BD Tritest™ CD4/CD8/CD3

50 Tests per kit with
BD Trucount™ Tubes—Catalog No. 342445

23-5333(10)
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English

CE 2797 IVD

1. INTENDED USE

BD Tritest™ CD4/CD8/CD3 reagent with BD Trucount™ Tubes is a three-color direct immunofluorescence reagent for use with a BD FACSLyric™ flow cytometer to identify and determine the percentages and absolute counts of CD4 and CD8 subpopulations of T cells, as well as absolute count of CD3+ T cells, in peripheral blood.

BD Tritest™ CD4/CD8/CD3 can be used with the BD FACS™ Universal Loader.

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 of $CD3^+CD4^+$ or $CD3^+CD8^+$ T lymphocytes 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 and B 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 Tritest™ CD4/CD8/CD3 with 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

The sample acquisition can be automated using the optional BD FACS™ Universal Loader when used with the BD FACSLyric™ flow cytometer. This assay is not for automated sample preparation. 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 Tritest™ CD4/CD8/CD3 reagent is composed of three monoclonal antibodies, each conjugated to a specific fluorochrome. The reagent and a precise volume of peripheral blood are added to a BD Trucount™ Tube and incubated, allowing each monoclonal antibody in the reagent to bind to a specific antigen on the surface of the cells. 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 incubation, BD FACS™ Lysing Solution is added to lyse the red blood cells in the sample. Cells are acquired on the BD FACSLyric™ flow cytometer using BD FACSuite™ Clinical application and the BD Tritest™ assay module. 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 Tritest™ 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. BD FACSuite™ Clinical application determines the absolute cell counts by comparing cellular events to bead events, and reports the absolute cell counts in the lab report.

For BD FACSLyric™ flow cytometer principles of operation, see the *BD FACSLyric™ System Instructions for Use*.

3. REAGENT

Reagent Composition

The reagent contains the following conjugated antibodies:

Table 1 Reagent composition

Antibody	Fluorochrome	Clone	Isotype	Concentration (µg/mL)
CD3	PerCP	SK7[4,5]	$IgG_{1,\kappa}$	6.25
CD4	FITC	SK3[6,7,8]	$IgG_{1,\kappa}$	1.0
CD8	PE	SK1[6,7]	$IgG_{1,\kappa}$	1.5

CD3 (SK7) recognizes the epsilon chain of the CD3 antigen/T-cell antigen receptor (TCR) complex.[9] The CD3 antigen is present on T lymphocytes and is noncovalently associated with either α/β or γ/δ TCR.[10] CD3 reacts minimally with other cell populations.[11]

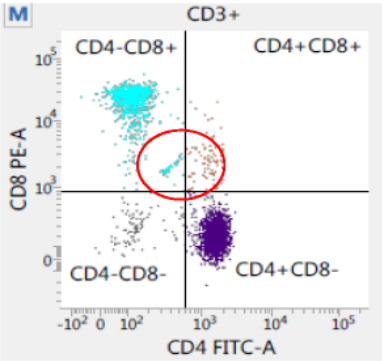
CD4 (SK3) recognizes an antigen that interacts with class II MHC molecules and is the primary receptor for HIV.[12,13] The CD4 antigen is present on helper/inducer T lymphocytes and is present in low density on the cell surface of monocytes and in the cytoplasm of monocytes.[10]

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.[14,15,16] The CD8 antigen is present on suppressor/cytotoxic T lymphocytes. CD8 also recognizes a subset of NK lymphocytes.[17]

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.

- Calibrate pipets to deliver exactly 50 µL of sample or perform the reverse pipetting technique (see Reverse Pipetting on page 5). 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.
- Some samples might show an artifact in the CD4 FITC-A vs CD8 PE-A dot plot. The artifact is seen as a population along a diagonal line in the lower right hand corner of the CD4–CD8+ quadrant. We recommend that you adjust the quadrant gate to exclude the artifact from the CD4–CD8+ quadrant.



- 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.
- 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™ system is outlined in the following table. See the corresponding reagent or instrument user documentation for details.

Table 2 BD FACSLyric™ system

Flow cytometer	Setup beads	Setup software	Analysis software	Assay module
BD FACSLyric™	BD® CS&T Beads BD® FC Beads 7-Color Kit	BD FACSuite™ Clinical application	BD FACSuite™ Clinical application	BD Tritest™

The BD FACS™ Universal Loader can be used with this product.

