BD Multitest™ CD3/CD8/CD45/CD4

50 Tests per kit—Catalog No. 342417

50 Tests per kit with
BD Trucount™ Tubes—Catalog No. 342447

23-5351(12)
2023-07
English

CE 2797 IVD

1. INTENDED USE

The BD Multitest™ CD3/CD8/CD45/CD4 reagent with optional BD Trucount™ Tubes is a four-color direct immunofluorescence reagent for use in identifying and determining the percentages and absolute counts of T cells, as well as the CD4 and CD8 subpopulations of T cells, in peripheral blood on a BD flow cytometer equipped with the following:

- At least a 488-nm blue laser and a 640-nm red laser
- The ability to detect forward scatter (FSC) and side scatter (SSC)
- At least 4-color fluorescence
- Software to acquire and analyze the data

Clinical Applications

Determining percentages or absolute counts of $CD3^+CD4^+$ T lymphocytes is used in monitoring human immunodeficiency virus (HIV)-infected individuals. Individuals with HIV typically exhibit a steady decrease of $CD3^+CD4^+$ T lymphocyte absolute counts as the infection progresses.[1]

Determining percentages or absolute counts of $CD3^+$, $CD3^+CD4^+$, or $CD3^+CD8^+$ T lymphocytes is used to characterize or monitor some forms of immune deficiency and autoimmune diseases.[1,2]

2. SUMMARY OF THE TEST

Human peripheral blood contains three types of lymphocytes: T, B, and NK lymphocytes. They have distinct biologic functions and can be identified by differences in their cell-surface antigen expression.

Subsets of antigen-specific T lymphocytes have different roles in the adaptive immune response. Helper/inducer T lymphocytes secrete cytokines that help regulate the activity of other T lymphocytes as well as B lymphocytes. Suppressor/cytotoxic T lymphocytes suppress the activity of other T lymphocytes, or recognize and lyse infected or abnormal cells.[3]

BD Multitest™ CD3/CD8/CD45/CD4 with or without BD Trucount™ Tubes is a quantitative assay intended for use by laboratory professionals to identify and enumerate the following T-lymphocyte subset populations:

- $CD3^+$ T lymphocytes
- $CD3^+CD4^+$ helper/inducer T lymphocytes
- $CD3^+CD8^+$ suppressor/cytotoxic T lymphocytes

Automated sample preparation and acquisition can be achieved using the BD FACSDuet™ Sample Preparation System and BD loaders, respectively. Data analysis can be performed using a pre-defined template and automated gating, which can be manually adjusted by the user, if needed.

Principle of Operation

The BD Multitest™ CD3/CD8/CD45/CD4 reagent is composed of four monoclonal antibodies, each conjugated to a specific fluorochrome. The reagent is added to peripheral blood and incubated, allowing each monoclonal antibody in the reagent to bind to a specific antigen on the surface of the cells. After incubation, BD FACS™ Lysing Solution is added to lyse the red blood cells in the sample. Cells are acquired on a BD flow cytometer using the appropriate software. During acquisition, the cells travel past the laser beam and scatter the laser light. The stained cells fluoresce. These scatter and fluorescence signals, detected by the instrument, provide information about the cell's size, internal complexity, and relative fluorescence intensity. BD Multitest™ reagents employ fluorescence triggering, allowing direct fluorescence gating of the lymphocyte population to reduce contamination of unlysed or nucleated red blood cells in the gate. The software and the BD Multitest™ 4-Color assay module are used to analyze the data and report the result.

When determining absolute cell counts, expressed as the number of cells/µL, a precise volume of specimen and the BD Multitest™ CD3/CD8/CD45/CD4 reagent are added to a BD Trucount™ Tube. The BD Trucount™ Tube contains a lyophilized pellet of fluorescent beads. During incubation of the reagent and the specimen, the bead pellet dissolves, releasing a known number of fluorescent beads, which are distinguished from cells by their fluorescence intensity. After lysing red blood cells, the sample is acquired on a BD flow cytometer. The software determines the absolute cell counts by comparing cellular events to bead events, and reports the absolute cell counts in the lab report.

For flow cytometer principles of operation, see the instructions for use (IFU) for your instrument.

3. REAGENT

Reagent Composition

The reagent contains the following conjugated antibodies:

Table 1 Reagent composition

Antibody	Fluorochrome	Clone	Isotype	Concentration (µg/mL)
CD3	FITC	SK7[4,5]	$IgG_{1,\kappa}$	2.3
CD8	PE	SK1[6,7]	$IgG_{1,\kappa}$	1.75
CD45	PerCP	2D1 [8]	$IgG_{1,\kappa}$	7.50
CD4	APC	SK3[6,7,9]	$IgG_{1,\kappa}$	0.92

CD3 (SK7) recognizes the epsilon chain of the CD3 antigen/T-cell antigen receptor (TCR) complex.[10] The CD3 antigen is present on T lymphocytes and is noncovalently associated with either α/β or γ/δ TCR.[11] CD3 reacts minimally with other cell populations.[12]

CD8 (SK1) recognizes an antigen that interacts with class I major histocompatibility complex (MHC) molecules, resulting in increased adhesion between the $CD8^+$ T lymphocytes and the target cells and enhanced activation of resting T lymphocytes.[13,14,15] The CD8 antigen is present on suppressor/cytotoxic T lymphocytes. CD8 also recognizes a subset of NK lymphocytes.[16]

CD45 (2D1) recognizes all isoforms of the leucocyte common antigen (LCA)/T200 family.[17] The CD45 antigen is present on all human leucocytes, including lymphocytes, monocytes, granulocytes, eosinophils, and basophils in peripheral blood.[17] CD45 has been reported to react weakly with mature circulating erythrocytes and platelets.[17,18]

CD4 (SK3) recognizes an antigen that interacts with class II MHC molecules and is the primary receptor for HIV.[19,20] The CD4 antigen is present on helper/inducer T lymphocytes and is present in low density on the cell surface of monocytes and in the cytoplasm of monocytes.[9]

Precautions

- The reagent should be clear. Do not use the reagent if you observe any change in appearance. Precipitation, cloudiness, or change in color indicates instability or deterioration.
- The antibody reagent contains sodium azide as a preservative. However, take care to avoid microbial contamination, which can cause erroneous results.
- If using BD Trucount™ Tubes, calibrate pipets to deliver exactly 50 µL of sample or perform the reverse pipetting technique (see Reverse Pipetting on page 7). See the pipet manufacturer's instructions for more information.
- Bead count varies by lot of BD Trucount™ Tubes. It is critical to use the bead count shown on the current lot of BD Trucount™ Tubes when entering this value in the software or when manually calculating absolute counts. Do not mix multiple lots of BD Trucount™ Tubes in the same run.
- BD Trucount™ Tubes are designed for use with a specific lyse/no-wash procedure. Do not attempt to threshold on forward scatter (FSC) for data collection.
- Go to regdocs.bd.com/regdocs/sdsSearch to download the Safety Data Sheet.

Storage and Handling

- Store the reagent at 2–8 °C. Reagent in opened or unopened vials is stable until the expiration date shown on the vial label. Do not use after this expiration date.
- Do not freeze the reagent or expose it to direct light during storage or incubation with cells. Keep the reagent vial dry.
- The reagent is stable if kept in the BD FACSDuet™ instrument for 8 hours per day for 5 days. Do not store the reagent overnight in the instrument. Use of any reagent remaining after being kept in the BD FACSDuet™ instrument for 5 days must be validated by the user.
- 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. Do not remove the desiccant pack from the pouch. Use tubes within 1 hour after removal from the foil pouch.
- BD Trucount™ Tubes in an unopened pouch are stable until the expiration date shown on the packaging. Do not use tubes after the expiration date.
- Tubes in an opened pouch are stable for 1 month after the date of opening, when stored as directed. Write the date when you first open the pouch in the space provided on the label.

4. INSTRUMENT

The BD FACSLyric™ and BD FACSCanto™ II systems are outlined in the following table. See the corresponding reagent or instrument user documentation for details.

Table 2 BD FACSLyric™ and BD FACSCanto™ II systems

Flow cytometer	Setup beads	Setup software	Analysis software	Assay module
BD FACSLyric™	BD® CS&T Beads[a] BD® FC Beads 7-Color Kit[b]	BD FACSuite™ Clinical application	BD FACSuite™ Clinical application	BD Multitest™ 4-Color
BD FACSCanto™ II	BD FACS™ 7-Color Setup Beads[c]	BD FACSCanto™ Clinical Software v2.4 or later	BD FACSCanto™ Clinical Software v2.4 or later	BD Multitest™ 4-Color

a. To perform daily cytometer quality control.

b. To calculate compensation.

c. To set photomultiplier tube (PMT) voltages and fluorescence compensation, and check instrument sensitivity before use.

