BD Leucocount™ Kit

Catalog No. 340523—50 Tests

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1. INTENDED USE

The BD Leucocount™ Kit is intended for counting residual white blood cells (rWBCs) in leucoreduced blood products. The kit is for in vitro diagnostic use on a BD flow cytometer equipped with:

- A 488-nm blue laser
- The ability to detect forward scatter (FSC) and side scatter (SSC)
- At least two-color fluorescence
- Software to acquire and analyze the data

Clinical Application

Determining absolute counts of rWBCs for use in screening of leucoreduced blood products for patients or individuals requiring platelet or red blood cell transfusions.

2. SUMMARY OF THE TEST

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. Flow cytometry is recommended as a counting method for evaluating leucoreduced blood products.[8]

The BD Leucocount™ Kit is intended for in vitro diagnostic use by laboratory professionals only. The sample acquisition can be automated using the optional BD loaders. This assay is not for automated sample preparation. Data analysis can be performed using pre-defined template and automated gating (BD FACSLyric™ flow cytometers only), which can be manually adjusted by the user, if needed.

Principle of Operation

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 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.

3. REAGENT

Reagent Composition

The BD Leucocount™ Kit is composed of:

- 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

- 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.
- Gently vortex samples immediately prior to running them on the flow cytometer to ensure thorough resuspension of cells and beads.
- BD Leucocount™ Reagent is classified as an environmental hazard according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and Regulation (EC) No 1272/2008.

Hazard	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.

- Go to regdocs.bd.com/regdocs/sdsSearch to download the Safety Data Sheet.

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.
- 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.

4. INSTRUMENTS

The BD Leucocount™ Kit is designed for use on the following BD systems. See the corresponding reagent, cytometer, or software user documentation for details.

Table 1 Recommended BD systems

Flow cytometer	Setup	Analysis software
BD FACSLyric™	BD FACSuite™ Clinical application v1.4 or later using BD® CS&T Beads[a]	BD FACSuite™ Clinical application v1.4 or later
BD FACSVia™	BD FACSVia™ clinical software	BD FACSVia™ clinical software
BD FACSCalibur™	BD FACSComp™ software	BD CellQuest™ Pro software

a. To perform daily cytometer quality control.

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

5. SPECIMEN COLLECTION AND PREPARATION

- Collect red blood cell (RBC) and platelet specimens according to manufacturer's instructions.
- 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 platelet 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.
- Lipemic specimens can interfere with the assay.[11,12]
- Triglycerides at 500 mg/dL interfere with the assay in leucoreduced RBCs.

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

6. PROCEDURE

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. 341001 or 341003)

  BD Leucocount™ PLT Control (Catalog No. 341002 or 341003)

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

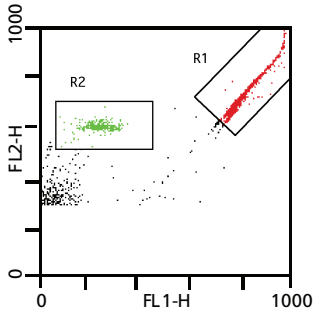
We recommend running process controls each day of use to provide absolute rWBC counts around your laboratory's critical cut-off values. In addition, it is advisable to run controls on every shift. We recommend using BD Leucocount™ RBC Control or BD Leucocount™ PLT Control for this purpose, stained as 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 FACSComp™ 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 10.

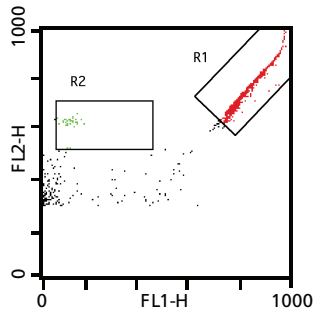
Quality control runs should produce results equivalent to the expected values.

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.

   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).

