BD Tritest™
CD3/CD4/CD45

50 Tests per kit—Catalog No. 342413
50 Tests per kit with
BD Trucount™ Tubes—Catalog No. 342444
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WARRANTY

REFERENCES
1. INTENDED USE

BD Tritest™ CD3 fluorescein isothiocyanate (FITC)/CD4 phycoerythrin (PE)/CD45 peridinin chlorophyll protein (PerCP) is a three-color direct immunofluorescence reagent for use with a suitably equipped flow cytometer to identify and determine the percentages and absolute counts of mature human T lymphocytes (CD3+) and helper/inducer (CD3+CD4+) T-lymphocyte subsets in erythrocyte-lysed whole blood. When used with BD Trucount™ tubes, absolute counts of these populations can be enumerated from a single tube.

This BD Tritest reagent and BD Trucount tubes can be used with the BD FACS™ Loader, BD FACSVia™ Loader, and BD FACS™ Universal Loader. For BD FACSCalibur™ systems, the reagent can be used with or without an isotype control.

2. SUMMARY AND EXPLANATION

Human lymphocytes can be divided into three major populations based on their biologic function and cell-surface antigen expression: T lymphocytes, B lymphocytes, and natural killer (NK) lymphocytes.

Clinical Applications

Helper/inducer lymphocytes are a subset of T lymphocytes (CD3+) that are CD4+. CD3+CD4+ percentages or counts are used to characterize and monitor some forms of immunodeficiency1–3 and autoimmune diseases.4,5 Determining percentages or counts of helper/inducer T lymphocytes can be useful in monitoring human immunodeficiency virus (HIV)-infected individuals.6 Individuals with HIV typically exhibit a steady decrease of helper/inducer T-lymphocyte counts as the infection progresses.7
The Centers for Disease Control (CDC) recommend using reagent combinations containing CD3 and CD4 antibodies for determining the percentage of CD4⁺ T lymphocytes in HIV-infected subjects. The BD Tritest CD3/CD4/CD45 reagent allows helper/inducer T lymphocytes to be identified and enumerated separately from contaminating CD3⁻CD4⁺ monocytes.

3. PRINCIPLES OF THE PROCEDURE

When whole blood is added to the reagent, the fluorochrome-labeled antibodies in the reagent bind specifically to leucocyte surface antigens. 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 use fluorescence triggering, allowing direct fluorescence gating of the lymphocyte population to reduce contamination of unlysed or nucleated red blood cells in the gate.

When BD Trucount tubes are used, a precise volume of sample is stained directly in a BD Trucount tube. The lyophilized pellet in the tube dissolves, releasing a known number of fluorescent beads. During analysis, the absolute number (cells/µL) of gated cells in the sample can be determined by comparing cellular events to bead events. If appropriate cytometer-specific BD software is used (see Table 1), absolute counts will be determined by the software. If manually performing data analysis using software such as BD CellQuest™ software, simply divide the number of positive cellular events by the number of bead events, and then multiply by the BD Trucount bead concentration.
4. REAGENT

Reagent Provided, Sufficient for 50 Tests

The BD Tritest CD3/CD4/CD45 reagent is provided in 1 mL of buffered saline with bovine serum albumin and 0.1% sodium azide. It contains FITC-labeled CD3, clone SK7; PE-labeled CD4, clone SK3; and PerCP-labeled CD45, clone 2D1 (HLe-1).

CD3 identifies T lymphocytes and recognizes the epsilon chain of the CD3 antigen/T-cell antigen receptor (TCR) complex. This complex is composed of at least six proteins that range in molecular weight from 20–30 kilodaltons (kDa). The antigen recognized by CD3 antibodies is noncovalently associated with either αβ or γδ TCR (70–90 kDa).

CD4 identifies helper/inducer T lymphocytes and recognizes the CD4 antigen, 55 kDa, which interacts with class II molecules of the major histocompatibility complex (MHC) and is the primary receptor for HIV. The cytoplasmic portion of the antigen is associated with the protein tyrosine kinase p56lck.

CD45 identifies leucocytes and recognizes a 180- to 220-kDa human leucocyte antigen that is a member of the leucocyte common antigen (LCA) family.

CD3, CD4, and CD45 antibodies are composed of mouse γ1 heavy chains and kappa light chains.