The BD FACS™ Loader and BD FACS™ Universal Loader can be used with this product. See the IFU for the cytometer used with your Loader for more information.

The BD FACSDuet™ sample preparation system can be used with this product. See the *BD FACSDuet™ Sample Preparation System Instructions for Use* for more information.

5. SPECIMEN COLLECTION AND PREPARATION

- Collect blood specimens aseptically by venipuncture into a BD Vacutainer® EDTA blood collection tube, or equivalent.[21]

  BD Multitest™ CD3/CD8/CD45/CD4 with BD Trucount™ Tubes has been validated with both liquid and dry formulations of EDTA. The reagent has not been validated by BD Biosciences for use with heparin or acid citrate dextrose (ACD) liquid anticoagulants in determining absolute counts with BD Trucount™ Tubes.

  The assay requires 50 µL of peripheral blood per test. We recommend starting with a minimum of 100 µL of blood to accommodate the excess volume needed to perform reverse pipetting.
- If using the dual platform method, obtain a white blood cell (WBC) count and a differential white cell count from the same whole blood sample before staining to calculate absolute counts from percentages. See Dual Platform Method on page 15.
- Store blood specimens at room temperature (20–25 °C).
- Stain specimens within 48 hours of draw.
- Acquire samples within 24 hours of staining.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[22,23] 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. Fixation has been reported to inactivate HIV.[24]

Interference

Substances present in the specimen might interfere with the assay:

- Specimens obtained from patients taking immunosuppressive drugs[25,26,27] or undergoing monoclonal antibody treatment[28,29,30,31,32,33] can yield erroneous results.
- Hemolyzed samples can interfere with the assay and should be rejected.[34] Do not use previously fixed and stored patient specimens. Whole blood samples refrigerated before staining can give aberrant results.
- Blast cells can interfere with test results.[35]
- Lipemic specimens can interfere with the assay.[36,37]
- Bilirubin interferes at an absorbance peak of 456 nm.[38]

Interfering Conditions

The following table lists the substances that were tested for interference with a similar reagent, the BD Multitest™ 6-Color TBNK reagent with optional BD Trucount™ Tubes.

Testing for interference was performed in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines.[39] There was no detectable interference at the following concentrations.

Table 3 Non-interfering substances

Analyte	Concentration tested
Acetaminophen	156 µg/mL
Acetylsalicylic acid (Aspirin)	30 µg/mL
Albuterol	0.015 µg/mL
Atenolol	3 µg/mL
Atorvastatin	0.25 µg/mL
Azithromycin	3.7 µg/mL
Bilirubin, conjugated	2 mg/dL
Cobicistat	3.6 µg/mL
Efavirenz	12 µg/mL
Enoxaparin	2 µg/mL
Guaifenesin	1.5 µg/mL
Hydroxychloroquine	0.2 µg/mL
Ibuprofen	73 µg/mL
Insulin	37 µU/mL
Kaletra	15.5 µg/mL
Lisinopril	0.082 µg/mL
Maraviroc	0.888 µg/mL
Oseltamivir	0.133 µg/mL
Raltegravir	15 µg/mL
Remdesivir	16.32 µg/mL
Ritonavir	15 µg/mL
Tenofovir	0.978 µg/mL
Tocilizumab	149.4 µg/mL
Vancomycin	40 µg/mL

The following substances interfered with the assay at the indicated concentration:

Table 4 Interfering substances

Analyte	Concentration tested
Albumin[a,e]	6 g/dL
Bilirubin, unconjugated[b,e]	2 mg/dL
Erythrocytes[c,e]	$6x10^3$ cells/µL
Hemoglobin[c,e]	1000 mg/dL
Triglycerides[d,e]	1500 mg/dL

a. Albumin interferes as a result of its comparatively large concentration in the peripheral blood and its ability to bind as well as to release large quantities of ligands.40

b. Unconjugated Bilirubin may induce autofluorescence.41

c. The presence of red blood cells (RBCs) in the sample preparation can cause light interference and non-specific interactions leading to erroneous test results.42 Hemolyzed samples should be rejected. The hemoglobin concentration refers to free hemoglobin.

d. Immunomodulatory drugs used for treatment of HIV infection may cause lipemia. Lipemia is known to interfere in assays that use the transmission of light and impact the scattering of light.43,44

e. The listed endogenous substances interfere with the assay at higher than normal concentrations, i.e. hyperalbuminemia, unconjugated hyperbilirubinemia, erythrocytosis, hemoglobinemia, and hypertriglyceridemia. Interference caused by these endogenous substances is not uncommon and has been described in the literature (see references listed in notes a–d).

6. PROCEDURE

Reagents and Materials

Reagents and materials provided

BD Multitest™ CD3/CD8/CD45/CD4 is provided in 1 mL of buffered saline with <0.1% sodium azide. The reagent is sufficient for 50 tests.

If calculating absolute counts, use BD Multitest™ CD3/CD8/CD45/CD4 with BD Trucount™ Tubes. The reagent comes with two pouches of BD Trucount™ Tubes. Each pouch contains 25 tubes, sufficient for 25 tests. The tubes contain a freeze-dried pellet of fluorescent beads in a single-use tube.

Reagents and materials required but not provided

- BD FACS™ Lysing Solution (Catalog No. 349202)

  The lysing solution is provided as a 10X concentrate and it contains diethylene glycol and formaldehyde. See the *BD FACS™ Lysing Solution* IFU for precautions and warnings.
- Disposable 12 × 75-mm capped polystyrene test tubes, or equivalent (if not using BD Trucount™ Tubes)
- Vortex mixer
- Micropipettor with tips
- Bulk dispenser or pipettor (450 µL) for dispensing 1X BD FACS™ Lysing Solution
- BD Multi-Check™ Control (Catalog Nos. 340911, 340912, 340913)
- BD Multi-Check™ CD4 Low Control (Catalog Nos. 340914, 340915, 340916)
- (Optional) BD Trucount™ Controls (Catalog No. 340335)
- (Optional) BD FACS™ Universal Loader
- (Optional) BD FACS™ Loader (used on the BD FACSCanto™ II flow cytometer)

Diluting BD FACS™ Lysing Solution

Dilute the 10X concentrate 1:10 with room temperature (20–25 °C) deionized water. The prepared solution is stable for 1 month when stored in a glass or high density polyethylene (HDPE) container at room temperature.

Reverse Pipetting

Accurate pipetting is critical when using a BD Trucount™ Tube. Use the reverse pipetting technique to add the sample to a BD Trucount™ Tube. For reverse pipetting, depress the button to the second stop. Release the button to draw excess sample into the tip. Press the button to the first stop to expel a precise volume of sample, leaving excess sample in the tip.

Performing Quality Control

Run two levels of process control material (for example, BD Multi-Check™ Control and BD Multi-Check™ CD4 Low Control) before acquiring patient specimens.[45] Control materials should provide established values for percent positive and absolute counts for the relevant cell populations. Process the controls like patient specimens to monitor the performance of the entire analytic process. This is done at least once each day when patient testing is performed.

NOTE BD Multi-Check™ Control and BD Multi-Check™ CD4 Low Control are validated as process controls on BD FACSLyric™ flow cytometers.

If needed, use BD Trucount™ Controls to verify pipetting accuracy and the bead count value of the BD Trucount™ Tubes.

Staining the Cells

If using the BD FACSDuet™ system to prepare the samples, see the *BD FACSDuet™ Sample Preparation System Instructions for Use*.

1. For each sample, remove a tube and label it with the appropriate sample identification.

   For calculating absolute counts and lymphocyte subset percentages, label a BD Trucount™ Tube. For calculating lymphocyte subset percentages only, label a 12 × 75-mm tube.

   NOTE For samples stained in BD Trucount™ Tubes, verify that the BD Trucount™ bead pellet is under the metal retainer at the bottom of the tube. If this is not the case, discard the BD Trucount™ Tube and replace it with another. Do not transfer beads to another tube.
2. Pipette 20 µL of BD Multitest™ CD3/CD8/CD45/CD4 reagent into the bottom of the tube.

   If using a BD Trucount™ Tube, pipette the reagent onto the side of the tube, just above the metal retainer, without touching the bead pellet.
3. Pipette 50 µL of well-mixed control material or anticoagulated peripheral blood onto the side of the tube.

   If using a BD Trucount™ Tube, pipette the sample onto the side of the tube, just above the metal retainer, without touching the bead pellet.

   NOTE Thoroughly mix the controls before pipetting them. See the BD Multi-Check™ Control or BD Multi-Check™ CD4 Low Control IFU for detailed instructions.