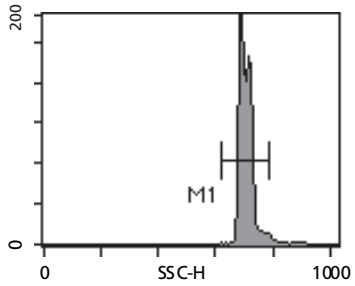
Running the Assay on BD FACSCalibur™ Flow Cytometers

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.

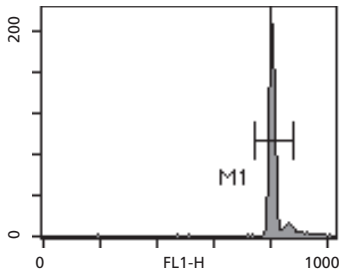
Figure 3 SSC histogram



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.

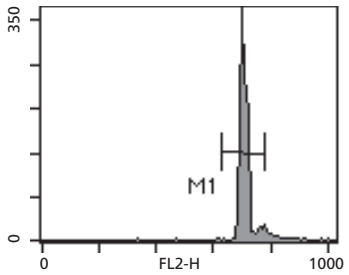
Figure 4 FL1 histogram



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



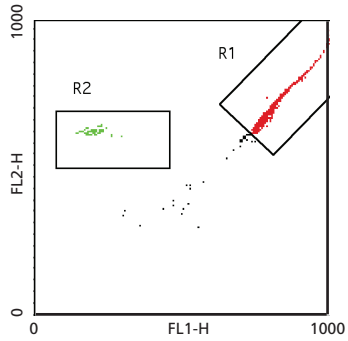
Marker	Events	Mean
All	8,340	701.04
M1	8,121	698.72

4. Save instrument settings.

To acquire the samples using BD FACSComp™ 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 FACSComp™ 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.

7. 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) x (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.

8. 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.
- When running the BD Leucocount™ Kit on a BD FACSVia™ flow cytometer, certain endogenous compounds might interfere with the results.

9. PERFORMANCE CHARACTERISTICS

Specimen Handling and Collection (AOB/AOS)

A study was performed to assess the Age of Blood (AOB) and Age of Stain (AOS) using the BD Leucocount™ Kit. The stability of leucoreduced specimens was evaluated by assessing the combined effect of:

- AOB: Time duration between leucoreduction and staining
- AOS: Time duration between staining specimen and acquiring the stained sample

RBC specimens were maintained at 1–6 °C before staining whereas platelet specimens were maintained at room temperature (20–24 °C).

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

Age of Blood	Age of Stain
Within 24 hours	24 hours
Within 48 hours	1 hour

The Age of Blood refers to the time post-leucoreduction.

Detection Capability (LOB, LOQ)

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.

BD FACSLyric™ Flow Cytometers

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

Leucoreduced PLT and RBC specimens were divided into two bins for analysis: Low (0 ≤ WBC/µL < 10) and High (10 ≤ WBC/µL < 350). Six replicates of each specimen were stained with two lots of BD Leucocount™ Reagent in BD Trucount™ Tubes. Two replicates were acquired on a BD FACSLyric™ flow cytometer using manual acquisition, two replicates were acquired on a BD FACSLyric™ flow cytometer using the BD FACS™ Universal Loader (UAL), and two replicates were acquired on a BD FACSVia™ flow cytometer using manual acquisition or the BD FACSVia™ Loader.

Two comparisons were conducted for each bin and specimen type:

- BD FACSLyric™ manual acquisition vs BD FACSVia™ flow cytometer
- BD FACSLyric™ manual acquisition vs BD FACSLyric™ with BD FACS™ Universal Loader (UAL)

The number of specimens (N) and absolute or relative difference (bias) are presented.

Table 3 Results for BD FACSLyric™ manual acquisition vs BD FACSVia™

Specimen	Bin	N	Mean absolute difference	Mean relative difference
RBC	High	34	N/A	4.69%
	Low	19	0.12	N/A
PLT	High	34	N/A	1.25%
	Low	13	0.08	N/A
N/A = Not applicable				

Table 4 Results for BD FACSLyric™ (UAL) vs BD FACSLyric™ (manual acquisition)

Specimen	Bin	N	Mean absolute difference	Mean relative difference
RBC	High	34	N/A	–0.70%
	Low	19	0.05	N/A
PLT	High	33	N/A	–2.18%
	Low	14	–0.25	N/A

Precision (repeatability) control material (BD FACSLyric™ flow cytometers)

A 21-day single-site precision study was performed to assess repeatability and 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 and the standard deviation (SD) or coefficient of variation (%CV) are shown.

