BD Leucocount™ Kit

Catalog No. 662415—50 Tests

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English

℞ Only IVD

1. INTENDED USE

The BD Leucocount™ Kit consists of BD Leucocount™ Reagent (propidium iodide fluorescent dye) and BD Trucount™ Tubes and is intended for use with the BD FACSCalibur™, BD FACSort™, BD FACScan™, BD FACSVia™ and BD FACSLyric™ flow cytometer systems, or for a flow cytometer equipped with a 488-nm laser able to threshold on propidium iodide fluorescence, for enumerating residual white blood cells (rWBCs) in leucoreduced blood products.

For in vitro diagnostic use.

2. SUMMARY AND EXPLANATION

The presence of white blood cells (WBCs) in blood and platelet products is associated with an increased incidence of febrile transfusion reactions, transmission of cytomegalovirus, and alloimmunization to HLA antigens in transfusion recipients.[1,2,3] Leucoreduction, the collection of platelets via apheresis, or post-collection processing with special filters, can lower the WBC count to 5×10^6 per unit or below, thus minimizing complications associated with transfusions.[4,5] The BD Leucocount™ Kit is designed to provide an efficient, sensitive method for enumerating rWBCs using flow cytometry while eliminating limitations associated with other methods.[6,7] The assay incorporates BD Trucount™ Tubes to determine absolute cell counts of rWBCs in a single tube. The FDA has recommended the use of flow cytometry as a counting method for evaluating leucoreduced blood products.[8]

3. PRINCIPLES OF THE PROCEDURE

The BD Leucocount™ Reagent contains propidium iodide (PI). PI is a nucleic acid dye which, when used with RNAse, stains only cellular DNA. White blood cells are nucleated cells that contain DNA and are therefore stained with the dye. Non-nucleated particles (including platelets and red blood cells) do not stain with this reagent. BD Trucount™ Tubes contain beads that act as an internal reference to accurately determine the absolute count of residual white cells. Appropriate samples are combined with the lyophilized bead pellet in the BD Trucount™ Tube before staining. After staining rWBCs, samples are acquired on a flow cytometer. Absolute rWBC counts are determined by using a simple calculation based on bead number and sample volume.

4. REAGENTS

Reagent Composition

The BD Leucocount™ Kit contains:

- BD Leucocount™ Reagent, containing:
  - PI, a nucleic acid dye

  - RNAse, for the enzymatic digestion of RNA in the specimen
  - Detergent, which permeabilizes the cell membrane to allow for entry of PI
  - Buffers, to stabilize the stained sample
  - 0.1% sodium azide
- BD Trucount™ Tubes

  The tubes contain a lyophilized pellet of 4.2-µm fluorescent beads used as an internal reference for calculating absolute counts of rWBCs.

Precautions

- For in vitro diagnostic use.
- The addition of a precise volume of sample is critical. Pipettors must be calibrated to deliver exactly 100 µL of sample. If necessary, perform the reverse pipetting technique according to the manufacturer's instructions.
- Care should be taken to avoid microbial contamination of the reagent, which could give aberrant results.
- Do not use the reagents if you observe any change in appearance. Precipitation or discoloration indicates instability or deterioration.
- Before use, inspect the BD Trucount™ Tube to make sure the pellet is intact and below the retainer.
- Bead count varies by lot of BD Trucount™ Tubes. It is critical to use the bead count shown on the lot of tubes that you are currently using when calculating absolute cell counts. Do not mix multiple lots of tubes in the same worklist.
- BD Trucount™ Tubes are designed for use with a specific lyse/no-wash procedure. Do not threshold on forward scatter (FSC) for data collection.
- Samples of poor quality might interfere with the results.
- Gently vortex samples immediately prior to running them on the flow cytometer to ensure thorough resuspension of cells and beads.
- BD Leucocount™ Reagent contains 0.49% 2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol (CAS number 9002-93-1). It is classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and 29 CFR 1910.1200. Go to regdocs.bd.com/regdocs/sdsSearch to download the Safety Data Sheet.

Hazards	H401: Toxic to aquatic life. H412: Harmful to aquatic life with long lasting effects.
Prevention	P273: Avoid release to the environment.
Disposal	P501: Dispose of contents/container to an approved facility in accordance with local, regional, national and international regulations.

Storage and Handling

- Store BD Leucocount™ Reagent at 2–8 °C. Do not use after the expiration date shown on the label.
- Avoid unnecessary exposure of the reagent to light.
- Do not freeze the reagent or expose it to direct light during storage or incubation with cells.
- Close the vial immediately after dispensing the reagent and return it to storage at 2–8 °C.
- Store BD Trucount™ Tubes in their original foil pouch at 2–25 °C. To avoid potential condensation, open the pouch only after it has reached room temperature and carefully reseal the pouch immediately after removing a tube. An unopened pouch is stable until the expiration date shown on the packaging. Use tubes within 1 hour after removal from the foil pouch. Use remaining tubes within 1 month after opening the pouch. Do not use tubes after the expiration date.

Special disposal instructions

Collect and dispose of all used and unused reagents and any other contaminated disposable materials following procedures for biohazardous or potentially biohazardous waste. It is the responsibility of each laboratory to handle solid and liquid waste according to their nature and degree of hazardousness and to adequately treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations. Do not discharge liquid waste down the drain where prohibited.

5. INSTRUMENTS

The BD Leucocount™ Kit is designed for use on a flow cytometer equipped with appropriate computer hardware and software. The cytometer must have a 488-nm laser capable of detecting forward scatter (FSC) and side scatter (SSC) and at least two-color fluorescence. It must also be able to threshold or discriminate using propidium iodide fluorescence. The BD Leucocount™ Kit can be used on the following BD systems. See the corresponding reagent, cytometer, or software user documentation for details.