   NOTE Use the reverse pipetting technique to pipette sample onto the side of the tube just above the retainer. See Reverse Pipetting on page 7. Avoid smearing sample down the side of the tube. If whole blood or control material remains on the side of the tube, it will not be stained with the reagent and can affect results.
4. Cap the tube and vortex gently to mix.
5. Incubate for 15–30 minutes in the dark at room temperature (20–25 °C).
6. Add 450 µL of 1X BD FACS™ Lysing Solution to the tube.
7. Cap the tube and vortex gently to mix.
8. Incubate for 15–30 minutes in the dark at room temperature (20–25 °C).

   The sample is now ready to be analyzed on the flow cytometer. Acquire the sample within 24 hours of staining. Store the stained sample in the dark at room temperature (20–25 °C) until acquisition.

Running the Assay on a BD FACSLyric™ Flow Cytometer

Before you begin:

1. Ensure that Characterization QC (CQC) and lyse/no wash reference settings have not expired.
2. Add reagent lots to library, if needed.

   See the *BD FACSLyric™System Instructions For Use* for information.
3. Perform daily Performance QC (PQC) using BD® CS&T Beads.

   See the *BD® CS&T Beads* IFU and the *BD FACSLyric™ System Instructions For Use* for information.

To run the assay:

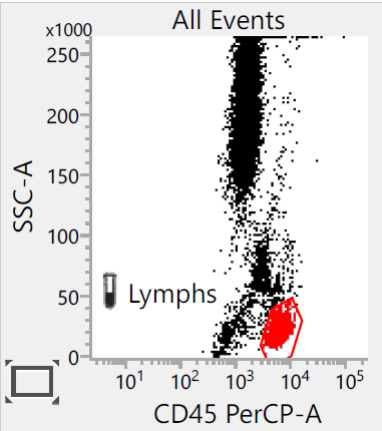
1. Create a worklist.
   - Create a Multi-Check™ Control task for each process control you are running.
   - Create an appropriate assay task for each patient specimen you are running.
2. Enter information in the worklist table.
   - If not using BD Trucount™ Tubes, enter the WBC count and the percentage of lymphocytes (WBC (x1000) and Lymphs (%), respectively), or the lymphocyte count (Lymphs (x1000)) in the appropriate column.

   NOTE Divide the WBC count or the lymphocyte count by 1,000 before entering it into the software.
   - If using BD Trucount™ Tubes, enter the lot ID for the tubes and the bead count, found on the pouch label, in the appropriate column (Trucount Lot ID and Beads Per Pellet, respectively).
3. Run the control tasks on the worklist.
4. Vortex each tube thoroughly at low speed immediately before acquiring it.[46]

   NOTE If you are using the BD FACS™ Universal Loader, vortex tubes immediately before placing them into the Loader racks.
5. After acquiring the control samples, click **Stop Tube**.

   NOTE This assumes that process control passes. Stop it to verify, then continue with samples of interest. If process control fails, restain samples and process controls because you cannot discriminate whether process control failure comes from staining or the instrument.
6. Review the lab report for the controls.
7. Visually inspect the CD45 PerCP-A vs SSC-A dot plot.

   The lymphocyte population should appear as a bright, compact cluster with low SSC. Monocytes and granulocytes should also appear as distinct clusters. Do not proceed with analysis if populations are diffuse and there is little or no separation between clusters.

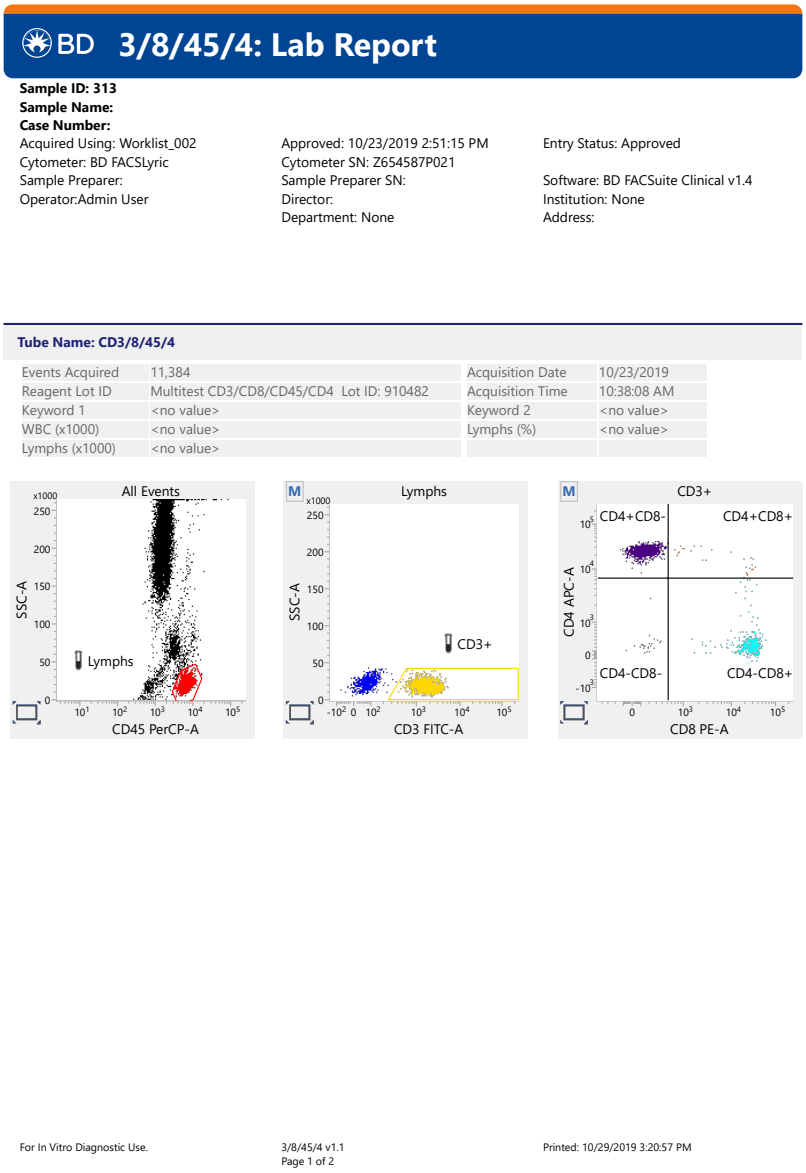


8. Verify that the results are within the values reported in the Assay Values sheet, provided with the controls.
9. Set the run pointer to the first patient specimen and select **Run from Pointer** from the **Run** menu.

Before acquiring samples, adjust the threshold to minimize debris and ensure populations of interest are included.

10. Review the assay lab report.

    Page 1 of the lab report shows dot plots to identify the cell populations. The lab report shown is for BD Multitest™ CD3/CD8/CD45/CD4 without BD Trucount™ Tubes.



BD 3/8/45/4: Lab Report

Sample ID: 313
Sample Name:
Case Number:

Acquired Using: Worklist_002
Cytometer: BD FACSLyric
Sample Preparer:
Operator:Admin User

Approved: 10/23/2019 2:51:15 PM
Cytometer SN: Z654587P021
Sample Preparer SN:
Director:
Department: None

Entry Status: Approved

Software: BD FACSuite Clinical v1.4
Institution: None
Address:

Tube Name: CD3/8/45/4

Events Acquired	11,384	Acquisition Date	10/23/2019
Reagent Lot ID	Multitest CD3/CD8/CD45/CD4 Lot ID: 910482	Acquisition Time	10:38:08 AM
Keyword 1	<no value>	Keyword 2	<no value>
WBC (x1000)	<no value>	Lymphs (%)	<no value>
Lymphs (x1000)	<no value>		

For In Vitro Diagnostic Use.