5. SPECIMEN COLLECTION AND PREPARATION

- Collect blood specimens aseptically by venipuncture into a BD Vacutainer® EDTA blood collection tube, or equivalent.[18]

BD Tritest™ CD4/CD8/CD3 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.

- Store blood specimens at room temperature (20–25 °C).
- Stain specimens within 48 hours of draw.
- Acquire samples within 6 hours of staining.

NOTE If samples are stained within 24 hours of draw, they can be analyzed 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[19,20] 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.[21]

Interference

Substances present in the specimen might interfere with the assay:

- Specimens obtained from patients taking immunosuppressive drugs[22,23,24] or undergoing monoclonal antibody treatment[25,26,27,28,29,30] can yield erroneous results.
- Hemolyzed samples can interfere with the assay and should be rejected.[31] 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.[32]
- Lipemic specimens can interfere with the assay.[33,34]
- Bilirubin interferes at an absorbance peak of 456 nm.[35]

6. PROCEDURE

Reagents and Materials

Reagents and materials provided

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

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* instructions for use (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

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 the 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.[36] 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.

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

Staining the Cells

1. For each sample, remove a BD Trucount™ Tube and label it with the appropriate sample identification.

   We recommend that you stain the control materials, acquire them, and verify that they passed before you start staining the patient specimens.

   NOTE 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 Tritest™ CD4/CD8/CD3 reagent onto the side 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 whole 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 Make sure to 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 5. 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 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 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 6 hours or 24 hours of staining, depending on the age of the specimen. Store the stained sample in the dark at room temperature (20–25 °C) until acquisition.

Acquiring the Sample

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.

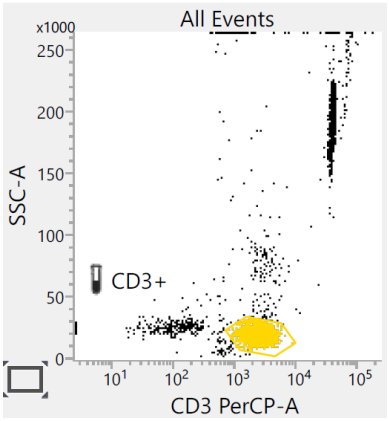
To run the assay:

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.

   Enter the lot ID for the BD Trucount™ 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.[37]

   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 CD3 PerCP-A vs SSC-A dot plot.