Table 1 Recommended BD systems

Flow cytometer	Setup beads	Setup software	Analysis software
BD FACSLyric™	BD® CS&T Beads[a]	BD FACSuite™ Clinical application v1.4 or later	BD FACSuite™ Clinical application v1.4 or later
BD FACSVia™	BD® CS&T Beads[b]	BD FACSVia™ clinical software	BD FACSVia™ clinical software
BD FACSCalibur™	BD Calibrite™ Beads 3-Color Kit BD Calibrite™ Beads 2-Color Kit	BD FACSComp™ software	BD CellQuest™ Pro software

a. To perform daily cytometer quality control.

b. For instrument QC, to check the instrument's measurements and performance.

The assay can be used with the BD FACS™ Universal Loader, the BD FACSVia™ Loader, and the BD FACS™ Loader.

Results can be achieved using flow cytometers manufactured by companies other than BD Biosciences. Set up the cytometer for two-color acquisition following the manufacturer's recommendations.

6. SPECIMEN COLLECTION AND PREPARATION

- Collect red blood cell (RBC) and platelet (PLT) specimens according to manufacturer's instructions. The BD Leucocount™ Kit can be used with single-donor or pooled PLT specimens.
- A minimum of 100 µL of specimen is required for this procedure.
- Prepare and run the samples within 48 hours following leucoreduction.
- Store RBC specimens at 2–8 °C until ready for staining.
- Store PLT specimens at room temperature (20–25 °C) until ready for staining.
- For specimens stained within 24 hours of leucoreduction, acquire the samples within 24 hours of staining.
- For specimens stained within 48 hours of leucoreduction, acquire the samples within 60 minutes of staining.
- The BD Leucocount™ Kit can be used with the following anticoagulants:

  ACD, CPD, CP2D, CPDA, heparin, and 4% sodium citrate

NOTE Labs must validate any deviations from the specimen collection and preparation conditions.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[9,10] and dispose of with proper precautions in accordance with

federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

Interfering Conditions

- Do not use previously fixed and stored samples.
- Samples without additive that are refrigerated before staining could give aberrant results.
- Lipemic specimens can interfere with the assay.[11,12]

The table lists the substances that were tested for interference with the BD Leucocount™ Kit. Testing for interference was performed using leucoreduced RBC and PLT specimens in accordance with CLSI guidelines.[13,14] With the exception of triglycerides in RBC specimens, there was no detectable interference at the following concentrations.

Table 2 Interferents tested

Analyte	Interferent type	Maximum concentration	
		RBC	PLT
Acetaminophen	Exogenous	15.6 mg/dL	15.6 mg/dL
Acetylsalicylic acid	Exogenous	3 mg/dL	3 mg/dL
Albumin	Endogenous	0.6 g/dL	0.6 g/dL
Albuterol	Exogenous	0.0045 mg/dL	0.0045 mg/dL
Bilirubin, conjugated	Endogenous	2 mg/dL	2 mg/dL
Bilirubin, unconjugated	Endogenous	2 mg/dL	2 mg/dL
Guaifenesin	Exogenous	0.45 mg/dL	0.45 mg/dL
Hemoglobin	Endogenous	1,000 mg/dL	1,000 mg/dL
Ibuprofen	Exogenous	21.9 mg/dL	21.9 mg/dL
Oseltamivir	Exogenous	0.0399 mg/dL	0.0399 mg/dL
Triglycerides	Endogenous	Interferes at 500 mg/dL	1,500 mg/dL
SAG-M	Exogenous (RBC additives used to extend shelf life)	3X	–
AS-1 (Adsol)		3X	–
AS-3 (Nutricell)		3X	–
AS-5 (Optisol)		3X	–
PAS-C (PAS-III, Intersol)	Exogenous (PLT additives used to extend shelf life)	–	3X
PAS-E (PAS-IIIM, SSP+)		–	3X
PAS-F (Plasmalyte-A, Isoplate)		–	3X

7. INSTRUCTIONS FOR USE

Reagents and Materials

Reagents and materials provided

- BD Leucocount™ Reagent, sufficient for 50 tests
- BD Trucount™ Tubes

  Two pouches are provided, each containing 25 single-use tubes. Each tube contains a freeze dried pellet of fluorescent beads.

Reagents and materials required but not provided

- Falcon® disposable 12 x 75-mm polypropylene test tubes or equivalent
- Vortex mixer
- Micropipettor with tips
- Process controls:

  BD Leucocount™ RBC Control (Catalog No. 662416 or 662418)

  BD Leucocount™ PLT Control (Catalog No. 662417 or 662418)

NOTE Labs must validate any deviations from the following procedures.

Reverse Pipetting

Accurate pipetting is critical when using a BD Trucount™ Tube. Use the reverse pipetting technique, or a positive displacement pipettor, to pipette specimen onto the side of the tube just above the retainer.

For reverse pipetting, depress the button to the second stop. Insert the pipettor into the specimen and release the button. When you release the button, excess specimen is drawn up into the tip. When dispensing, press the button to the first stop to expel a precise volume of specimen. This leaves excess specimen in the tip.

Performing Quality Control

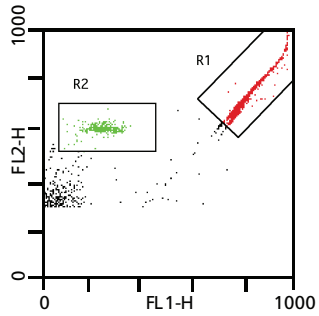
We recommend running process controls each day of use to provide absolute rWBC counts around your laboratory's critical cut-off values. In addition, it is advisable to run controls on every shift. We recommend using BD Leucocount™ RBC Control or BD Leucocount™ PLT Control for this purpose, stained as a leucoreduced specimen. Other process controls must be validated by the lab.

The fluorescence intensity of BD Leucocount™ control cells might differ slightly from that of unpreserved WBCs. See Figure 1, Figure 2, and Figure 6.

If you are using BD CellQuest™ Pro software, and if necessary, create and save an acquisition/analysis template for control samples, with R2 appropriately adjusted. A gating aid sample can be useful in defining the expected fluorescence intensity of WBCs present in blood products. For details, see Creating a gating aid (optional) on page 11.