Concentration values of the conjugated antibodies are listed in the following table:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3 FITC</td>
<td>2.0</td>
</tr>
<tr>
<td>CD4 PE</td>
<td>0.2</td>
</tr>
<tr>
<td>CD45 PerCP</td>
<td>6.25</td>
</tr>
</tbody>
</table>
BD Trucount tubes contain a freeze-dried pellet of fluorescent beads in a single-use tube. Each BD Trucount pouch contains 25 tubes, sufficient for 25 tests.

**Cross-Reacti**
y

The CD4 antibody reacts with monocytes as well as with helper/inducer T lymphocytes.\textsuperscript{17}

**Precautions**

- Do not use the reagent if you observe any change in appearance. Precipitation or discoloration 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 13). 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. We recommend that you do not mix multiple lots of tubes in the same run.
- BD Trucount tubes are designed for use with a specific lysis/no-wash procedure. Do not attempt to threshold on forward scatter (FSC) for data collection.
- Lyse red blood cells following staining using diluted (1X) BD FACSTM lysing solution. Use care to protect the tubes from direct light. Perform the procedure at room temperature (20°C–25°C).

**WARNING** All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection\textsuperscript{27,28} 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.29

Storage and Handling
- Store the reagent at 2°C–8°C. Do not use after the expiration date shown on the label.
- 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°C–25°C. To avoid potential condensation, open the pouch only after it has reached room temperature and carefully reseal the pouch immediately after removing a tube. An unopened pouch is stable until the expiration date shown on the packaging. Do not open the pouch and use tubes after the expiration date. Use tubes within 1 hour after removal from the foil pouch. Use remaining tubes within 1 month after opening the pouch.

5. INSTRUMENTS
The BD Tritest CD3/CD4/CD45 reagent and BD Trucount tubes are designed for use on a flow cytometer equipped with appropriate computer hardware and software. We recommend the BD systems listed in Table 1 for cytometer setup, acquisition, and analysis. See the corresponding reagent or cytometer instructions for use (IFUs) for details.

The BD FACS Loader, BD FACSVia Loader, and BD FACS Universal Loader can also be used with this product.

Ensure that the instrument is properly set up and passes daily quality control before use.
Use the specified software with:

- BD Trucount tubes to automatically calculate lymphocyte subset percentages and absolute counts
- 12 × 75-mm polystyrene tubes to automatically calculate lymphocyte subset percentages only

Alternatively, enter lymphocyte data obtained from another instrument or method to enable the software to calculate absolute counts in addition to lymphocyte subset percentages.

Users of flow cytometers manufactured by companies other than BD should refer to the manufacturer’s instructions for setting up three-color immunophenotyping. The flow cytometer must be equipped with a 488-nm laser capable of detecting light scatter (forward and side) and three-color fluorescence with emission detectable in three ranges:

- 515–545 nm
- 562–607 nm
- >650 nm

### Table 1 Recommended BD systems

<table>
<thead>
<tr>
<th>Flow cytometer</th>
<th>Setup beads</th>
<th>Setup software</th>
<th>Analysis software</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD FACSLyric™</td>
<td>BD™ CS&amp;CT beads</td>
<td>BD FACSuite™ Clinical software</td>
<td>BD FACSuite Clinical software</td>
</tr>
<tr>
<td>BD FACSVia™</td>
<td>BD CS&amp;CT beads</td>
<td>BD FACSvia™ clinical software</td>
<td>BD FACSvia clinical software</td>
</tr>
<tr>
<td>BD FACSCalibur</td>
<td>BD Calibrite™ 3-color beads</td>
<td>BD FACSComp™ software</td>
<td>BD Multiset™ software</td>
</tr>
</tbody>
</table>

Use the specified software with:

- BD Trucount tubes to automatically calculate lymphocyte subset percentages and absolute counts
- 12 × 75-mm polystyrene tubes to automatically calculate lymphocyte subset percentages only

Alternatively, enter lymphocyte data obtained from another instrument or method to enable the software to calculate absolute counts in addition to lymphocyte subset percentages.