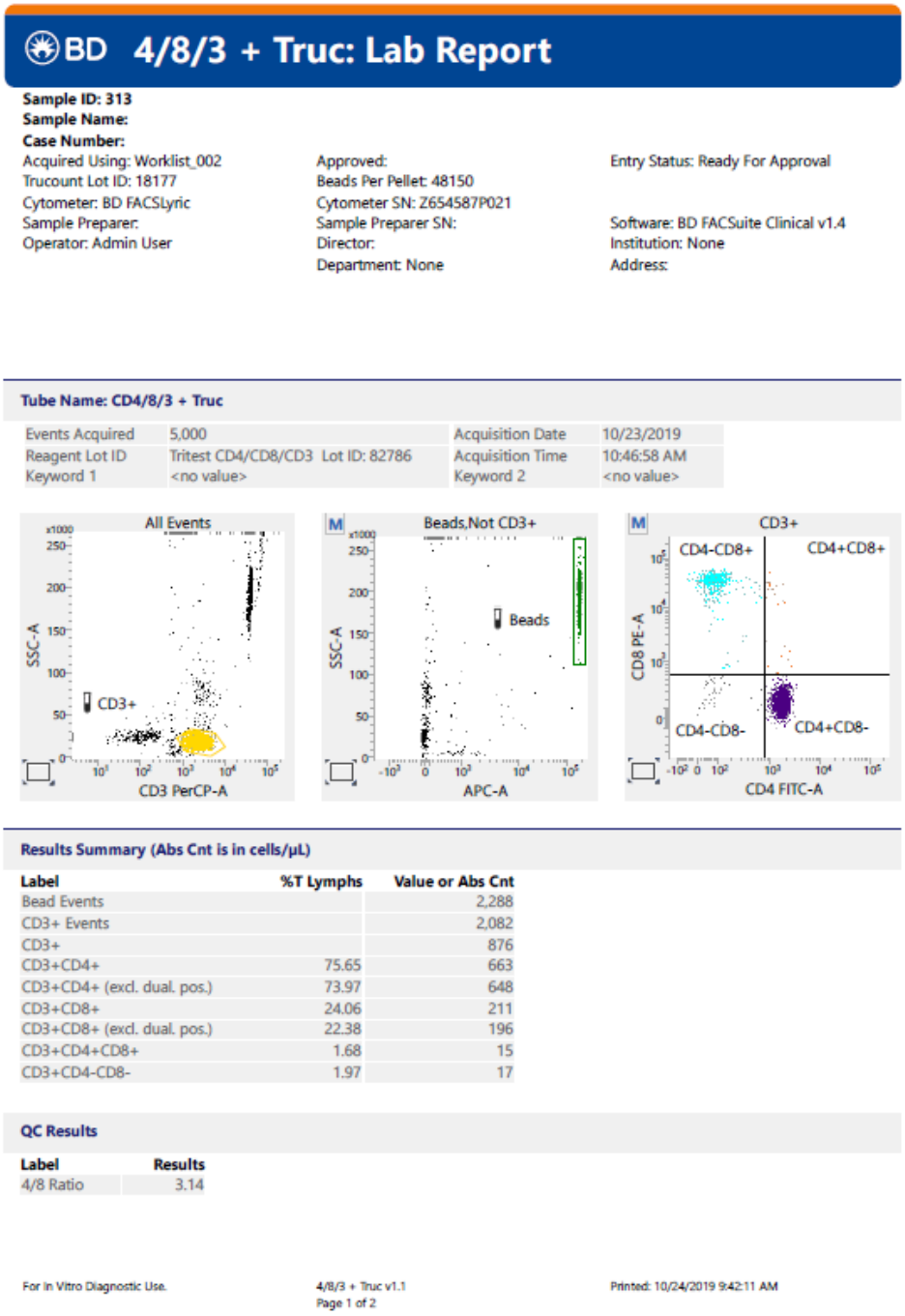


   The CD3$^+$ population should appear as a bright, compact cluster with low SSC. Do not proceed with analysis if the population is diffuse and there is little or no separation between clusters.
8. Verify that the results are within the values reported in the Assay Values sheet, provided with the controls.
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, summarizes the results, and presents QC results for the assay.



BD 4/8/3 + Truc: Lab Report

Sample ID: 313
Sample Name:
Case Number:
Acquired Using: Worklist_002
Trucount Lot ID: 18177
Cytometer: BD FACSLyric
Sample Preparer:
Operator: Admin User

Approved:
Beads Per Pellet: 48150
Cytometer SN: Z654587P021
Sample Preparer SN:
Director:
Department: None

Entry Status: Ready For Approval

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

Tube Name: CD4/8/3 + Truc

Events Acquired	5,000	Acquisition Date	10/23/2019
Reagent Lot ID	Tritest CD4/CD8/CD3 Lot ID: 82786	Acquisition Time	10:46:58 AM
Keyword 1	<no value>	Keyword 2	<no value>

Results Summary (Abs Cnt is in cells/µL)

Label	%T Lymphs	Value or Abs Cnt
Bead Events		2,288
CD3+ Events		2,082
CD3+		876
CD3+CD4+	75.65	663
CD3+CD4+ (excl. dual. pos.)	73.97	648
CD3+CD8+	24.06	211
CD3+CD8+ (excl. dual. pos.)	22.38	196
CD3+CD4+CD8+	1.68	15
CD3+CD4-CD8-	1.97	17

QC Results

Label	Results
4/8 Ratio	3.14

For In Vitro Diagnostic Use.
4/8/3 + Truc v1.1
Page 1 of 2
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Page 2 of the lab report presents any QC messages that were triggered.