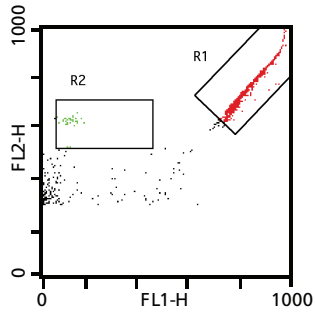
Quality control runs should produce results that are within the values found on the Residual WBC Assay Values and Expected Ranges sheet provided with the controls.

Figure 1 High-level control samples



Region	Events
R1	10,000
R2	320

Figure 2 Low-level control samples



Region	Events
R1	10,000
R2	34

Staining the Specimens

1. Carefully dispense 200–400 µL of well-mixed RBC or platelet specimens into clean 12 x 75-mm polypropylene tubes.

   NOTE Use polypropylene tubes for sample storage, not for counting rWBCs in RBC or platelet specimens.

2. For each specimen, remove a BD Trucount™ Tube.

   We recommend that you stain the process controls, acquire them, and verify that the results are within the values reported in the Residual WBC Assay Values and Expected Ranges sheet, provided with the controls, before you start staining the specimens.

   NOTE Verify that the bead pellet is intact under the metal retainer at the bottom of the BD Trucount™ Tube. If this is not the case, discard the tube and replace it with another. Do not transfer beads to another tube.

3. Reseal the pouch immediately.

4. Label each tube with the appropriate sample identification.

   NOTE Start staining the specimens within 1 hour of removing the BD Trucount™ Tube from the pouch.

5. Add 100 µL of well-mixed specimen (platelet, RBC, or control) to the labeled BD Trucount™ Tube.

   NOTE Pipette the specimen or control onto the side of each tube just above the metal retainer. Do not touch the bead pellet. If the specimen remains on the side of the tube, it will not be stained with the reagent.

6. Add 400 µL of BD Leucocount™ Reagent to each tube.
7. Cap the tubes and gently vortex.

   Do not vortex longer than 15 seconds.

8. Incubate the tubes for 5 minutes in the dark at room temperature.
9. Store the tubes at room temperature in the dark until ready for acquisition.
   - Samples stained within 24 hours of leucoreduction can be acquired up to 24 hours after staining.
   - Samples stained within 48 hours of leucoreduction should be acquired within 60 minutes of staining.

Running the Assay on BD FACSLyric™ Flow Cytometers

Before you begin:

- Verify that the BD Leucocount™ Reagent, BD® CS&T Beads, and BD Trucount™ Tubes have not expired. Add reagent, bead, and tube lots to library, if needed.
- Verify that Characterization QC (CQC) has not expired. Perform CQC, if needed.
- Verify that the reference settings have not expired. Create or update reference settings using BD® FC Beads, if needed.

  NOTE Reference settings are not required to run the BD Leucocount™ Assay. However, in BD FACSuite™ Clinical application v1.4 and v1.5, a QC message is generated. If you want to to avoid generating a QC message, see the *BD Leucocount™ Application Guide for BD FACSLyric™ Flow Cytometers*.

See the *BD FACSLyric™ System Instructions for Use* and the *BD Leucocount™ Application Guide for BD FACSLyric™ Flow Cytometers* for more information.

To run the assay:

1. Perform daily Performance QC (PQC) using BD® CS&T Beads.
2. Perform Assay/Tube Settings Setup for Leucocount Tube Settings.

   We recommend selecting the Run Setup and Generate Reports checkboxes. A QC message will be generated in BD FACSuite™ Clinical application v1.4 and v1.5. See the *BD Leucocount™ Application Guide for BD FACSLyric™ Flow Cytometers*.

3. Create a worklist.

   Create a Leucocount task for each process control and specimen that you are running.

4. Enter the Sample ID, pack volume, and other information.

   See the *BD Leucocount™ Application Guide for BD FACSLyric™ Flow Cytometers*.

5. Run the PLT or RBC control specimens on the worklist.

We recommend selecting the Run Selected option to run the process controls first. See the *BD FACSLyric™ System Instructions for Use*.

6. Vortex each tube thoroughly at low speed immediately before acquiring it.

   Do not vortex longer than 15 seconds. If using the BD FACS™ Universal Loader, vortex tubes immediately before placing them in the Loader racks.

7. Review the lab report and confirm that the values are within the ranges shown on the Residual WBC Assay Values and Expected Ranges sheet, provided with the process controls.
8. Run the specimens on the worklist.

   NOTE If using the BD FACS™ Universal Loader, make sure that all of the tubes in the rack are acquired within the recommended age of stain. If not, you must validate tubes acquired outside the recommended time.

   See the *BD Leucocount™ Application Guide for BD FACSLyric™ Flow Cytometers* for more information.

Running the Assay on BD FACSVia™ Flow Cytometers

1. Perform daily cytometer Quality Control (QC).

   Additional setup is not required for the BD FACSVia™ flow cytometer because test definitions for each assay define default acquisition and gate settings.

   For instructions on how to run a sample on this cytometer, see the *BD FACSVia™ System Instructions For Use* (IFU).

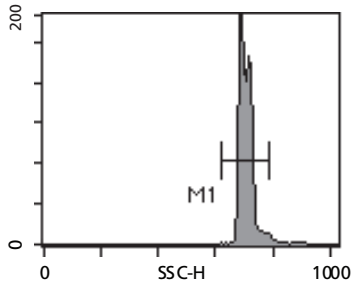
Running the Assay on BD FACSCalibur™ Flow Cytometers

For instructions on performing BD Leucocount™ setup automatically using BD FACSComp™ software (version 4.1 or later), refer to the *BD FACSComp™ Software User's Guide*.