Users of flow cytometers manufactured by companies other than BD should refer to the manufacturer’s instructions for setting up three-color immunophenotyping. The flow cytometer must be equipped with a 488-nm laser capable of detecting light scatter (forward and side) and three-color fluorescence with emission detectable in three ranges:

- 515–545 nm
- 562–607 nm
- >650 nm
6. SPECIMEN COLLECTION AND PREPARATION
Collect blood aseptically by venipuncture into a sterile BD Vacutainer® EDTA (ethylenediaminetetraacetic acid) blood collection tube or equivalent. The BD Tritest CD3/CD4/CD45 reagent and BD Trucount tubes have been validated with both liquid and dry formulations of EDTA blood collection tubes.
A minimum of 100 µL of whole blood is required for this procedure. Follow the collection tube manufacturer’s guidelines for the minimum volume of blood to be collected to ensure proper specimen staining, especially when determining absolute counts using BD Trucount tubes.
Obtain a white blood cell (WBC) count and a differential white cell count from the same whole blood sample before staining to ensure that the WBC count is within the linear range for the appropriate instrument, or to calculate absolute counts from percentages. Anticoagulated blood stored at room temperature (20°C–25°C) must be stained within 72 hours of draw and then analyzed within 6 hours of staining. If samples are stained within 24 hours of draw, they can be analyzed within 24 hours of staining.

Interfering Conditions
Do not use previously fixed and stored patient specimens. Whole blood samples refrigerated before staining can give aberrant results. Samples obtained from patients taking immunosuppressive drugs can yield poor resolution. Blast cells can interfere with test results. Hemolyzed samples should be rejected.

7. REAGENTS AND MATERIALS
Provided
- BD Tritest CD3/CD4/CD45 (Catalog No. 342413), or
- BD Tritest CD3/CD4/CD45 with BD Trucount tubes (Catalog No. 342444)
Required but Not Provided

- For BD FACSLyric flow cytometers:
  
  BD CS&T beads (Catalog Nos. 656504, 656505)
  BD FC beads 7-color kit (Catalog No. 656867)

- For BD FACSVia flow cytometers:

  BD CS&T beads (Catalog Nos. 656504, 656505)
  Filtered deionized (DI) water

  **CAUTION** For the BD FACSVia flow cytometer, use only DI water to dilute BD CS&T beads.

- For BD FACSCalibur flow cytometers:

  BD Calibrite 3-color kit (Catalog No. 340486)

  BD FACS lysing solution (10X), 100 mL (Catalog No. 349202)

  BD FACS lysing solution contains diethylene glycol and formaldehyde. See the *BD FACS Lysing Solution IFU* for precautions and warnings.

- Reagent-grade (distilled or deionized) water

- BD FACSFlow™ sheath fluid (Catalog No. 342003) or equivalent

  **CAUTION** Use only BD FACSFlow sheath fluid to dilute BD Calibrite 3-color beads, and BD CS&T beads.

  **NOTE** Use BD™ FC beads dilution buffer, supplied with the kit, to reconstitute the BD FC beads.

- BD Vacutainer EDTA blood collection tubes or equivalent

- Disposable 12 x 75-mm Falcon® capped polystyrene test tubes, or equivalent (if not using BD Trucount tubes)

- Vortex mixer

* Falcon is a registered trademark of Corning Incorporated.
• Micropipettor with tips
• Bulk dispenser or pipettor (450 µL) for dispensing 1X BD FACS lysing solution
• Lysable whole blood control (available commercially)
• BD Trucount™ controls (Catalog No. 340335), necessary if using BD Trucount tubes

8. PROCEDURE

Dilution Instructions for BD FACS Lysing Solution
Dilute the 10X concentrate 1:10 with room temperature (20°C–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.

Staining the Cells
NOTE For BD FACSCalibur systems, BD Tritest CD3/CD4/CD45 can be used with or without an isotype control to assess the amount of nonspecific antibody binding. If you want to use a control, BD Tritest™ γ/γ/CD45 isotype control reagent (Catalog No. 342415) is available.

1. For each patient sample, label a 12 × 75-mm tube with the sample identification number.
For absolute counts, label a BD Trucount tube in place of the 12 × 75-mm tube.

**NOTE** Before using a BD Trucount tube, verify that the BD Trucount bead pellet is intact and within 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.


   If using a BD Trucount tube, pipette just above the stainless steel retainer. Do not touch the pellet.