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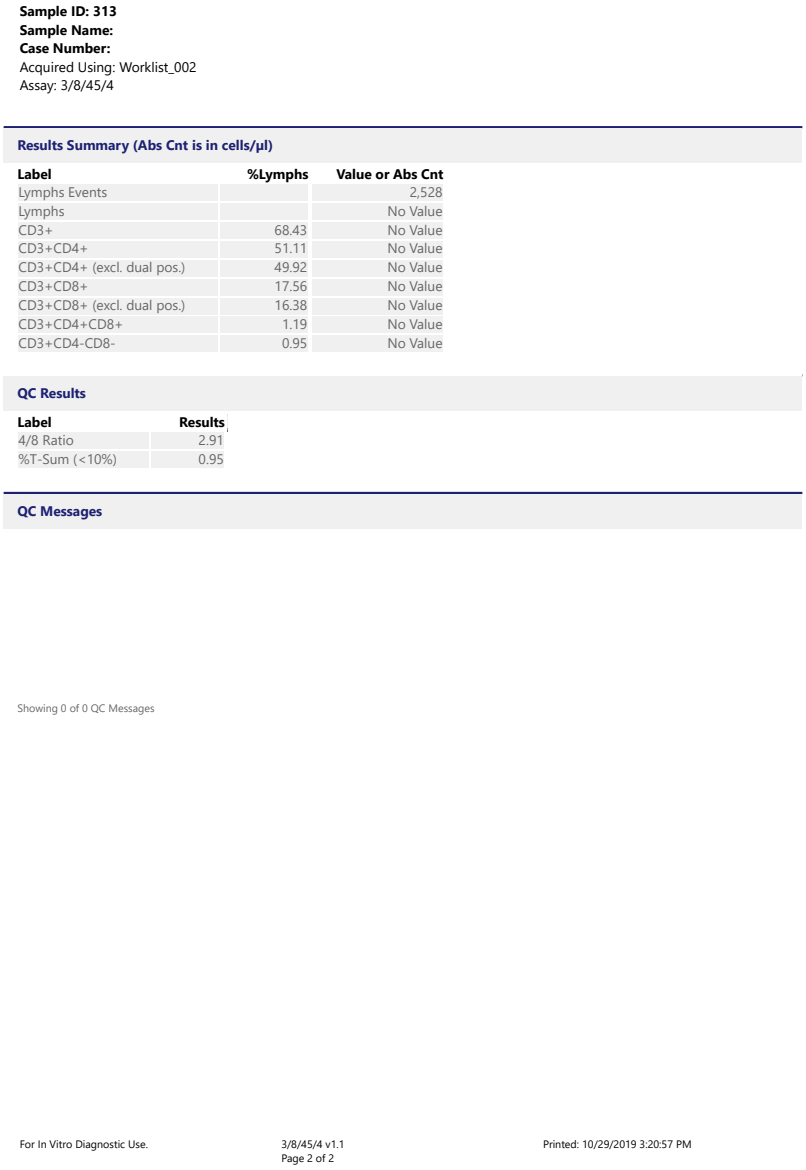
Page 2 of the lab report summarizes the results, presents QC results for the assay, and presents any QC messages that were triggered.



Sample ID: 313
Sample Name:
Case Number:
Acquired Using: Worklist_002
Assay: 3/8/45/4

Results Summary (Abs Cnt is in cells/µl)

Label	%Lymphs	Value or Abs Cnt
Lymphs Events		2,528
Lymphs		No Value
CD3+	68.43	No Value
CD3+CD4+	51.11	No Value
CD3+CD4+ (excl. dual pos.)	49.92	No Value
CD3+CD8+	17.56	No Value
CD3+CD8+ (excl. dual pos.)	16.38	No Value
CD3+CD4+CD8+	1.19	No Value
CD3+CD4-CD8-	0.95	No Value

QC Results

Label	Results
4/8 Ratio	2.91
%T-Sum (<10%)	0.95

QC Messages

Showing 0 of 0 QC Messages

For In Vitro Diagnostic Use. 3/8/45/4 v1.1 Page 2 of 2 Printed: 10/29/2019 3:20:57 PM

See the *BD FACSLyric™ System Instructions for Use* or the *BD FACSLyric™ Clinical Reference System* for more information.

Running the Panel on a BD FACSCanto™ II Flow Cytometer

1. Run Setup using BD FACS™ 7-Color Setup Beads.
   See the *BD FACSCanto™ II Instructions for Use* for more information.
2. Add a BD Multitest™ CD3/CD8/CD45/CD4 panel entry for each process control and patient sample.

   NOTE The word "Control" must appear in the sample name of the process controls.

3. Acquire the process control samples.
4. Vortex each tube thoroughly at low speed immediately before acquiring it. It is important to reduce aggregation before running samples on the flow cytometer.

   NOTE If you are using the BD FACS™ Loader, vortex tubes immediately before placing them into the Loader racks.
5. Verify that the process control values are within the manufacturer's expected ranges.
6. Acquire the patient samples.

7. Review the assay lab report.

   The lab report shows dot plots to identify the cell populations, a table summarising the results, QC results, and any QC messages that were triggered. The lab report shown is for BD Multitest™ CD3/CD8/CD45/CD4 without BD Trucount™ Tubes.

d7670ii

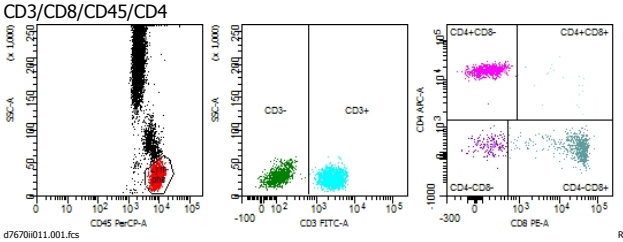
Director:

Panel: 3/8/45/4
Acquired: 10/28/2015 3:17:20 PM
Analyzed: 10/28/2015 3:17:20 PM
Status: OK
Operator: Lab Manager
Reviewer: 28102015.csv
Results:

WBC Count (x1000): | Lymphs (%): | Lymphs (x1000): 1.5

BD FACSCanto II V96300004 | BD FACSCanto v.3.1.5773.39345

CD3/CD8/CD45/CD4 | Total Events: 10058



d7670ii011.001.fcs | Reagent Lot ID: 00000

Parameter	Percent	Value/AbsCnt
Lymph Events		2600
CD3+	61.15	917.31
CD3+CD8+ (Excl. dual pos.)	**27.96**	**419.42**
CD3+CD4+ (Excl. dual pos.)	**26.23**	**393.46**
CD3+CD4-CD8-	**6.12**	**91.73**
CD3+CD4+CD8+	0.85	12.69
CD3+CD8+	28.81	432.12
CD3+CD4+	27.08	406.15
CD45+		1500
4/8 Ratio (Excl. dual pos.)		**0.94**
4/8 Ratio		0.94

QC Messages
% T-Sum is: 5.27
4/8 ratio is: 0.94
% T-Sum (Excl. dual pos.) is: 6.12
4/8 ratio (Excl. dual pos.) is: 0.94

Comments

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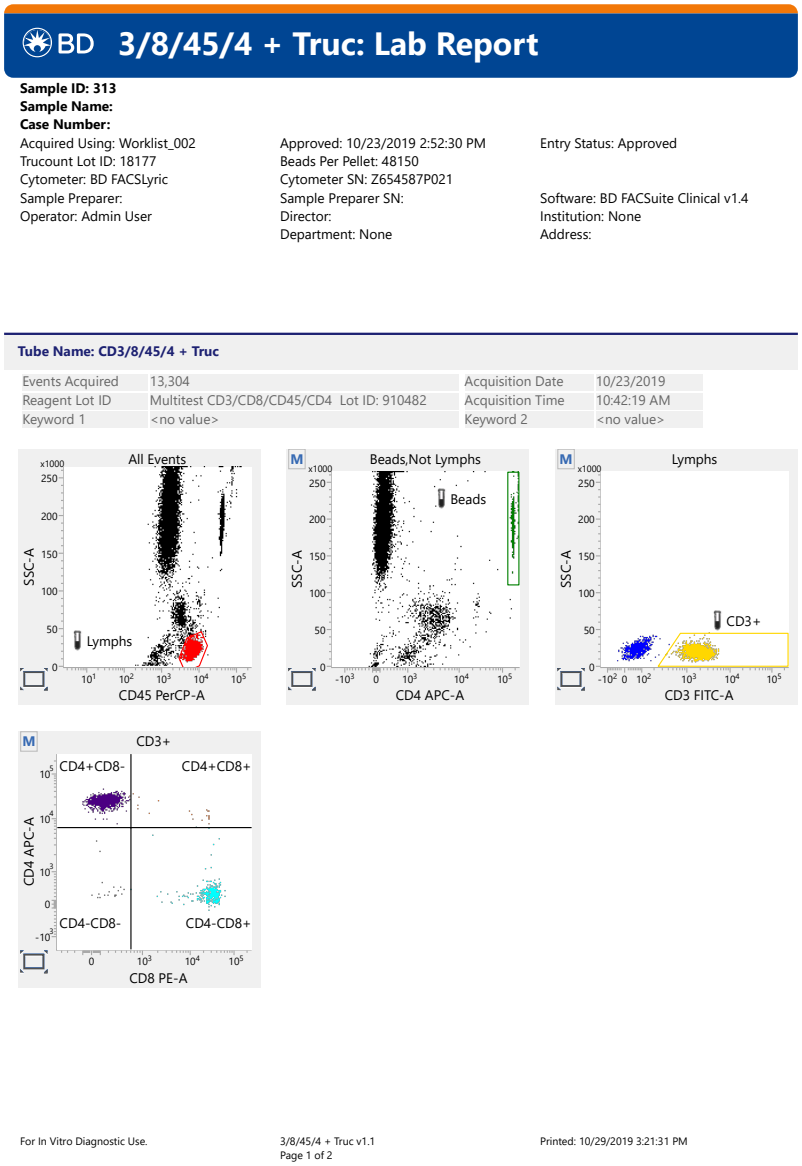
7. RESULTS