To perform manual setup using BD FACSComp™ software:

1. Prepare an instrument setup tube by adding 500 µL of phosphate buffered saline (PBS) to a labeled BD Trucount™ Tube.
2. Run the instrument setup tube in Setup mode and make the following adjustments:
   - Turn all compensation settings to 0.0%.
   - Set FSC to LINEAR amplification.
   - Set FL1, FL2, and SSC to LOG amplification. Use channel values.
   - Adjust FL2 threshold to approximately 300 to eliminate debris.
   - Under Acquisition and Storage, verify that the instrument resolution is 1,024.
3. Adjust the SSC, FL1, and FL2 photomultiplier tube (PMT) voltages to place the BD Trucount™ beads in the appropriate mean channel values as follows.
   - While viewing the SSC histogram (Figure 3), adjust the SSC PMT voltage to place the beads in channel 700 ± 20.

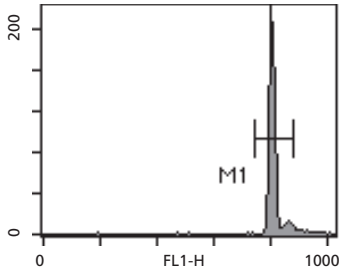
Figure 3 SSC histogram



Marker	Events	Mean
All	8,340	696.54
M1	8,249	695.38

- While viewing the FL1 histogram (Figure 4), adjust the FL1 PMT voltage to place the beads in channel 800 ± 20.

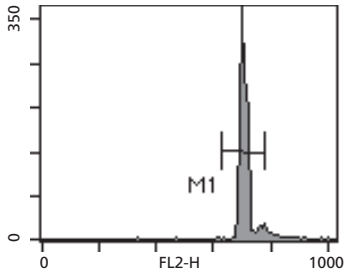
Figure 4 FL1 histogram



Marker	Events	Mean
All	8,340	799.46
M1	8,203	797.95

- While viewing the FL2 histogram (Figure 5), adjust the FL2 PMT voltage so the beads are in channel 700 ± 20.

Figure 5 FL2 histogram



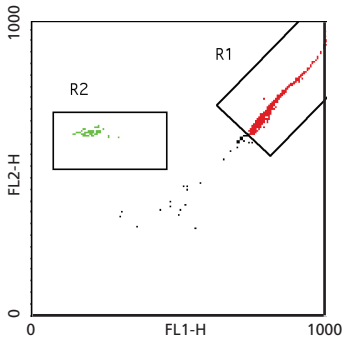
Marker	Events	Mean
All	8,340	701.04
M1	8,121	698.72

4. Save instrument settings.

To acquire the samples using BD CellQuest™ Pro software:

1. Create an FL1 vs FL2 dot plot.
2. Begin acquisition of the prepared sample. If using the BD FACS™ Loader, a 10-second start-of-rack mix and a 3-second interim mix after every tube is recommended. Vortex the tubes immediately before placing them into the BD FACS™ Loader racks. For complete instructions on the BD FACS™ Loader, refer to the *BD FACS™ Loader User's Guide*.
3. Without storing data, create regions R1 and R2, which contain BD Trucount™ Tubes beads and rWBCs respectively (Figure 6).

Figure 6 FL1 vs FL2 dot plot with data from a leucoreduced platelet unit



Region	Events
R1	10,000
R2	83

4. Confirm that the FL2 threshold is set appropriately.
5. Acquire and store all events. Stop acquisition when 10,000 events have been collected in R1 (the bead region).

Analyzing the Data

Review the laboratory report for the assay. See the *BD Leucocount™ Application Guide for BD FACSLyric™ Flow Cytometers* or the *BD Leucocount™ Application Guide for the BD FACSVia™ System* for more information.

To manually analyze the data using BD CellQuest™ Pro software:

1. To begin analysis, create an FL1 vs FL2 dot plot with statistics and regions R1 and R2 (Figure 6).
2. Obtain region statistics on sample data.
3. Perform the calculations as discussed in the Results section.

Creating a gating aid (optional)

NOTE The BD FACSLyric™ and BD FACSVia™ flow cytometers do not use gating aids.

A gating aid sample can be prepared by making a 1:100 dilution of an ABO-matched, non-leucoreduced RBC segment using filtered red cells, plasma, PBS with 2% fetal bovine serum (FBS), or BD FACSFlow™ Sheath Fluid as the diluent. (We recommend ABO matching to avoid red-cell agglutination).

1. Follow the BD Leucocount™ Kit staining procedure to prepare the gating aid sample.
2. Install the gating aid sample on the sample injection port.
3. Acquire the gating aid sample in setup mode.
4. As events are displayed, adjust R1 and R2, as needed.

8. RESULTS

See the *BD Leucocount™ Application Guide for BD FACSLyric™ Flow Cytometers* or the *BD Leucocount™ Application Guide for the BD FACSVia™ System* for examples of lab reports.

Calculated Values

Specimens are stained in BD Trucount™ Tubes and the absolute count of rWBCs in the sample can be determined by comparing cellular events to bead events. The software calculates absolute counts using the following formula:

$A = (B/C) \times (D/E)$

where:

A = absolute count of rWBC (cells/µL)

B = number of WBC events

C = number of bead events

D = bead count per test

E = stained sample volume

The bead count per test is found on the BD Trucount™ Tubes foil pouch label and varies from lot to lot.

Multiplying this result by the volume of the pack (in µL) results in the total number of WBCs in the entire pack.

9. LIMITATIONS

- Nucleated red cells contain nucleic acid and could be detected as rWBCs in this assay. However, nucleated red cells are not present in detectable quantities in blood from normal individuals.[15]
- Using a collection tube containing EDTA when sampling from a leucoreduced source might result in a reduction of WBC counts.

10. PERFORMANCE CHARACTERISTICS

BD FACSLyric™ Flow Cytometers

Specimen handling and collection (AOB/AOS) (BD FACSLyric™ flow cytometers)

A study was performed at one site to assess the Age of Blood (AOB) and Age of Stain (AOS) using the BD Leucocount™ Kit. The stability of leucoreduced RBC and PLT (single-donor and pooled) specimens was evaluated by assessing the combined effect of:

- AOB: Time duration between leucoreduction and staining (or time duration between pooling and staining for pooled PLT specimens)
- AOS: Time duration between staining specimen and acquiring the stained sample

RBC specimens were maintained at 2–8 °C before staining whereas platelet specimens were maintained at room temperature (20–25 °C).