3. Pipette 50 µL of well-mixed, anticoagulated whole blood into the bottom of the tube.

   **NOTE** Use the reverse pipetting technique to pipette sample onto the side of the tube just above the retainer. See Reverse Pipetting on page 13. Avoid smearing blood down the side of the tube. If whole blood 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. Incubate for 15 minutes in the dark at room temperature (20°C–25°C).

5. Add 450 µL of 1X BD FACS lysing solution to the tube.

6. Cap the tube and vortex gently to mix. Incubate for 15 minutes in the dark at room temperature (20°C–25°C).

   The sample is now ready to be analyzed on the flow cytometer.

**Flow Cytometry**

- If samples are not to be analyzed immediately after preparation, store them in the dark at room temperature (20°C–25°C).
- Vortex the cells thoroughly (at low speed) to reduce aggregation before running them on the flow cytometer.33
• If using the Loader, vortex tubes immediately before placing them into the Loader racks.
• Acquire and analyze data using the appropriate cytometer-specific BD software. See Table 1.
• Before acquiring samples, adjust the threshold to minimize debris and ensure populations of interest are included.

**Quality Control**

Run a control sample daily from a normal adult subject or a commercially available whole blood control to optimize instrument settings and as a quality control check of the system.³¹

Use commercial controls providing established values for percent positive and absolute counts with each run to assess system performance.

Visually inspect the CD45 vs SSC 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.

**Representative Data**

**BD FACSlyric flow cytometer**

A hematologically normal adult sample stained with BD Tristest CD3/CD4/CD45 in a BD Trucount tube was acquired with BD FACSuite Clinical software using a BD FACSlyric flow cytometer. See Figure 1. Panel A shows CD45+ lymphocytes (1) identified in the CD45 PerCP-A vs SSC-A dot plot. Panel B shows BD Trucount absolute count bead events (2) in the APC-A vs SSC-A dot plot. Panel C shows T lymphocytes (CD3+CD4+ and CD3+CD4–) in the CD3 FITC-A vs CD4 PE-A dot plot.
Figure 1  Representative data from a hematologically normal adult sample stained with BD Tritest CD3/CD4/CD45 in a BD Trucount tube (BD FACSLyric)

A hematologically normal adult sample stained with BD Tritest CD3/CD4/CD45 in a BD Trucount tube was acquired with BD FACSVia clinical software using a BD FACSVia flow cytometer. See Figure 2. Panel A shows CD45+ lymphocytes identified in the CD45 PerCP vs SSC dot plot. Panel B shows BD Trucount absolute count bead events in the APC vs SSC dot plot. Panel C shows T lymphocytes (3+4+ and 3+4−) in the CD3 FITC vs CD4 PE dot plot.

Figure 2  Representative data from a hematologically normal adult sample stained with BD Tritest CD3/CD4/CD45 in a BD Trucount tube (BD FACSVia)
BD FACSCalibur flow cytometer

A hematologically normal adult sample stained with BD Tritest CD3/CD4/CD45 in a BD Trucount tube was acquired using a BD FACSCalibur flow cytometer. See Figure 3. Panel A shows CD45+ lymphocytes (1) identified in the CD45 PerCP vs SSC dot plot. Panel B shows BD Trucount absolute count bead events (2) in the CD3 FITC vs CD4 PE dot plot. Panel C shows gated T lymphocytes in the CD3 FITC vs CD4 PE dot plot.

Figure 3  Representative data from a hematologically normal adult sample stained with BD Tritest CD3/CD4/CD45 in a BD Trucount tube (BD FACSCalibur)

9. RESULTS

Results are reported as the percentage of positive cells per lymphocyte population or as the number of positive cells per microliter of blood (absolute count).