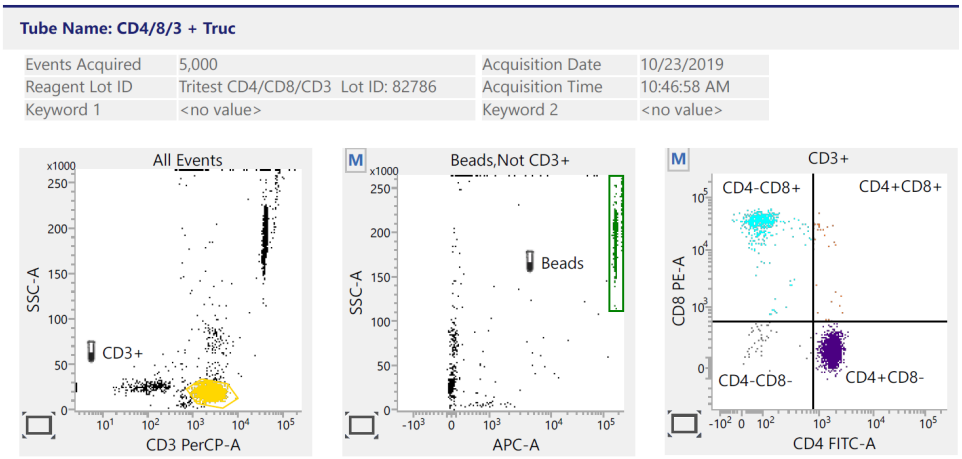
See the *BD FACSLyric™ System Instructions for Use* or the *BD FACSLyric™ Clinical Reference System* for more information.

7. RESULTS

Representative Data

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

Figure 1 Representative data from a hematologically normal adult sample stained with BD Tritest™ CD4/CD8/CD3 in a BD Trucount™ Tube.



The lymphocyte subsets are identified using the following gating strategies:

Table 3 Gating strategy for BD Tritest™ CD4/CD8/CD3

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

Calculating Absolute Counts

BD FACSuite™ Clinical application presents results showing positive cells as a percentage of T lymphocytes. In addition, the application calculates absolute counts of positive cells per microliter of blood (cells/µL) using the following method.

The absolute number of positive cells in the sample can be determined by comparing cellular events to bead events. BD FACSuite™ Clinical application calculates absolute counts using the following formula:

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

* This value is found on the BD Trucount™ Tube foil pouch label and can vary from lot to lot.

8. LIMITATIONS

- Laboratories must establish their own normal reference intervals for the lymphocyte subsets identified using BD Tritest™ CD4/CD8/CD3. Age, gender, clinical characteristics, and ethnicity of patients should be known when a reference interval is determined.[38] The provided reference intervals are for information only.
- BD Tritest™ CD4/CD8/CD3 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 Tritest™ CD4/CD8/CD3 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 Tritest™ CD4/CD8/CD3 with BD Trucount™ Tubes were determined in a study using the BD FACSLyric™ flow cytometer.[38] Subjects were hematologically normal adults. See the first limitation (in the preceding section) for more information about reference intervals.

Table 4 Representative reference intervals for BD Tritest™ CD4/CD8/CD3

T-lymphocyte subset	N[a]	Mean (cells/µL)	Lower 2.5 percentile	Upper 97.5 percentile
$CD3^+$	134	1,562.89	803	2,552
$CD3^+CD4^+$	134	999.70	524	1,655
$CD3^+CD8^+$	134	511.30	157	1,085

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 Tritest™ CD4/CD8/CD3 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 either up to 51 hours post draw with stained samples tested up to 8 hours post stain, or up to 27 hours post draw with stained samples tested up to 27 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 6 hours of staining; or staining samples within 24 hours of draw and analyzing within 24 hours of staining.

Method Comparison, BD FACSLyric™ vs BD FACSCalibur™ Flow Cytometer

Whole blood specimens were collected from normal donors and HIV-infected individuals at four sites. Specimens were stained using BD Tritest™ CD4/CD8/CD3 in BD Trucount™ Tubes and acquired on the BD FACSLyric™ flow cytometer using BD FACSuite™ Clinical application. T-lymphocyte subset percentages and absolute counts were analyzed. The results were compared with results from stained specimens analyzed on the BD FACSCalibur™ flow cytometer using BD Multiset™ software version 1.1 or later.

Method comparison statistics are reported for all cell subsets.[39] See the following table.