Table 5 Repeatability and within-site precision for rWBC absolute count

Specimen	Mean	SD	
		Repeatability	Within-site
BD Leucocount™ RBC Control Low	2.48	0.32	0.34
BD Leucocount™ PLT Control Low	2.02	0.28	0.29

Table 6 Repeatability and within-site precision for rWBC absolute count

Specimen	Mean	%CV	
		Repeatability	Within-site
BD Leucocount™ RBC Control High	21.30	5.22	5.31
BD Leucocount™ PLT Control High	19.67	6.12	6.99

Precision (repeatability), leucoreduced specimens (BD FACSLyric™ flow cytometers)

Leucoreduced RBC or PLT specimens with matched WBCs were stained in nine replicates with one of two lots of BD Leucocount™ Reagent in BD Trucount™ Tubes. The repeatability and within-site precision were evaluated across three 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 samples (N), mean, standard deviation (SD), and coefficient of variation (CV) for within-site precision are presented in the following tables.

Table 7 Within-site precision using clinical specimens (low bin)

Specimen	N	Mean	SD
RBC	19	2.57	0.42
PLT	15	4.59	0.67

Table 8 Within-site precision using clinical specimens (high bin)

Specimen	N	Mean	%CV
RBC	34	183.01	4.73%
PLT	26	167.68	4.83%

Linearity (BD FACSLyric™ flow cytometers)

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

Measuring range (BD FACSLyric™ flow cytometers)

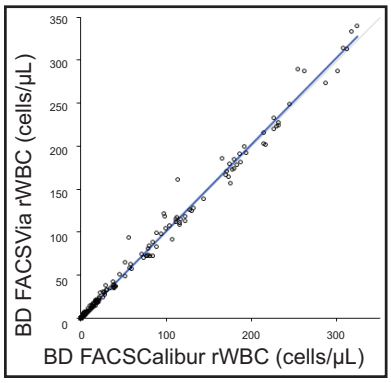
The analytical measuring range of the BD Leucocount™ Kit was established based on data from the method comparison study, the linearity study, and the LOQ study. The lower end of the range was defined by the LOQ study and the linearity study and the upper end of the range was supported by data from the method comparison study and the linearity study. The measuring range is 0.7–300 rWBCs/µL.

BD FACSVia™ Flow Cytometers

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

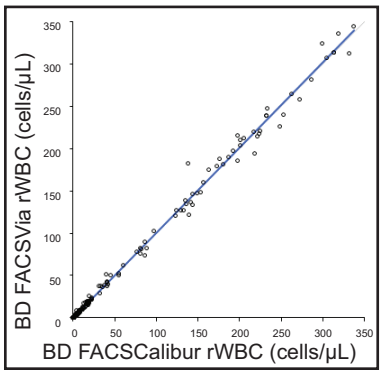
The BD Leucocount™ Assay on the BD FACSVia™ and BD FACSCalibur™ flow cytometers was compared for accuracy of residual white cell enumeration. This comparison was performed using both RBC and platelet samples at four clinical sites. The results are shown in Figure 7 for RBC samples (n = 278) and Figure 8 for platelet samples (n = 252).

Figure 7 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 8 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

Method comparison (BD FACSVia™ with BD FACSVia™ Loader vs manual acquisition)

The equivalency of manual acquisition on the BD FACSVia™ flow cytometer vs acqusition using the BD FACSVia™ Loader was determined. Leucoreduced PLT and RBC specimens were divided into two bins for analysis: Low (0 ≤ WBC/µL < 10) and High (10 ≤ WBC/µL < 350). Four replicates of each specimen were stained using the BD Leucocount™ Kit. Two replicates were acquired on a BD FACSVia™ flow cytometer using manual acquisition and two replicates were acquired on a BD FACSVia™ flow cytometer using the BD FACSVia™ Loader.