Calculating Absolute Counts

During analysis, the absolute number (cells/µL) of positive cells in the sample can be determined by comparing cellular events to bead events. If BD Multiset software, BD FACSVia clinical software, or BD FACSuite Clinical software is used, absolute counts will be determined by the software.
For manual data analysis using BD CellQuest or other software, the absolute count of the cell population (A) can be calculated using the following equation:

\[ A = \frac{X}{Y} \times \frac{N}{V}, \]

where:

- \(X\) is the number of positive cell events
- \(Y\) is the number of bead events
- \(N\) is the number beads per test, which is found on the BD Trucount tubes foil pouch and can vary from lot to lot
- \(V\) is the sample volume (50 µL)

10. LIMITATIONS

- Laboratories must establish their own normal reference intervals for the BD Tritest CD3/CD4/CD45 reagent parameters that can be affected by gender of patient, age of patient, and preparative technique. Race of patient\(^\text{34}\) and individual variations of epitope expression\(^\text{35}\) can also have an effect, although sufficient data is not available to establish this. Age, gender, clinical characteristics, and race of patients should be known when a reference interval is determined.\(^\text{36}\) Reference intervals provided are for information only.

- The BD Tritest CD3/CD4/CD45 reagent has not been validated for use with heparin or acid citrate dextrose (ACD) liquid anticoagulants in determining absolute counts with BD Trucount tubes.

- The BD Tritest CD3/CD4/CD45 reagent is not intended for screening samples for the presence of leukemic cells or for use in phenotyping samples from leukemia patients.

- Absolute counts are not comparable between laboratories using different manufacturer’s equipment.
11. EXPECTED VALUES

Reference Intervals

Reference intervals for lymphocyte subsets for BD Tritest CD3/CD4/CD45 were determined. Subjects were hematologically normal adults between the ages of 18 and 65 years. See the first limitation in the previous section for more information about reference intervals.

<table>
<thead>
<tr>
<th>Subset</th>
<th>N*</th>
<th>Unit</th>
<th>Mean</th>
<th>Lower 2.5 percentile</th>
<th>Upper 97.5 percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helper/inducer T lymphocytes</td>
<td>523</td>
<td>%</td>
<td>45</td>
<td>31</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cells/µL</td>
<td>880</td>
<td>410</td>
<td>1,590</td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>516</td>
<td>%</td>
<td>72</td>
<td>55</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cells/µL</td>
<td>1,410</td>
<td>690</td>
<td>2,540</td>
</tr>
</tbody>
</table>

a. N = number of samples
b. Absolute counts are rounded to the nearest 10 cells/µL.

12. PERFORMANCE CHARACTERISTICS

BD FACSLyric Flow Cytometer

Method comparison (BD FACSLyric flow cytometer)

Lymphocyte subset percentages and absolute counts were enumerated with the BD Tritest CD3/CD4/CD45 reagent in BD Trucount tubes and analyzed on the BD FACSLyric flow cytometer using BD FACSuite Clinical software version 1.0. The results were compared with results from the reagents analyzed on the BD FACSCalibur flow cytometer using BD Multiset software version 1.1 or later.

Whole blood samples were collected at random at one clinical study site. Method comparison statistics are reported for all cell subsets. See Table 3.
Within-site precision (BD FACSLyric flow cytometer)

An 11-day study was conducted at one site, BD Biosciences, to assess within-site precision. 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®† CD4 Low control and CD-Chex Plus® control, stained in duplicate with four lots of BD Tritest CD3/CD4/CD45. Two separate runs were analyzed during each of the 11 tested days for a total of 22 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 3 Method comparison statistics for lymphocyte subsets (BD FACSLyric flow cytometer)

<table>
<thead>
<tr>
<th>Lymphocyte Subset</th>
<th>N</th>
<th>Unit</th>
<th>( R^2 )</th>
<th>Slope</th>
<th>Intercept</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+CD4+</td>
<td>106</td>
<td>%</td>
<td>0.98</td>
<td>1.02</td>
<td>-0.261</td>
<td>9.71–57.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cells/µL</td>
<td>0.98</td>
<td>1.023</td>
<td>2.248</td>
<td>112–1,319</td>
</tr>
<tr>
<td>CD3+</td>
<td>106</td>
<td>%</td>
<td>0.94</td>
<td>1.011</td>
<td>0.13</td>
<td>48.1–89.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cells/µL</td>
<td>0.97</td>
<td>0.985</td>
<td>61.316</td>
<td>728–3,969</td>
</tr>
</tbody>
</table>

† CD-Chex Plus is a registered trademark of Streck, Inc.
### Table 4 Within-site precision of lymphocyte subset percentages in low analyte concentration (CDL) (BD FACSLyric flow cytometer)