Based on the results of this study, we recommend the following:

Age of Blood	Age of Stain
Within 24 hours post-leucoreduction	24 hours
Within 48 hours post-leucoreduction	1 hour

Method comparison, BD FACSLyric™ vs BD FACSVia™ flow cytometers

A method comparison study between the investigational BD FACSLyric™ system and the predicate BD FACSVia™ system using three lots of the BD Leucocount™ Kit was conducted at four clinical sites. The BD FACSLyric™ system comprises a BD FACSLyric™ flow cytometer (10- or 12-color configuration) with BD FACSuite™ Clinical application, the BD Leucocount™ assay module, BD® CS&T Beads, and BD® FC Beads 7-Color Kit with a BD FACS™ Universal Loader. The BD FACSVia™ system comprises the BD FACSVia™ flow cytometer with BD FACSVia™ Clinical Software, and BD® CS&T Beads with a BD FACSVia™ Loader.

Regression statistics between the values for rWBC absolute counts in leucoreduced specimens on the BD FACSLyric™ and BD FACSVia™ systems are summarized. The single-donor and pooled PLT samples were combined in the analysis.

Table 3 Regression statistics for rWBC absolute counts using the BD Leucocount™ Kit on the BD FACSLyric™ vs BD FACSVia™ system

Specimen	N	R^2	Slope (95% of CI)	Intercept
PLT (combined single-donor and pooled)	290	0.998	1.01 (0.98, 1.04)	–0.03
RBC	212	0.995	1.10 (1.05, 1.14)	0.01

Regression plots for rWBC absolute counts (cells/µL) on the BD FACSLyric™ flow cytometer vs the BD FACSVia™ flow cytometer are shown in the following figures. The solid line is the fitted line. The dotted line is the line where the results from the BD FACSVia™ system are equal to the results from the BD FACSLyric™ system.

Figure 7 Deming plot of rWBC absolute counts on BD FACSLyric™ vs BD FACSVia™ flow cytometers for combined single-donor and pooled PLT samples

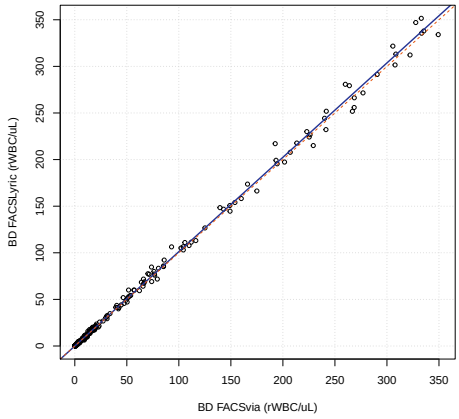
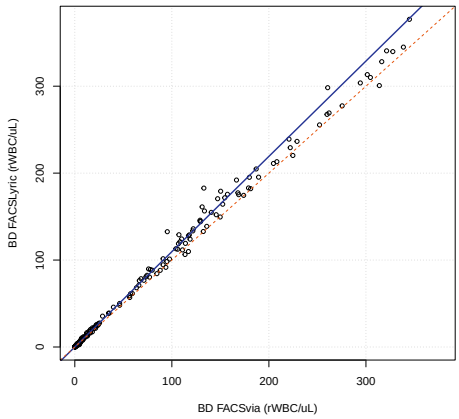


Figure 8 Deming plot of rWBC absolute counts on BD FACSLyric™ vs BD FACSVia™ flow cytometers for RBC samples



Precision (within-site), control material (BD FACSLyric™ flow cytometers)

A 21-day single-site precision study was performed to assess within-site precision using control material. Estimates of precision were determined across three BD FACSLyric™ flow cytometers and three operators acquiring two levels of each analyte, BD Leucocount™ RBC Control and BD Leucocount™ PLT Control, stained in duplicate using three lots of BD Leucocount™ Reagent. Two separate runs were analyzed during each of the 21 tested days.

The mean, the standard deviation (SD), and coefficient of variation (%CV) for within-site precision are shown in the following table.

Table 4 Within-site precision for rWBC absolute counts

Specimen	Mean	SD	%CV
BD Leucocount™ RBC Control High	21.30	1.13	5.31
BD Leucocount™ RBC Control Low	2.48	0.34	13.67
BD Leucocount™ PLT Control High	19.67	1.38	6.99
BD Leucocount™ PLT Control Low	2.02	0.29	14.19

Precision (within-site), leucoreduced specimens (BD FACSLyric™ flow cytometers)

Leucoreduced RBC or PLT (single-donor and pooled) specimens with matched WBCs were stained in nine replicates with one of four lots of BD Leucocount™ Reagent in BD Trucount™ Tubes. The repeatability and within-site precision were evaluated across seven BD FACSLyric™ flow cytometers acquiring three replicates per instrument. Specimens were divided into two bins for analysis: Low (0 ≤ WBC/µL < 10) and High (10 ≤ WBC/µL ≤ 350).

The number of measurements (N), mean, standard deviation (SD), and coefficient of variation (%CV) for within-site precision are presented in the following table.

Table 5 Within-site precision using leucoreduced specimens

Specimen	Bin	N	Mean (WBCs/µL)	SD	%CV
RBC	High	279	164.78	5.65	3.43
	Low	144	4.02	0.48	11.88
PLT (single-donor)	High	279	145.64	5.45	3.74
	Low	180	4.21	0.47	11.21
PLT (pooled)	High	315	157.57	5.55	3.53
	Low	144	4.30	0.48	11.14

Precision (reproducibility), control material (BD FACSLyric™ flow cytometers)

A study was performed at three sites to assess the reproducibility of the system. One lot of each control material (custom PLT Controls Low, custom PLT Controls High, custom RBC Controls Low, and custom RBC Controls High) and three lots of BD Leucocount™ Kit were provided to each site. For each type of control material, three replicates were stained using BD Leucocount™ Reagent in BD Trucount™ Tubes. Testing was performed twice per day for 15 non-consecutive days, with at least one operator and one BD FACSLyric™ flow cytometer at each site.