Representative Data

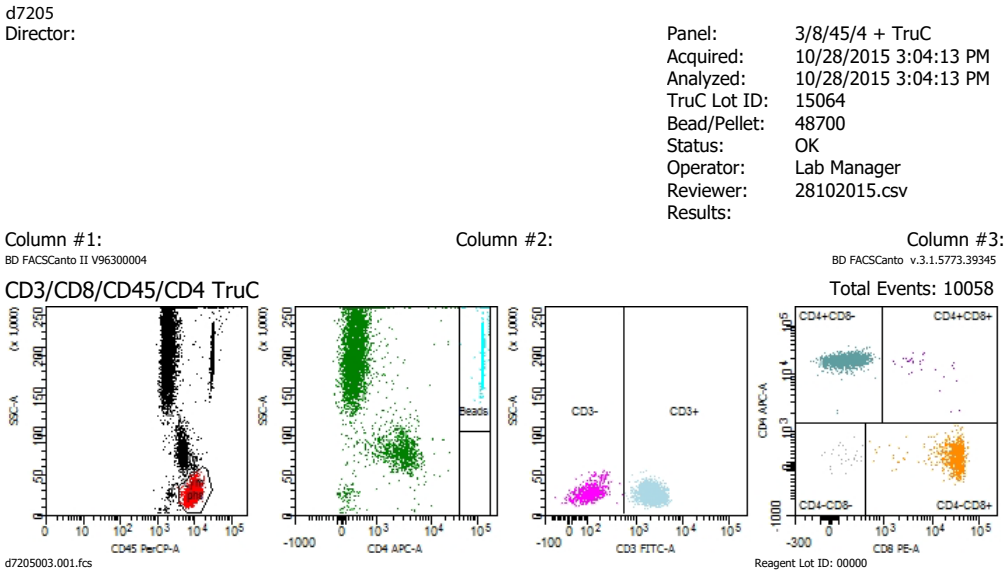
A hematologically normal adult sample stained with BD Multitest™ CD3/CD8/CD45/CD4 in a BD Trucount™ Tube was acquired on a BD FACSLyric™ flow cytometer. See Figure 1.

Figure 1 BD FACSLyric™ laboratory report showing data collected with BD Trucount™ Tubes.



A similar sample was acquired on a BD FACSCanto™ II flow cytometer.

Figure 2 BD FACSCanto™ II laboratory report showing data collected with BD Trucount™ Tubes.



The lymphocyte subsets are identified using the following gating strategy:

Table 5 Gating strategy for BD Multitest™ CD3/CD8/CD45/CD4

Dot plot	Parent population	Gate	Populations identified
CD45 PerCP-A vs SSC-A	All Events	Lymphs	Lymphocytes
CD4 APC-A vs SSC-A	Beads, Not Lymphs	Beads	Trucount beads
CD3 FITC-A vs SSC-A	Lymphs	$CD3^+$	$CD3^+$ T lymphocytes
CD8 PE-A vs CD4 APC-A	$CD3^+$	Quadrant	$CD4^+CD8^-$ $CD4^+CD8^+$ $CD4^-CD8^+$ $CD4^-CD8^-$

The second dot plot, used to identify Trucount™ beads, is present in the 3/8/45/4 + Truc Lab Report only.

See the *BD FACSLyric™ Clinical Reference System*, which provides information on gating and troubleshooting.

Calculating Absolute Counts

When using cytometer-specific BD software, results show positive cells as a percentage of lymphocytes. In addition, the software uses one of two methods to calculate absolute counts of positive cells per microliter of blood (cells/µL).

Single Platform Method

When BD Trucount™ Tubes are used, the absolute number of positive cells in the sample can be determined by comparing cellular events to bead events. The software calculates absolute counts using the following formula:

$$\frac{\text{\# events in cell population}}{\text{\# events in absolute count bead region}} \times \frac{\text{\# beads/test}}{\text{test volume}} = \text{cell population absolute count}$$

The # beads/test is found on the BD Trucount™ Tubes foil pouch label and varies from lot to lot.

Dual Platform Method

This method is used when using 12 × 75-mm polystyrene tubes (or equivalent) instead of BD Trucount™ Tubes. When creating the worklist, enter values for either the lymphocyte count, or the WBC count and the percentage of lymphocytes, as determined by a hematology analyzer or another method. See the instructions for use for your instrument for more information. The software uses one of the following formulas to calculate absolute counts:

- User provides lymphocyte count per µL.

$$\frac{\text{\# events in cell population} \times \text{lymphocyte count per µL}}{\text{\# lymphocytes acquired}} = \text{cell population absolute count}$$

- User provides WBC count per µL and percentage of lymphocytes.

$$\frac{\text{\# events in cell population} \times \text{WBC count} \times (\%\text{lymphocytes}/100)}{\text{\# lymphocytes acquired}} = \text{cell population absolute count}$$

NOTE The accuracy of the absolute counts determined with the Dual Platform Method depends upon the accuracy of the values entered into the software.

8. LIMITATIONS

- Laboratories must establish their own normal reference intervals for the lymphocyte subsets identified using BD Multitest™ CD3/CD8/CD45/CD4. Age, gender, clinical characteristics, and ethnicity of patients should be known when a reference interval is determined.[47] The provided reference intervals are for information only.
- BD Multitest™ CD3/CD8/CD45/CD4 is not intended for screening samples for the presence of leukemic cells or for immunophenotyping samples from leukemia patients.
- Absolute counts are not comparable between laboratories using different manufacturers' equipment.
- BD Multitest™ CD3/CD8/CD45/CD4 with BD Trucount™ Tubes has not been validated by BD Biosciences for use with heparin or acid citrate dextrose (ACD) liquid anticoagulants to determine absolute counts.

9. REFERENCE INTERVALS

Reference intervals for BD Multitest™ CD3/CD8/CD45/CD4 with and without BD Trucount™ Tubes were determined in a study using the BD FACSLyric™ flow cytometer.[41] The study objective was to establish device reference interval values in stained peripheral blood from a healthy cohort of male and female adults that are free of hematological abnormality. Device reference interval refers to a specified interval of the distribution of lymphocyte subset absolute count and percent values taken from a biological reference population. Blood from a population of healthy control subjects was stained with the BD Multitest™ CD3/CD8/CD45/CD4 with BD Trucount™ Tubes, and then acquired and analyzed on a BD FACSLyric™ flow cytometer using BD FACSuite™ Clinical application. See the first limitation (in the preceding section) for more information about reference intervals.

Table 6 Representative reference intervals for BD Multitest™ CD3/CD8/CD45/CD4

Lymphocyte subset	N[a]	Units	Mean	95% range
$CD3^+$	130	%	72.00	56.65–83.36
		cells/µL	1,551.28	840–2,641
$CD3^+CD4^+$	130	%	46.51	32.42–63.19
		cells/µL	1,003.50	488–1,711
$CD3^+CD8^+$	130	%	23.25	8.99–38.99
		cells/µL	514.19	154–1,097
a. N = number of samples				

10. 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 BD Multitest™ CD3/CD8/CD45/CD4 with BD Trucount™ Tubes. The stability of EDTA-anticoagulated blood was evaluated by assessing the combined effect of:

- AOB: Time duration between specimen draw and staining
- AOS: Time duration between staining specimen (end of lysis) and acquiring stained sample

Whole blood specimens were tested to at least 51 hours post draw and stained samples were tested to at least 26 hours post stain. All samples were maintained at room temperature (20–25 °C) before staining or acquisition.

Based on the results of this study, we recommend staining samples within 48 hours of draw and analyzing samples within 24 hours of staining.

Limit of blank and limit of detection

The detection capability of the BD Multitest™ CD3/CD8/CD45/CD4 reagents on the BD FACSLyric™ flow cytometer was assessed at one site. Samples were prepared manually or using the BD FACSDuet™ system. Limit of Blank (LOB) refers to the highest apparent absolute count values that can be detected in a stained sample containing no lymphocytes. Limit of Detection (LOD) refers to the lowest absolute count values that can be detected above zero in a stained sample containing a very low $CD3^+CD4^+$ lymphocyte concentration.

Cell-free plasma samples were used to estimate LOB. Plasma samples containing 10 ±5 $CD3^+CD4^+$ cells/µL were used to estimate LOD. Sixty replicates of each sample type were stained manually or using the BD FACSDuet™ system with each of three reagent lots.