<table>
<thead>
<tr>
<th>Lymphocyte Subset (%)</th>
<th>Mean</th>
<th>SD (Repeatability)</th>
<th>SD (Within-site precision)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+CD4+</td>
<td>13.35</td>
<td>0.66</td>
<td>0.68</td>
</tr>
<tr>
<td>CD3+</td>
<td>61.18</td>
<td>1.07</td>
<td>1.07</td>
</tr>
</tbody>
</table>

a. CDL = CD-Chex Plus CD4 Low control

### Table 5 Within-site precision of lymphocyte subset percentages in normal analyte concentration (CDC) (BD FACSLyric flow cytometer)

<table>
<thead>
<tr>
<th>Lymphocyte Subset (%)</th>
<th>Mean</th>
<th>SD (Repeatability)</th>
<th>SD (Within-site precision)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+CD4+</td>
<td>51.23</td>
<td>1.12</td>
<td>1.13</td>
</tr>
<tr>
<td>CD3+</td>
<td>78.22</td>
<td>0.96</td>
<td>0.97</td>
</tr>
</tbody>
</table>

a. CDC = CD-Chex Plus control

### Table 6 Within-site precision of lymphocyte subset absolute counts in low analyte concentration (CDL) (BD FACSLyric flow cytometer)

<table>
<thead>
<tr>
<th>Lymphocyte Subset (cells/µL)</th>
<th>Mean</th>
<th>%CV (Repeatability)</th>
<th>%CV (Within-site precision)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+CD4+</td>
<td>182.37</td>
<td>6.58</td>
<td>7.30</td>
</tr>
<tr>
<td>CD3+</td>
<td>835.57</td>
<td>4.70</td>
<td>5.18</td>
</tr>
</tbody>
</table>
Stability (BD FACSLyric flow cytometer)

A study was conducted to assess blood sample stability and stained sample stability using the BD Tritest CD3/CD4/CD45 reagent. The study measured:

- Changes associated with the storage of whole blood before staining
- Changes as a result of time between staining and data acquisition
- The combined effect of the two

Whole blood samples were tested either up to 74 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°C–25°C) before staining or acquisition.

Based on the results of this study, we recommend staining samples within 72 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.

Linearity (BD FACSLyric flow cytometer)

Linearity was assessed for the BD FACSLyric flow cytometer using triplicate measurements of 11 equally spaced concentrations of WBCs. Lymphocyte subsets were observed to be linear across the following ranges. See Table 8.

Table 7 Within-site precision of lymphocyte subset absolute counts in normal analyte concentration (CDC) (BD FACSLyric flow cytometer)

<table>
<thead>
<tr>
<th>Lymphocyte Subset (cells/µL)</th>
<th>Mean</th>
<th>%CV (Repeatability)</th>
<th>%CV (Within-site precision)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+CD4+</td>
<td>1,058.05</td>
<td>5.61</td>
<td>6.38</td>
</tr>
<tr>
<td>CD3+</td>
<td>1,615.71</td>
<td>5.35</td>
<td>6.15</td>
</tr>
</tbody>
</table>

Status: Released
EFFECTIVE

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Method comparison (BD FACSVia flow cytometer)
Lymphocyte subset percentages and absolute counts were enumerated with the BD Triteq CD3/CD4/CD45 reagent in BD Trucount tubes and analyzed on the BD FACSVia flow cytometer using BD FACSVia clinical software version 2.0. The results were compared with results from the reagents analyzed on the BD FACSCalibur flow cytometer using BD Multiset software version 2.2 or later.
Whole blood samples were collected at random at 2 clinical study sites. Method comparison statistics are reported for all cell subsets. See Table 9.

Table 8 Linear ranges of lymphocyte subsets (BD FACSLyric flow cytometer)