Table 5 Method comparison statistics for lymphocyte subsets

Lymphocyte subset	N	Units	R^2	Slope	Intercept	Range
$CD3^+$	299	cells/µL	1.00	1.05	3.31	10-9,019
$CD3^+CD4^+$	299	%	1.00	1.00	0.12	0.57–95.8
		cells/µL	1.00	1.05	–0.24	1–5,257
$CD3^+CD8^+$	299	%	0.99	1.02	1.69	4.49–91.52
		cells/µL	0.99	1.04	–1.26	1–4,612

Method Comparison, BD FACS™ Universal Loader vs Manual Acquisition

A study was performed at one site to demonstrate equivalency between acquisition using the BD FACS™ Universal Loader and manual acquisition. Whole blood specimens were collected from normal donors and HIV-infected individuals and stained using BD Tritest™ CD4/CD8/CD3 in BD Trucount™ Tubes. A total of 106 stained samples were acquired using the BD FACS™ Universal Loader and 76 stained samples were acquired manually.

The mean bias, the lower 95% confidence level (CL) of the mean bias, and the upper 95% CL of the mean bias between acquisition using the BD FACS™ Universal Loader vs manual acquisition were determined for lymphocyte subset percentages and absolute counts.

Table 6 BD FACS™ Universal Loader vs manual acquisition

Lymphocyte subset	Units	Mean (automated acquisition)	Mean % bias	Lower 95% CL of mean % bias	Upper 95% CL of mean % bias
$CD3^+$	cells/µL	1,587.6	3.6	1.6	5.6
$CD3^+CD4^+$	cells/µL	750.8	2.4	–0.5	5.2
	%	47.4	–1.2	–3.0	0.5
$CD3^+CD8^+$	cells/µL	780.2	4.3	2.1	6.5
	%	49.3	0.5	–0.5	1.4

Precision (Repeatability), Control Material

An 11-day single-site precision study was performed to assess repeatability and within-site precision using control material. Estimates of precision for the enumeration of T-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 with three lots of BD Tritest™ CD4/CD8/CD3. Two separate runs were analyzed during each of the 11 tested days.

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

Table 7 Repeatability and within-site precision of lymphocyte subset percentages in normal analyte concentration (CDN)

Lymphocyte subset	Mean (%)	Repeatability (SD)	Within-site precision (SD)
$CD3^+CD4^+$	65.13	1.19	1.20
$CD3^+CD8^+$	32.98	1.18	1.21

Table 8 Repeatability and within-site precision of lymphocyte subset percentages in low analyte concentration (CDL)

Lymphocyte subset	Mean (%)	Repeatability (SD)	Within-site precision (SD)
$CD3^+CD4^+$	22.35	1.11	1.17
$CD3^+CD8^+$	72.76	1.23	1.31

Table 9 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)
$CD3^+$	1,615.83	6.26	6.56
$CD3^+CD4^+$	1,052.39	6.62	6.88
$CD3^+CD8^+$	532.87	7.47	7.67

Table 10 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)
$CD3^+$	835.30	4.29	4.81
$CD3^+CD4^+$	186.70	6.20	6.92
$CD3^+CD8^+$	607.80	4.73	5.20

Precision (Reproducibility), Control Material

A study was performed at three sites to assess reproducibility of the BD Tritest™ CD4/CD8/CD3 reagent. A single lot of each control material, CD-Chex Plus® control and CD-Chex Plus® CD4 Low control, was provided to each of the sites. For each type of control material, three replicates were stained using BD Tritest™ CD4/CD8/CD3. Two separate runs were analyzed during each of 5 nonconsecutive tested days.

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

Table 11 Reproducibility of lymphocyte subset percentages in normal analyte concentration (CDN)

Lymphocyte subset	Mean (%)	SD
$CD3^+CD4^+$	67.10	1.22
$CD3^+CD8^+$	30.43	1.30

Table 12 Reproducibility of lymphocyte subset percentages in low analyte concentration (CDL)

Lymphocyte subset	Mean (%)	SD
$CD3^+CD4^+$	21.68	1.15
$CD3^+CD8^+$	71.36	1.57

Table 13 Reproducibility of lymphocyte subset absolute counts in normal analyte concentration (CDN)

Lymphocyte subset	Mean (cells/µL)	%CV
$CD3^+$	1,721.76	5.62
$CD3^+CD4^+$	1,155.21	5.90
$CD3^+CD8^+$	523.90	7.16

Table 14 Reproducibility of lymphocyte subset absolute counts in low analyte concentration (CDL)

Lymphocyte subset	Mean (cells/µL)	%CV
$CD3^+$	877.3	5.14
$CD3^+CD4^+$	190.17	7.09
$CD3^+CD8^+$	626.07	5.47

Limit of Blank and Limit of Detection

The detection capability of BD Tritest™ CD4/CD8/CD3 on the BD FACSLyric™ flow cytometer was assessed at one site. 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. Cell-free plasma samples reconstituted with peripheral blood mononuclear cells (PBMC) to a concentration of 10 ± 5 $CD3^+CD4^+$ cells/µL were used to estimate LOD. Fifteen replicates were evaluated for each of three reagent lots across 4 days. Three BD FACSLyric™ flow cytometers were used. Absolute count values for LOB and LOD are shown in the following table.