Three BD FACSLyric™ flow cytometers were used to acquire the manually prepared samples. A minimum of one BD FACSDuet™ system integrated with a BD FACSLyric™ flow cytometer was used in the other study. Absolute count values for LOB and LOD are shown in the following table.

Table 7 Detection capability of BD Multitest™ CD3/CD8/CD45/CD4 (LOB and LOD)

	Manual sample preparation		Sample preparation with BD FACSDuet™ system	
Lymphocyte subset	**LOB (cells/µL)**	**LOD (cells/µL)**	**LOB (cells/µL)**	**LOD (cells/µL)**
$CD3^+$	4	9	2	8
$CD3^+CD4^+$	4	8	2	6
$CD3^+CD8^+$	4	11	2	6

Limit of quantitation

The limit of quantitation (LOQ) of the BD Multitest™ CD3/CD8/CD45/CD4 reagents on the BD FACSLyric™ flow cytometer was assessed at one site. Samples were prepared manually or using the BD FACSDuet™ system. LOQ refers to the lowest lymphocyte absolute count values that can be quantitatively detected with stated accuracy in samples containing a range of very low $CD3^+CD4^+$ concentration. Plasma samples containing 10, 20, 30, or 50 $CD3^+CD4^+$ cells/µL were used to estimate LOQ.

In the study on the BD FACSLyric™ flow cytometer, 40 replicates of samples from each of the four concentration levels were stained using two lots of the BD Multitest™ CD3/CD8/CD45/CD4 reagents. For the comparator system, 10 of the 40 replicates from each concentration level were stained and acquired on a BD FACSCanto™ II flow cytometer. Three BD FACSLyric™ flow cytometers and one BD FACSCanto™ II flow cytometer were used in the study.

In the study using the BD FACSDuet™ system, 10 replicates from each concentration level were stained with three lots of the reagents using the BD FACSDuet™ system and acquired using an integrated BD FACSLyric™ flow cytometer. For the comparator system, five replicates from each concentration level were stained manually with three lots of the reagents and acquired on a BD FACSLyric™ flow cytometer. Three integrated BD FACSDuet™–BD FACSLyric™ systems and one standalone BD FACSLyric™ flow cytometer were used in the study. Absolute count values for LOQ are shown in the following table.

Table 8 Detection capability of BD Multitest™ CD3/CD8/CD45/CD4 (LOQ)

	Manual sample preparation (first study)	Sample preparation with BD FACSDuet™ system (second study)
Lymphocyte subset	**LOQ (cells/µL)**	**LOQ (cells/µL)**
$CD3^+$	15	17
$CD3^+CD4^+$	10	11
$CD3^+CD8^+$	11	10

BD FACSLyric™ Flow Cytometer

Method comparison, BD FACSLyric™ vs BD FACSCanto™ II flow cytometer

A study was performed at five sites to demonstrate equivalency between acquisition using the BD FACSLyric™ flow cytometer and the BD FACSCanto™ II flow cytometer. Peripheral blood specimens were collected from normal donors and HIV-infected individuals using BD Vacutainer® EDTA blood collection

tubes. Specimens were stained using BD Multitest™ CD3/CD8/CD45/CD4 in BD Trucount™ Tubes and acquired on a BD FACSLyric™ flow cytometer using the BD FACSuite™ Clinical application. Lymphocyte subset percentages and absolute counts were enumerated. The results were compared with results from the same samples acquired on a BD FACSCanto™ II flow cytometer using BD FACSCanto™ Clinical Software.

Method comparison statistics are reported for all cell subsets.[48] See the following table.

Table 9 Method comparison statistics for lymphocyte subsets (BD FACSLyric™ flow cytometer)

Lymphocyte subset	N	Units	R^2	Slope	Intercept	Range
$CD3^+$	362	%	0.99	1.00	0.68	1.29–98.35
		cells/µL	0.99	1.03	3.18	6–9,197
$CD3^+CD4^+$	362	%	1.00	1.01	–0.22	0.12–97.72
		cells/µL	1.00	1.03	–0.05	1–7,739
$CD3^+CD8^+$	362	%	1.00	1.00	–0.08	0.22–82.93
		cells/µL	0.99	1.02	–1.35	1–5,774

Method comparison, BD FACS™ Universal Loader vs manual acquisition

A single-site study was performed to demonstrate equivalency between acquisition using the BD FACS™ Universal Loader and manual acquisition. Peripheral blood specimens were stained in duplicate using BD Multitest™ CD3/CD8/CD45/CD4 with BD Trucount™ Tubes. Stained samples were acquired on one of three BD FACSLyric™ flow cytometers using either the BD FACS™ Universal Loader or manual acquisition.

The mean, difference, and relative difference for acquisition using the BD FACS™ Universal Loader vs manual acquisition were determined for lymphocyte subset percentages and absolute counts. See the following table.

Table 10 BD FACS™ Universal Loader vs manual acquisition

Lymphocyte subset	N	Units	Mean		Difference	Relative difference
			Loader	Manual		
$CD3^+$	72	%	74.09	73.93	0.16	0.28
		cells/µL	1,504.39	1,501.17	3.22	0.48
$CD3^+CD4^+$	72	%	28.46	28.55	–0.09	–0.78
		cells/µL	567.62	572.35	–4.72	–0.41
$CD3^+CD8^+$	72	%	43.04	42.92	0.12	0.46
		cells/µL	887.74	882.47	5.26	0.60

Method comparison, standalone BD FACSLyric™ vs BD FACSLyric™ with BD FACSDuet™ system

Peripheral blood specimens were collected at three clinical study sites. An aliquot of each specimen was stained with BD Multitest™ CD3/CD8/CD45/CD4 in a BD Trucount™ Tube using the BD FACSDuet™ system. Stained samples were automatically transferred to an integrated BD FACSLyric™ flow cytometer and acquired using a BD FACS™ Universal Loader and BD FACSuite™ Clinical application. A second aliquot of each specimen was stained manually with BD Multitest™ CD3/CD8/CD45/CD4 in a BD Trucount™ Tube. Stained samples were acquired on a standalone BD FACSLyric™ flow cytometer using a BD FACS™ Universal Loader and BD FACSuite™ Clinical application.

Results were compared between samples prepared using the BD FACSDuet™ system and samples prepared manually. See the following table.

Table 11 Method comparison statistics for lymphocyte subsets

Lymphocyte subset	N	Units	R^2	Slope	Intercept	Range
$CD3^+$	373	%	0.98	0.99	0.54	45.3–99.21
		cells/µL	0.98	1.00	5.73	93–11,138
$CD3^+CD4^+$	373	%	0.99	1.00	–0.02	0.37–91.86
		cells/µL	0.99	1.00	–0.15	4–7,911
$CD3^+CD8^+$	373	%	0.99	0.99	–0.01	2.52–86.68
		cells/µL	0.98	0.99	3.16	52–5,796

Precision (repeatability), control material (standalone BD FACSLyric™ flow cytometer)

A 21-day single-site precision study was performed to assess repeatability and within-site precision using control material.[49] Estimates of precision for the enumeration of lymphocyte subset percentages and absolute counts were determined across four BD FACSLyric™ flow cytometers and four operators by acquiring two concentrations of analyte, CD-Chex Plus® control (CDN) and CD-Chex Plus® CD4 Low control (CDL), stained in duplicate using four lots of BD Multitest™ CD3/CD8/CD45/CD4. Two separate runs were analyzed during each of the 21 tested days.

The following tables present the standard deviation (SD) or coefficient of variation (%CV) for repeatability and within-site precision of lymphocyte subset percentages and absolute counts using control material, respectively.