The mean, standard deviation (SD), and coefficient of variation (%CV) for reproducibility of rWBC absolute counts are presented in the following table.

Table 6 Reproducibility of rWBC counts

Sample type	Mean (rWBCs/µL)	SD	%CV
Custom RBC High	18.35	1.13	6.15
Custom RBC Low	1.22	0.35	28.96

Sample type	Mean (rWBCs/µL)	SD	%CV
Custom PLT High	21.98	1.17	5.34
Custom PLT Low	2.05	0.30	14.91

Detection capability (LOB, LOQ) (BD FACSLyric™ flow cytometers)

The limit of blank (LOB) was evaluated using plasma extracted from 16 leucoreduced RBC specimens. Five replicates were stained with three lots of BD Leucocount™ Reagent in BD Trucount™ Tubes and acquired on one of three BD FACSLyric™ flow cytometers (one lot per instrument). The LOB is 0 cells/µL.

The limit of quantitation (LOQ) was evaluated using leucoreduced RBC specimens with autologous WBCs added to achieve final concentrations of 1, 2, 3, and 4 cells/µL. Three lots of BD Leucocount™ Reagent with BD Trucount™ Tubes were used to stain 20 replicates of each concentration pool. A total of three BD FACSLyric™ flow cytometers and two BD FACSVia™ flow cytometers were used for the study. The LOQ is 0.7 cells/µL.

Linearity (BD FACSLyric™ flow cytometers)

Linearity of the BD Leucocount™ Kit was determined using nine evenly spaced concentrations of autologous rWBCs spiked into leucoreduced RBCs or leucoreduced platelets. Two sets of dilutions were tested, one covering the low end of the range (0.5–24 rWBCs/µL) and one covering the high end of the range (0.5–420 rWBCs/µL). Four replicates of each dilution level were stained using three lots of BD Leucocount™ Reagent and acquired on one of three BD FACSLyric™ flow cytometers. The BD Leucocount™ Kit provides linear results from 0.7–350 rWBCs/µL.

Measuring range (BD FACSLyric™ flow cytometers)

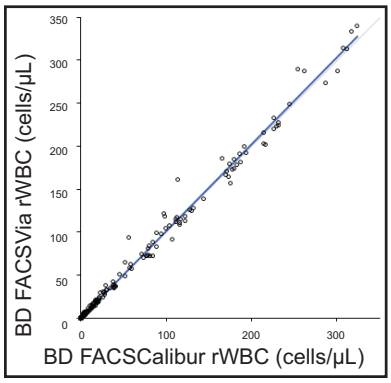
The analytical measuring range of the BD Leucocount™ Kit was established based on data from the method comparison study, the linearity study, and the LOQ study. The lower end of the range was defined by the LOQ study and the linearity study and the upper end of the range was supported by data from the method comparison study and the linearity study. The measuring range is 0.7–350 rWBCs/µL.

BD FACSVia™ Flow Cytometers

Method comparison (BD FACSVia™ vs BD FACSCalibur™ flow cytometers)

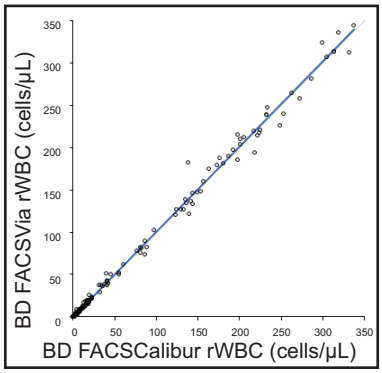
The BD Leucocount™ Assay on the BD FACSVia™ and BD FACSCalibur™ flow cytometers was compared for accuracy of residual white cell enumeration. This comparison was performed using both RBC and platelet samples at four clinical sites. The results are shown in Figure 9 for RBC samples (n = 278) and Figure 10 for platelet samples (n = 252).

Figure 9 Accuracy of the BD Leucocount™ Kit on BD FACSVia™ vs BD FACSCalibur™ flow cytometer for RBCs.



Statistic	Value
R^2	0.99
Slope	1.01
Intercept	0.13

Figure 10 Accuracy of the BD Leucocount™ Kit on BD FACSVia™ vs BD FACSCalibur™ flow cytometers for platelets.



Statistic	Value
R^2	1.00
Slope	1.01
Intercept	0.09

Precision (within-site), control material (BD FACSVia™ flow cytometers)

A 21-day study was conducted at one site, BD Biosciences, to assess within-site precision. Performance for the enumeration of rWBC absolute counts was determined across three BD FACSVia™ flow cytometers (two with an automated loader and one manual) and three operators by acquiring two levels of manipulated

BD Leucocount™ RBC Control and BD Leucocount™ PLT Control cells as test samples stained in duplicate with two lots of BD Leucocount™ Kit.

The mean, standard deviation (SD), and coefficient of variation (%CV) are presented in the following table.

Table 7 Summary of within-site precision results

Sample Type	Mean	SD	%CV
RBC High	16.50	1.12	6.81
RBC Low	7.58	0.68	9.03
Platelet High	15.11	0.92	6.12
Platelet Low	7.32	0.58	7.96

Precision (reproducibility), control material (BD FACSVia™ flow cytometers)

A 20-day study was conducted at three sites (two external sites and one site at BD Biosciences) to assess the reproducibility of the system. Two levels of manipulated BD Leucocount™ RBC Control and BD Leucocount™ PLT Control were stained in duplicate using the BD Leucocount™ Kit and then acquired each day on a BD FACSVia™ flow cytometer. At least two operators were included in the study at each site.

The mean, standard deviation (SD), and coefficient of variation (%CV) are presented in the following table.

