Total Error (TE) Criteria

Target rWBC (cells/μL)	Absolute Total Error criteria
1	1.1
2	1.3
3	1.6
4	2

Table 3. LOQ Results of BD Leucocount Assay on BD FACSLyric System

Day	LOQ (cells/μL)
LOQ Day 1	1.06
LOQ Day 2	1.03
LOQ Day 3	1.02
Final LOQ	1.06

Table 4. Four Levels of rWBCs on BD FACSLyric™ vs. BD FACSVia™ System

Day	Level	BD FACSLyric™ WBC Mean (cells/μL)	Absolute Total Error (TE)	Absolute Total Error Criteria	Results
Day 1	L1	1.06	0.28	1.1	Pass
	L2	2.05	0.32	1.3	Pass
	L3	2.97	0.34	1.6	Pass
	L4	4.04	0.43	2.0	Pass
Day 2	L1	1.03	0.27	1.1	Pass
	L2	1.96	0.34	1.3	Pass
	L3	3.04	0.49	1.6	Pass
	L4	4.09	0.4	2.0	Pass
Day 3	L1	1.02	0.26	1.1	Pass
	L2	2.15	0.35	1.3	Pass
	L3	3.14	0.4	1.6	Pass
	L4	4.12	0.37	2.0	Pass



Conclusions

- Comparison of absolute rWBC counts of leukoreduced PLT and RBC samples between the BD FACSLyric™ System and the BD FACSCalibur™ System demonstrated equivalent results.
- Limit of Quantitation of the BD Leucocount™ Assay on the BD FACSLyric™ System was measured as 1.06 cells/μL.

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