The number of specimens (N) and absolute or relative difference (bias) are presented.

Table 9 Results for BD FACSVia™ with the BD FACSVia™ Loader vs BD FACSVia™ manual acquisition

Specimen	Bin	N	Mean absolute difference	Mean relative difference
RBC	High	46	N/A	1.13%
	Low	42	0.17	N/A
PLT	High	41	N/A	1.09%
	Low	40	0.08	N/A

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 coefficients of variation (%CV) are presented in the following table.

Table 10 Summary of within-site precision results

Sample Type	%CV
RBC High	6.81%
RBC Low	9.03%
Platelet High	6.12%
Platelet Low	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 coefficients of variation (%CV) are presented in the following table.

Table 11 rWBC in leucoreduced platelets

Sample Type	%CV
RBC High	7.51%
RBC Low	10.76%
Platelet High	6.46%
Platelet Low	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 RBC. The BD Leucocount™ Kit provides linear results from 0–350 rWBCs/µL.

Measuring range (BD FACSVia™ flow cytometers)

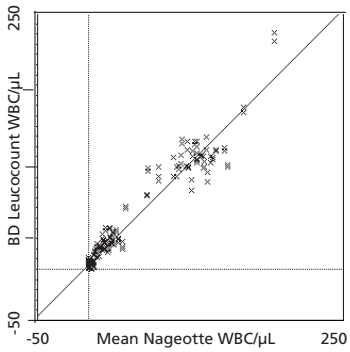
The analytical measuring range of the BD Leucocount™ Kit was established based on data from the method comparison study, the linearity study, and the LOQ study. The lower end of the range was defined by the LOQ study and the linearity study and the upper end of the range was supported by data from the method comparison study and the linearity study. The measuring range is 0.7–300 rWBCs/µL.

BD FACSCalibur™ Flow Cytometers

Method comparison (BD FACSCalibur™ flow cytometers)

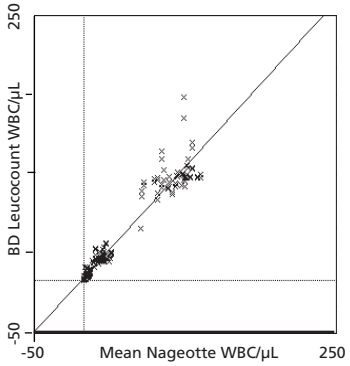
The BD Leucocount™ Assay and the Nageotte method were compared for accuracy of residual white cell enumeration. This comparison was performed using both RBC and platelet samples at three blood bank sites. The results are shown in Figure 9, where n = 226 for RBCs and Figure 10 where n = 217 for platelets.

Figure 9 Accuracy of the BD Leucocount™ Assay vs Nageotte in RBC product



Slope	**0.993**
Intercept	5.364
R^2	0.942

Figure 10 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 1.

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

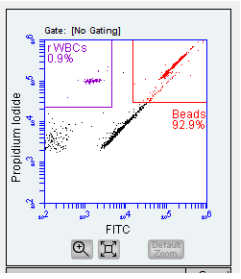
Linearity (BD FACSCalibur™ flow cytometers)

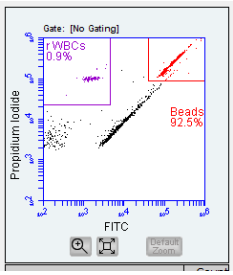
The BD Leucocount™ Kit provides linear results from 0–350 rWBCs/µL.