Table 12 Repeatability and within-site precision of lymphocyte subset percentages in normal analyte concentration (CDN)

Lymphocyte subset	Mean (%)	Repeatability (SD)	Within-site precision (SD)
$CD3^+$	76.81	0.80	0.83
$CD3^+CD4^+$	50.74	1.01	1.02
$CD3^+CD8^+$	22.22	0.80	0.80

Table 13 Repeatability and within-site precision of lymphocyte subset percentages in low analyte concentration (CDL)

Lymphocyte subset	Mean (%)	Repeatability (SD)	Within-site precision (SD)
$CD3^+$	57.31	1.13	1.18
$CD3^+CD4^+$	11.66	0.62	0.64
$CD3^+CD8^+$	40.36	1.04	1.06

Table 14 Repeatability and within-site precision of lymphocyte subset absolute counts in normal analyte concentration (CDN)

Lymphocyte subset	Mean (cells/µL)	Repeatability (%CV)	Within-site precision (%CV)
$CD3^+$	1,729.61	3.85	4.03
$CD3^+CD4^+$	1,142.52	4.04	4.18
$CD3^+CD8^+$	500.42	5.56	5.67

Table 15 Repeatability and within-site precision of lymphocyte subset absolute counts in low analyte concentration (CDL)

Lymphocyte subset	Mean (cells/µL)	Repeatability (%CV)	Within-site precision (%CV)
$CD3^+$	869.06	4.24	4.32
$CD3^+CD4^+$	176.91	6.59	6.67
$CD3^+CD8^+$	612.12	4.55	4.65

Precision (repeatability), control material (BD FACSLyric™ flow cytometer with BD FACSDuet™ system)

A 21-day single-site precision study was performed to assess repeatability and within-site precision when samples were prepared and acquired on the BD FACSLyric™ flow cytometer with BD FACSDuet™ sample preparation system using control material. Estimates of precision for the enumeration of lymphocyte subset percentages and absolute counts were determined across three BD FACSDuet™ systems, each integrated with a BD FACSLyric™ flow cytometer, and at least three operators by acquiring two concentrations of analyte, CD-Chex Plus control (CDN) and CD-Chex Plus CD4 Low control (CDL), stained in duplicate using three lots of BD Multitest™ CD3/CD8/CD45/CD4. Two separate runs were analyzed during each of the 21 tested days for a total of 42 runs.

The following tables present standard deviations (SDs) and coefficients of variation (%CVs) for within-site precision and repeatability of lymphocyte subset percentages and absolute counts, respectively.

Table 16 Repeatability and within-site precision of lymphocyte subset percentages in normal analyte concentration (CDN)

Lymphocyte subset	Mean (%)	Repeatability (SD)	Within-site precision (SD)
$CD3^+$	77.43	0.88	0.88
$CD3^+CD4^+$	48.79	0.96	0.96
$CD3^+CD8^+$	26.77	0.91	0.91

Table 17 Repeatability and within-site precision of lymphocyte subset percentages in low analyte concentration (CDL)

Lymphocyte subset	Mean (%)	Repeatability (SD)	Within-site precision (SD)
$CD3^+$	63.69	1.02	1.03
$CD3^+CD4^+$	14.94	0.68	0.70
$CD3^+CD8^+$	44.04	1.11	1.12

Table 18 Repeatability and within-site precision of lymphocyte subset absolute counts in normal analyte concentration (CDN)

Lymphocyte subset	Mean (cells/µL)	Repeatability (%CV)	Within-site precision (%CV)
$CD3^+$	1,751.48	5.10	6.86
$CD3^+CD4^+$	1,103.49	5.23	6.88
$CD3^+CD8^+$	605.62	6.23	7.70

Table 19 Repeatability and within-site precision of lymphocyte subset absolute counts in low analyte concentration (CDL)

Lymphocyte subset	Mean (cells/µL)	Repeatability (%CV)	Within-site precision (%CV)
$CD3^+$	738.14	4.06	4.92
$CD3^+CD4^+$	173.18	5.97	6.57
$CD3^+CD8^+$	510.44	4.40	5.28

Precision (repeatability), peripheral blood (standalone BD FACSLyric™ flow cytometer)

A single-site precision study was performed to evaluate system repeatability and within-site precision using 44 donor samples. Each donor sample was stained in duplicate using BD Multitest™ CD3/CD8/CD45/CD4 in BD Trucount™ Tubes and run on 12 instruments for a total of 24 runs per sample.

The following tables present the standard deviation (SD) or coefficient of variation (%CV) for repeatability and within-site precision of lymphocyte subset percentages and absolute counts using peripheral blood, respectively.

Table 20 Repeatability and within-site precision of lymphocyte subset percentages

Lymphocyte subset	Mean (%)	Repeatability (SD)	Within-site precision (SD)
$CD3^+$	73.54	0.96	0.96
$CD3^+CD4^+$	33.46	0.83	0.83
$CD3^+CD8^+$	37.93	0.93	0.93

Table 21 Repeatability and within-site precision of lymphocyte subset absolute counts

Lymphocyte subset	Mean (cells/µL)	Repeatability (%CV)	Within-site precision (%CV)
$CD3^+$	1,400.10	4.49	4.61
$CD3^+CD4^+$	633.59	5.32	5.40
$CD3^+CD8^+$	726.59	5.42	5.53

Precision (repeatability), peripheral blood (BD FACSLyric™ flow cytometer with BD FACSDuet™ system)

A single-site precision study was performed to evaluate system repeatability and within-site precision using 27 donor specimens. Each donor specimen was stained in duplicate using three lots of BD Multitest™ CD3/CD8/CD45/CD4 in BD Trucount™ Tubes and run on three BD FACSDuet™ instruments, each integrated with a BD FACSLyric™ flow cytometer, for a total of 18 runs per sample.

Table 22 Repeatability and within-site precision of lymphocyte subset percentages

Lymphocyte subset	Mean (%)	Repeatability (SD)	Within-site precision (SD)
$CD3^+$	76.62	0.91	0.91
$CD3^+CD4^+$	31.18	0.89	0.89
$CD3^+CD8^+$	44.04	1.01	1.05

Table 23 Repeatability and within-site precision of lymphocyte subset absolute counts

Lymphocyte subset	Mean (cells/µL)	Repeatability (%CV)	Within-site precision (%CV)
$CD3^+$	1,566.95	4.13	4.43
$CD3^+CD4^+$	636.55	5.12	5.38
$CD3^+CD8^+$	905.37	5.17	5.65

Precision (reproducibility), control material (standalone BD FACSLyric™ flow cytometer)

A study was performed at four clinical sites to assess reproducibility of BD Multitest™ CD3/CD8/CD45/CD4. A single lot of each process control, CD-Chex Plus control (CDN) and CD-Chex Plus CD4 Low control (CDL), was provided to each site. The control samples were stained using BD Multitest™ CD3/CD8/CD45/CD4. Two separate runs were analyzed during each of five non-consecutive tested days for a total of 10 runs.

The following tables present the standard deviation (SD) or coefficient of variation (%CV) for reproducibility of lymphocyte subset percentages and absolute counts, respectively.

Table 24 Reproducibility of BD Multitest™ CD3/CD8/CD45/CD4 for lymphocyte subset percentages in normal analyte concentration (CDN)

Lymphocyte subset	Mean (%)	SD
$CD3^+$	76.64	0.91
$CD3^+CD4^+$	51.67	1.58
$CD3^+CD8^+$	23.23	0.85

Table 25 Reproducibility of lymphocyte subset percentages in low analyte concentration (CDL)

Lymphocyte subset	Mean (%)	SD
$CD3^+$	57.14	1.21
$CD3^+CD4^+$	12.12	0.61
$CD3^+CD8^+$	40.74	1.12

Table 26 Reproducibility of BD Multitest™ CD3/CD8/CD45/CD4 for lymphocyte subset absolute counts in normal analyte concentration (CDN)

Lymphocyte subset	Mean (cells/µL)	%CV
$CD3^+$	1,746.97	4.65
$CD3^+CD4^+$	1,177.59	5.17
$CD3^+CD8^+$	529.63	6.05

Table 27 Reproducibility of BD Multitest™ CD3/CD8/CD45/CD4 for lymphocyte subset absolute counts in low analyte concentration (CDL)

Lymphocyte subset	Mean (cells/µL)	%CV
$CD3^+$	881.62	5.03
$CD3^+CD4^+$	187.01	7.30
$CD3^+CD8^+$	628.51	5.23

Precision (reproducibility), control material (BD FACSLyric™ flow cytometer with BD FACSDuet™ system)

A study was performed at three clinical sites to assess reproducibility of BD Multitest™ CD3/CD8/CD45/CD4. A single lot of each process control, CD-Chex Plus control (CDN) and CD-Chex Plus CD4 Low control (CDL), was provided to each site. The control samples were stained using three lots of BD Multitest™ CD3/CD8/CD45/CD4 with one lot of BD Trucount™ Tubes using the BD FACSDuet™ system and automatically transferred to an integrated BD FACSLyric™ flow cytometer and acquired using the BD FACS™ Universal Loader. Two separate runs were performed each day. Results obtained over 15 non-consecutive test days were analyzed.

The following tables present standard deviations (SDs) and coefficients of variation (%CVs) for reproducibility of lymphocyte subset percentages and absolute counts, respectively.