Table 8 Summary of reproducibility results

Sample Type	Mean	SD	%CV
RBC High	17.10	1.28	7.51
RBC Low	6.76	0.73	10.76
Platelet High	16.49	1.07	6.46
Platelet Low	7.30	0.69	9.49

Linearity (BD FACSVia™ flow cytometers)

Linearity of the BD Leucocount™ Kit was determined using triplicate measurements of 11 evenly spaced concentrations of autologous rWBCs spiked into leucoreduced platelet and RBCs. The BD Leucocount™ Kit provides linear results from 0–350 rWBCs/µL.

Stability (BD FACSVia™ flow cytometers)

A study at one clinical site was conducted to assess leucoreduced sample and stained sample stability. Sample stability and stained sample stability were evaluated by comparing the baseline sample (stained within 6 hours after leucoreduction and acquired within 30 minutes after staining) to a sample aliquot held for 24 or 48 hours, as shown in Table 9.

Table 9 Platelets and RBC time points

Age of Sample	Age of Stain
Within 24 hours	24 hours + 15 minutes
Within 48 hours	1 hour + 15 minutes

These stability results are shown in the following tables.

Table 10 Change from baseline of samples stored up to 24 hours following leucoreduction

Sample Type	n	Mean rWBC/µL at Baseline	Mean Change (rWBC/µL) at Stained Sample Age of 24 hours
RBCs	50	57.3	–0.3
Platelets	48	60.1	1.8

Table 11 Change from baseline of samples stored up to 48 hours following leucoreduction

Sample Type	n	Mean rWBC/µL at Baseline	Mean Change (rWBC/µL) at Stained Sample Age of 1 hour
RBCs	50	57.3	0.8
Platelets	48	60.1	–0.3

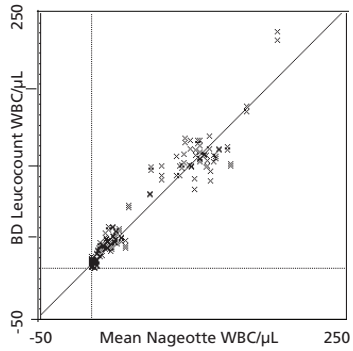
Both platelet and RBC samples can be tested up to 48 hours post leucoreduction. Platelets should be stored at room temperature (20–25 °C), and RBCs should be stored at 2–8 °C. Fresh to 24-hour old samples can be acquired up to 24 hours after staining. Alternatively, samples stored up to 48 hours, and then stained, are stable for 60 minutes after staining.

BD FACSCalibur™ Flow Cytometers

Method comparison (BD FACSCalibur™ flow cytometers)

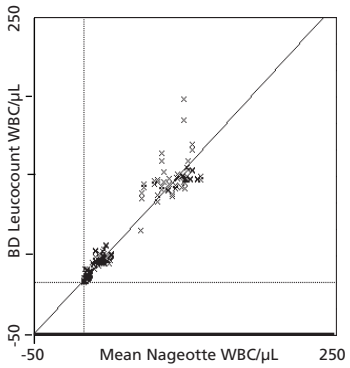
The BD Leucocount™ Assay and the Nageotte method were compared for accuracy of residual white cell enumeration. This comparison was performed using both RBC and platelet samples at three blood bank sites. The results are shown in Figure 11, where n = 226 for RBCs and Figure 12 where n = 217 for platelets.

Figure 11 Accuracy of the BD Leucocount™ Assay vs Nageotte in RBC product



Slope	0.993
Intercept	5.364
R^2	0.942

Figure 12 Accuracy of the BD Leucocount™ Assay vs Nageotte in platelet product



Slope	1.044
Intercept	2.433
R^2	0.940

Precision (BD FACSCalibur™ flow cytometers)

A study was conducted at two sites to assess stain-to-stain precision. Red blood cell samples and platelet samples were prepared and then acquired.

The mean, standard deviation (SD) and coefficient of variation (%CV) were calculated. Results are shown in Table 12.

Table 12 Stain-to-stain precision of the BD Leucocount™ Kit in RBC and platelet units

Sample Type	Range of WBC/µL	No. of Samples	Mean	SD	%CV
RBCs	0–1	15	0.4	0.17	43
	1–5	22	2.2	0.4	19
	5–25	13	9.5	1.1	11
	25–300	19	97.0	5.6	6
Platelets	0–1	21	0.4	0.13	34
	1–5	13	2.4	0.35	14
	5–25	13	11.0	0.87	8
	25–300	16	96.0	4.96	5

Linearity (BD FACSCalibur™ flow cytometers)

The BD Leucocount™ Kit provides linear results from 1–350 rWBCs/µL.

Stability (BD FACSCalibur™ flow cytometers)

A study at one site was conducted to assess sample and stained sample stability. Sample stability was evaluated by comparing the baseline sample (stained and acquired within 1 hour after leucoreduction) to a sample aliquot held for 24 or 48 hours (stained and acquired within 5 minutes). See Table 13.

Table 13 Change from baseline of samples stored up to 48 hours following leucoreduction

Sample Type	N	Mean WBC/µL at Baseline	Mean Change (WBC/µL) at Sample Age of 24 hours	Mean Change (WBC/µL) at Sample Age of 48 hours
RBCs	7	15.5	–0.45	–0.8
Platelets	7	14.8	0.71	0.53

Stained sample stability was evaluated by comparing the baseline sample (stained and acquired within 1 hour of leucoreduction) to samples stained at 24 hours and then held for 24 hours prior to acquisition. See Table 14.

Table 14 Change from baseline of samples stored up to 48 hours following leucoreduction

Sample Type	N	Mean WBC/µL at Baseline	Mean Change (WBC/µL) at Stain Age of 24 hours
RBCs	7	15.5	–0.25
Platelets	7	14.8	0.53

Both platelet and RBC samples can be tested up to 48 hours post leucoreduction. Platelets should be stored at room temperature, and RBCs should be refrigerated. Fresh to 24-hour old samples can be acquired up to 24 hours after staining. Alternatively, samples stored for 48 hours, and then stained, are stable for at least 60 minutes.