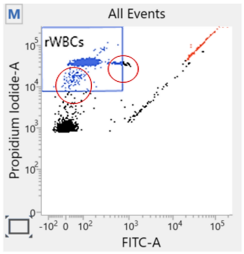
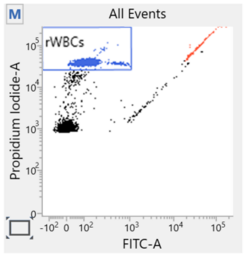
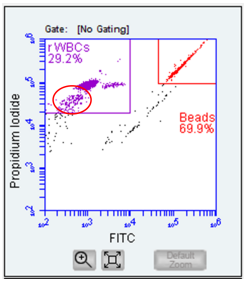
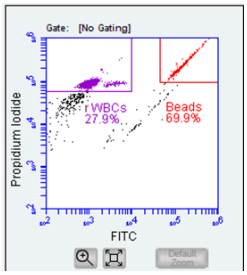
Measuring range (BD FACSCalibur™ flow cytometers)

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 0.7–300 rWBCs/µL.

10. TROUBLESHOOTING

Problem	Possible Cause	Solution
Excessive debris in plots	Threshold manually adjusted and set too low.	Increase the threshold.
	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 contains high levels of certain endogenous compounds or 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: 

Problem	Possible Cause	Solution
		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, certain endogenous compounds, 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, certain endogenous compounds, 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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NOTICE

EU Only: Users shall report any serious incident related to the device to the Manufacturer and National Competent Authority.

Outside EU: Contact your local BD representative for any incident or inquiry related to this device.

WARRANTY

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

THE PRODUCTS SOLD HEREUNDER ARE WARRANTED ONLY TO CONFORM TO THE QUANTITY AND CONTENTS STATED ON THE LABEL OR IN THE PRODUCT LABELING AT THE TIME OF DELIVERY TO THE CUSTOMER. BD DISCLAIMS HEREBY ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY AND FITNESS FOR ANY PARTICULAR PURPOSE AND NONINFRINGEMENT. BD'S SOLE LIABILITY IS LIMITED TO EITHER REPLACEMENT OF THE PRODUCTS OR REFUND OF THE PURCHASE PRICE. BD IS NOT LIABLE FOR PROPERTY DAMAGE OR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING PERSONAL INJURY, OR ECONOMIC LOSS, CAUSED BY THE PRODUCT.

TRADEMARKS

HISTORY

Revision	Date	Changes made
23-3434(12)	2022-11	Updated to meet requirements of Regulation (EU) 2017/746 and to add BD FACSLyric™ as a supported instrument.

SYMBOLS GLOSSARY [L006715(06) 2021-08]

Some symbols listed below may not apply to this product.
US Customers only: For symbol glossary, refer to **bd.com/symbols-glossary**

Symbol	Meaning
	Manufacturer
EC REP	Authorized representative in the European Community
CH REP	Authorised representative in Switzerland
	Date of manufacture
	Use-by date
LOT	Batch code
REF	Catalogue number
SN	Serial number
STERILE	Sterile
STERILE A	Sterilized using aseptic processing techniques
STERILE EO	Sterilized using ethylene oxide
STERILE R	Sterilized using irradiation
STERILE	Sterilized using steam or dry heat
	Do not resterilize
	Non-sterile
	Do not use if package is damaged and consult *instructions for use*
STERILE	Sterile fluid path
STERILE EO	Sterile fluid path (ethylene oxide)
STERILE R	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
LATEX	Contains or presence of natural rubber latex
IVD	In vitro diagnostic medical device
CONTROL -	Negative control
CONTROL +	Positive control
Σ	Contains sufficient for <*n*> tests
	For IVD performance evaluation only
	Non-pyrogenic

Symbol	Meaning
#	Patient number
	This way up
	Do not stack
	Single sterile barrier system
PHT DEHP BBP	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	CE marking; Signifies European technical conformity
	Device for near-patient testing
	Device for self-testing
R_x Only	This only applies to US: "Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner."
CC	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
H_2	Hydrogen gas is generated
	Perforation
	Start panel sequence number
	End panel sequence number
	Internal sequence number
MD	Medical device
	Contains hazardous substances
	Ukrainian conformity mark
FC	Meets FCC requirements per 21 CFR Part 15
c UL us	UL product certification for US and Canada
UDI	Unique device identifier

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