Table 28 Reproducibility of lymphocyte subset percentages in normal analyte concentration (CDN)

Lymphocyte subset	Mean (%)	SD
$CD3^+$	75.99	0.85
$CD3^+CD4^+$	49.83	0.91
$CD3^+CD8^+$	24.60	0.73

Table 29 Reproducibility of lymphocyte subset percentages in low analyte concentration (CDL)

Lymphocyte subset	Mean (%)	SD
$CD3^+$	57.15	1.02
$CD3^+CD4^+$	9.97	0.61
$CD3^+CD8^+$	42.94	1.05

Table 30 Reproducibility of lymphocyte subset absolute counts in normal analyte concentration (CDN)

Lymphocyte subset	Mean (cells/µL)	%CV
$CD3^+$	1,987.36	6.39
$CD3^+CD4^+$	1,303.23	6.62
$CD3^+CD8^+$	643.31	7.28

Table 31 Reproducibility of lymphocyte subset absolute counts in low analyte concentration (CDL)

Lymphocyte subset	Mean (cells/µL)	%CV
$CD3^+$	957.91	6.32
$CD3^+CD4^+$	167.16	8.47
$CD3^+CD8^+$	719.77	6.68

Linearity (BD FACSLyric™ flow cytometer with and without BD FACSDuet™ system)

Linearity was assessed for the BD FACSLyric™ flow cytometer, with and without an integrated BD FACSDuet™ system, using triplicate measurements of 11 equally spaced concentrations of WBCs. Lymphocyte subsets were observed to be linear across the following ranges.

Table 32 Linear ranges of lymphocyte subsets

	Range (cells/µL)	
Lyphocyte subset	**BD FACSLyric™**	**BD FACSLyric™ with BD FACSDuet™**
$CD3^+$	3–5,148	4–5,318
$CD3^+CD4^+$	1–3,184	3–3,016
$CD3^+CD8^+$	7–3,480	2–3,130

Measuring range (BD FACSLyric™ flow cytometer with and without BD FACSDuet™ system)

The analytical measurement range (AMR) for BD Multitest™ CD3/CD8/CD45/CD4 on the BD FACSLyric™ flow cytometer was determined. To establish the measuring range of the BD Multitest™ CD3/CD8/CD45/CD4, data was taken from the following:

- The LOQ studies using the BD FACSLyric™ flow cytometer with and without the BD FACSDuet™ system.
- The method comparison study between the BD FACSLyric™ and the BD FACSCanto™ II flow cytometers.
- The method comparison study between the standalone BD FACSLyric™ flow cytometer and the BD FACSLyric™ with BD FACSDuet™ system.

The lower end of the AMR was determined based on results from the limit of quantitation (LoQ) studies and the upper end of the AMR was determined based on results from the method comparison studies.

Table 33 BD Multitest™ CD3/CD8/CD45/CD4 measuring range

Lymphocyte subset	Analytical measuring range (cells/µL)
$CD3^+$	17–5,000
$CD3^+CD4^+$	11–3,000
$CD3^+CD8^+$	11–3,000

BD FACSCanto™ II Flow Cytometer

Method comparison, BD FACSCanto™ II vs BD FACSCanto™ flow cytometer

Lymphocyte subset percentages and absolute counts were enumerated with BD Multitest™ CD3/CD8/CD45/CD4 in BD Trucount™ Tubes and analyzed on the BD FACSCanto™ II flow cytometer using BD FACSCanto™ Clinical Software v2.1. The results were compared with results from the same samples analyzed on the BD FACSCanto™ flow cytometer using BD FACSCanto™ Clinical Software v2.0.

Peripheral blood samples were collected at random at two clinical laboratories. Method comparison statistics are reported in the following table.

Table 34 Method comparison statistics for subset percentages and absolute counts (BD FACSCanto™ II vs BD FACSCanto™ flow cytometer)

Lymphocyte subset	N	Units	R^2	Slope	Intercept	Range
Average $CD3^+$	104	%	0.984	0.97	2.72	51–92
		cells/µL	0.991	0.97	27.59	221–3,872
$CD3^+CD4^+$	104	%	0.994	1.01	0.20	1–57
		cells/µL	0.986	0.95	18.25	11–1,905
$CD3^+CD8^+$	104	%	0.993	1.00	0.34	11–81
		cells/µL	0.988	0.95	28.36	68–3,577

Precision (repeatability), control material (BD FACSCanto™ II flow cytometer)

A 21-day single-site study was conducted assess repeatability precision. Estimates of precision for the enumeration of lymphocyte subset percentages and absolute counts were determined across three instruments and at least three operators by acquiring two concentrations of analyte, CD-Chex Plus control (CDN) and CD-Chex Plus CD4 Low (CDL) control, stained in duplicate using one lot of BD Multitest™ CD3/CD8/CD45/CD4. Two separate runs were analyzed during each of the 21 tested days for a total of 42 runs.

The following tables provide SDs and %CVs for subset percentages and absolute counts for repeatability and within-site precision.

Table 35 Repeatability and within-site precision of lymphocyte subset percentages in normal analyte concentration (CDN)

Lymphocyte subset	Mean (%)	Repeatability (SD)	Within-site precision (SD)
Average $CD3^+$	73.0	0.63	0.67
$CD3^+CD4^+$	46.8	0.81	0.82
$CD3^+CD8^+$	25.4	0.78	0.80

Table 36 Repeatability and within-site precision of lymphocyte subset percentages in low analyte concentration (CDL)

Lymphocyte subset	Mean (%)	Repeatability (SD)	Within-site precision (SD)
Average $CD3^+$	54.1	0.96	0.98
$CD3^+CD4^+$	10.3	0.53	0.53
$CD3^+CD8^+$	43.2	1.33	1.34

Table 37 Repeatability and within-site precision of lymphocyte subset absolute counts in normal analyte concentration (CDN)

Lymphocyte subset	Mean (cells/µL)	Repeatability (%CV)	Within-site precision (%CV)
Average $CD3^+$	2,105.4	2.7	2.9
$CD3^+CD4^+$	1,347.1	3.6	3.8
$CD3^+CD8^+$	731.4	4.5	4.7

Table 38 Repeatability and within-site precision of lymphocyte subset absolute counts in low analyte concentration (CDL)

Lymphocyte subset	Mean (cells/µL)	Repeatability (%CV)	Within-site precision (%CV)
Average $CD3^+$	1,086.0	3.5	3.6
$CD3^+CD4^+$	205.6	5.9	5.9
$CD3^+CD8^+$	866.1	3.8	3.9

Linearity (BD FACSCanto™ II flow cytometer)

Linearity of the BD Multitest™ CD3/CD8/CD45/CD4 assay using BD Trucount™ Tubes was assessed for the BD FACSCanto™ II system within a WBC concentration of 0 to 3.8×10^4 cells/µL. Results were observed to be linear across the following range.

Table 39 Linear ranges of lymphocyte subsets

Lymphocyte subset	Range (cells/µL)
Average $CD3^+$	4–5,998
$CD3^+CD4^+$	1–3,669
$CD3^+CD8^+$	2–2,324

11. TROUBLESHOOTING

Problem	Possible Cause	Solution
Poor resolution between debris and lymphocytes.	Cell interaction with other cells and platelets.	Prepare and stain another sample.
	Rough handling during cell preparation.	Check cell viability. Centrifuge cells at lower speed.
	Inappropriate instrument settings.	Follow proper instrument setup procedures. Optimize instrument settings as required.

Problem	Possible Cause	Solution
Staining dim or fading.	Cell concentration too high at staining step.	Check and adjust cell concentration or sample volume. Stain with fresh sample.
	Insufficient reagent.	Repeat staining with increased amount of antibody.
	Cells not analyzed within 24 hours of staining.	Repeat staining with fresh sample. Analyze promptly.
Few or no cells.	Cell concentration too low.	Resuspend fresh sample at a higher concentration. Repeat staining and analysis.
	Cytometer malfunctioning.	Troubleshoot instrument.

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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.

Refer to the Eudamed website: https://ec.europa.eu/tools/eudamed for Summary of Safety and Performance.

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HISTORY

Revision	Date	Changes made
23-5351(11)	2022-12	Updated to meet requirements of Regulation (EU) 2017/746.
23-5351(12)	2023-07	Updated legal manufacturer address. Added EU and Swiss importer addresses and importer symbol. Updated symbols glossary.

Symbols Glossary

Please refer to product labeling for applicable symbols.

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
	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
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
Rx 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
12	Collection date
	Keep away from light
H_2	Hydrogen gas is generated
	Perforation
	Start panel sequence number
	End panel sequence number
	Internal sequence number
/	<Box #> / <Total Boxes>
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
	Importer
	Place patient label in framed area only
MR	Magnetic resonance (MR) safe
MR	Magnetic resonance (MR) conditional
MR	Magnetic resonance (MR) unsafe
For use with	For use with
This Product Contains Dry Natural Rubber	This Product Contains Dry Natural Rubber
For Export Only	For Export Only
Instruments	Instruments

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

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