Table 15 Detection capability of BD Tritest™ CD4/CD8/CD3 (LOB and LOD)

Lymphocyte subset	LOB (cells/µL)	LOD (cells/µL)
$CD3^+$	6	10
$CD3^+CD4^+$	4	6
$CD3^+CD8^+$	4	6

Limit of Quantitation

The limit of quantitation (LOQ) of BD Tritest™ CD4/CD8/CD3 on the BD FACSLyric™ flow cytometer was assessed at one site. 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 $CD3^+CD4^+$ concentrations.

Cell-free plasma samples reconstituted with peripheral blood mononuclear cells (PBMC) to a final concentration of 10, 20, 30, and 50 $CD3^+CD4^+$ cells/µL were used to estimate LOQ. Forty replicates of samples from each of the four concentration ranges were evaluated using two lots of BD Tritest™ CD4/CD8/CD3. Three BD FACSLyric™ flow cytometers and one BD FACSCalibur™ flow cytometer were used in the study. Absolute count values for LOQ are shown in the following table.

Table 16 Detection capability of BD Tritest™ CD4/CD8/CD3 (LOQ)

Lymphocyte subset	LOQ (cells/µL)
$CD3^+$	18
$CD3^+CD4^+$	11
$CD3^+CD8^+$	7

Linearity

The linear ranges of BD Tritest™ CD4/CD8/CD3 were assessed using triplicate measurements of 11 equally spaced concentrations of WBCs. Linearity was evaluated using four lots of the reagents and four BD FACSLyric™ flow cytometers. Lymphocyte subsets were observed to be linear across the following ranges. See the following table.

Table 17 Linear ranges of lymphocyte subsets

Lymphocyte subset	Range (cells/µL)
$CD3^+$	10–4,960
$CD3^+CD4^+$	4–2,782
$CD3^+CD8^+$	5–3,300

Measuring Range

Data from the LOQ study and from the method comparison study between the BD FACSLyric™ and the BD FACSCalibur™ flow cytometers was used to establish the measuring range of BD Tritest™ CD4/CD8/CD3. The lower end of the range was defined by the LOQ study, and the upper end of the range was supported by data from the method comparison study. See the following table.

Table 18 Measuring range of BD Tritest™ CD4/CD8/CD3

Lymphocyte subset	Analytical measuring range (cells/µL)
$CD3^+$	18–5,000
$CD3^+CD4^+$	11–3,000
$CD3^+CD8^+$	7–3,000

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.
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.

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.

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PATENTS AND TRADEMARKS

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

HISTORY

Revision	Date	Changes made
23-5333(09)	2022-06	Updated to meet requirements of Regulation (EU) 2017/746.
23-5333(10)	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
	Authorized representative in the European Community
	Authorised representative in Switzerland
	Date of manufacture
	Use-by date
	Batch code
	Catalogue number
	Serial number
	Sterile
	Sterilized using aseptic processing techniques
	Sterilized using ethylene oxide
	Sterilized using irradiation
	Sterilized using steam or dry heat
	Do not resterilize
	Non-sterile
	Do not use if package is damaged and consult *instructions for use*
	Sterile fluid path
	Sterile fluid path (ethylene oxide)
	Sterile fluid path (irradiation)
	Fragile, handle with care
	Keep away from sunlight
	Keep dry
	Lower limit of temperature
	Upper limit of temperature
	Temperature limit
	Humidity limitation
	Biological risks
	Do not re-use
	Consult *instructions for use* or consult electronic *instructions for use*
	Caution
	Contains or presence of natural rubber latex
	In vitro diagnostic medical device
	Negative control
	Positive control
	Contains sufficient for *<n>* tests
	For IVD performance evaluation only
	Non-pyrogenic
	Patient number
	This way up
	Do not stack

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

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

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