Comparison of Flow Cytometers

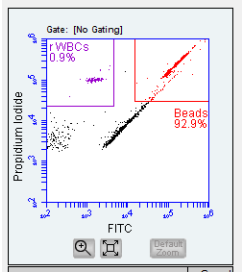
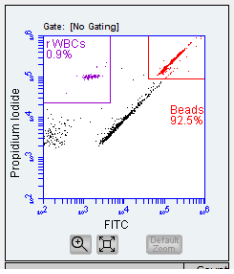
BD FACSCalibur™ flow cytometer

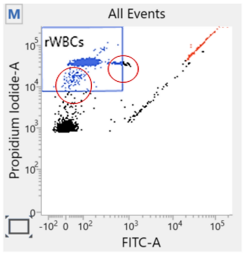
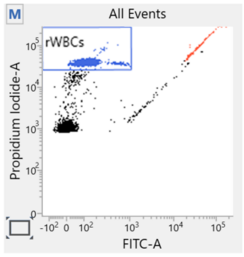
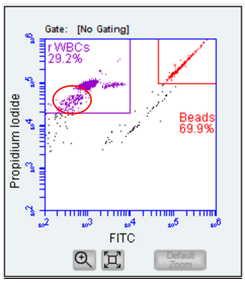
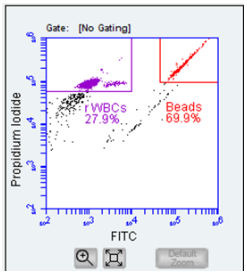
A study was conducted at one site to compare performance of the BD Leucocount™ Kit on flow cytometers from two different manufacturers. Thirty each of RBC and platelet samples were evaluated by regression analysis. Table 15 illustrates the results of that analysis.

Table 15 Comparison of BD Leucocount™ results on BD FACSCalibur™ flow cytometer vs another marketed flow cytometer

Sample Type	n	Slope	Intercept	Correlation
RBCs	30	1.06	–5.65	0.998
Platelets	30	0.99	–0.28	0.999

11. TROUBLESHOOTING

Problem	Possible Cause	Solution
Excessive debris in plots	Threshold manually adjusted and set too low on BD FACSVia™ or BD FACSCalibur™ flow cytometers.	Increase the threshold on BD FACSVia™ or BD FACSCalibur™ flow cytometers.
	Stained sample was too old.	Acquire the sample within recommended times.
	Improper sample preparation.	Verify the sample preparation procedure and technique.
Platelet or RBC streak	Specimen is of poor quality.	When running the BD Leucocount™ Kit on a BD FACSVia™ flow cytometer, resize the Beads gate to exclude non-bead events. Before:  After: 
		Re-stain and re-acquire the sample.
		If the streak persists, contact BD Biosciences.

Problem	Possible Cause	Solution
rWBC populations need regating (BD FACSLyric™ flow cytometer)	Specimen contains high levels of rWBC or is of poor quality.	When running the BD Leucocount™ Kit on a BD FACSLyric™ flow cytometer, resize the rWBCs gate to include the positive PI population(s) and exclude streaks in the rWBCs gate. Before:  After: 
rWBC populations need regating (BD FACSVia™ flow cytometer)	Specimen contains high levels of rWBC or is of poor quality.	When running the BD Leucocount™ Kit on a BD FACSVia™ flow cytometer, resize the rWBCs gate to include the positive Propidium Iodide population(s) and exclude streaks in the rWBCs gate. Before:  After: 

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PATENTS AND TRADEMARKS

For US patents that may apply, see bd.com/patents.

Symbols Glossary

Please refer to product labeling for applicable symbols.

Symbol	Meaning
	Manufacturer
	Authorized representative in the European Community
	Authorised representative in Switzerland
	Date of manufacture
	Use-by date
	Batch code
	Catalogue number
	Serial number
	Sterile
	Sterilized using aseptic processing techniques
	Sterilized using ethylene oxide
	Sterilized using irradiation
	Sterilized using steam or dry heat
	Do not resterilize
	Non-sterile
	Do not use if package is damaged and consult *instructions for use*
	Sterile fluid path
	Sterile fluid path (ethylene oxide)
	Sterile fluid path (irradiation)
	Fragile, handle with care
	Keep away from sunlight
	Keep dry
	Lower limit of temperature
	Upper limit of temperature
	Temperature limit
	Humidity limitation
	Biological risks
	Do not re-use
	Consult *instructions for use* or consult electronic *instructions for use*
	Caution
	Contains or presence of natural rubber latex
	In vitro diagnostic medical device
	Negative control
	Positive control
	Contains sufficient for <*n*> tests
	For IVD performance evaluation only
	Non-pyrogenic
	Patient number
	This way up
	Do not stack

Symbol	Meaning
	Single sterile barrier system
	Contains or presence of phthalate: combination of bis(2-ethylhexyl) phthalate (DEHP) and benzyl butyl phthalate (BBP)
	Collect separately Indicates separate collection for waste of electrical and electronic equipment required.
	CE marking; Signifies European technical conformity
	Device for near-patient testing
	Device for self-testing
	This only applies to US: "Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner."
	Country of manufacture "CC" shall be replaced by either the two letter or the three letter country code.
	Collection time
	Cut
	Peel here
	Collection date
	Keep away from light
	Hydrogen gas is generated
	Perforation
	Start panel sequence number
	End panel sequence number
	Internal sequence number
	<Box #> / <Total Boxes>
	Medical device
	Contains hazardous substances
	Ukrainian conformity mark
	Meets FCC requirements per 21 CFR Part 15
	UL product certification for US and Canada
	Unique device identifier
	Importer
	Place patient label in framed area only
	Magnetic resonance (MR) safe
	Magnetic resonance (MR) conditional
	Magnetic resonance (MR) unsafe
	For use with
	This Product Contains Dry Natural Rubber
	For Export Only
	Instruments

Note: Text layout in symbols is determined by label design.

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HISTORY

Revision	Date	Changes made
23-19381-00	2017-02	Initial release
23-19381(01)	2023-08	Updated to add BD FACSLyric™ as a supported instrument. Updated document to use new template. Updated legal manufacturer address. Added Australia and New Zealand addresses.