<table>
<thead>
<tr>
<th>Lymphocyte Subset</th>
<th>Range (cells/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+CD4⁺</td>
<td>5-7,584</td>
</tr>
<tr>
<td>CD3⁺</td>
<td>6-12,659</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Lymphocyte Subset</th>
<th>N</th>
<th>Unit</th>
<th>R²</th>
<th>Slope</th>
<th>Intercept</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+CD4⁺</td>
<td>165</td>
<td>%</td>
<td>0.99</td>
<td>1.02</td>
<td>-0.51</td>
<td>0.2–63.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cells/µL</td>
<td>0.99</td>
<td>0.95</td>
<td>2.00</td>
<td>2–1,862</td>
</tr>
<tr>
<td>CD3⁺</td>
<td>165</td>
<td>%</td>
<td>0.98</td>
<td>1.01</td>
<td>-0.04</td>
<td>0.2–94.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cells/µL</td>
<td>0.98</td>
<td>0.97</td>
<td>3.00</td>
<td>3–3,553</td>
</tr>
</tbody>
</table>
Within-site precision (BD FACSVia flow cytometer)

A 21-day study was conducted at one site, BD Biosciences, to assess within-site precision. Estimates of precision for the enumeration of lymphocyte subset percentages and absolute counts were determined across three BD FACSVia flow cytometers and three operators by acquiring two concentrations of analyte, BD Multi-Check CD4 Low Control and BD Multi-Check Control, stained in duplicate with four lots of BD Tritest CD3/CD4/CD45. Two separate runs were analyzed during each of the 21 tested days for a total of 42 runs.

The following tables present SDs and CVs for within-site precision and repeatability of lymphocyte subset percentages and absolute counts, respectively.

**Table 10** Within-site precision of lymphocyte subset percentages in low analyte concentration (MCL$^a$) (BD FACSVia flow cytometer)

<table>
<thead>
<tr>
<th>Lymphocyte Subset (%)</th>
<th>Mean (SD)</th>
<th>Repeatability (SD)</th>
<th>Within-site precision (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD$^+$CD$^+$</td>
<td>10.2</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>CD$^+$</td>
<td>55.0</td>
<td>1.0</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*a. MCL = BD Multi-Check CD4 Low control

**Table 11** Within-site precision of lymphocyte subset percentages in normal analyte concentration (MCN$^a$) (BD FACSVia flow cytometer)

<table>
<thead>
<tr>
<th>Lymphocyte Subset (%)</th>
<th>Mean (SD)</th>
<th>Repeatability (SD)</th>
<th>Within-site precision (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD$^+$CD$^+$</td>
<td>43.2</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>CD$^+$</td>
<td>72.3</td>
<td>1.3</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*a. MCN = BD Multi-Check control
Stability (BD FACSVia flow cytometer)

A study was conducted to assess blood sample stability and stained sample stability using the BD Tritest CD3/CD4/CD45 reagent. The study measured:

- Changes associated with the storage of whole blood before staining
- Changes as a result of time between staining and data acquisition
- The combined effect of the two

Whole blood samples were tested either up to 74 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°C–25°C) before staining or acquisition.

### Table 12: Within-site precision of lymphocyte subset absolute counts in low analyte concentration (MCL) (BD FACSVia flow cytometer)

<table>
<thead>
<tr>
<th>Lymphocyte Subset (cells/µL)</th>
<th>Mean</th>
<th>%CV (Repeatability)</th>
<th>%CV (Within-site precision)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+CD4+</td>
<td>146.2</td>
<td>7.5</td>
<td>8.4</td>
</tr>
<tr>
<td>CD3+</td>
<td>791.8</td>
<td>5.2</td>
<td>6.2</td>
</tr>
</tbody>
</table>

### Table 13: Within-site precision of lymphocyte subset absolute counts in normal analyte concentration (MCN) (BD FACSVia flow cytometer)

<table>
<thead>
<tr>
<th>Lymphocyte Subset (cells/µL)</th>
<th>Mean</th>
<th>%CV (Repeatability)</th>
<th>%CV (Within-site precision)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+CD4+</td>
<td>779.1</td>
<td>5.3</td>
<td>6.5</td>
</tr>
<tr>
<td>CD3+</td>
<td>1,304.0</td>
<td>5.3</td>
<td>6.4</td>
</tr>
</tbody>
</table>
Based on the results of this study, we recommend staining samples within 72 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.

**Linearity (BD FACSVia flow cytometer)**

Linearity was assessed for the BD FACSVia flow cytometer using triplicate measurements of 11 equally spaced concentrations of WBCs. Results were observed to be linear across the following ranges. See Table 14.

**Table 14 Linear ranges of lymphocyte subsets (BD FACSVia flow cytometer)**

<table>
<thead>
<tr>
<th>Lymphocyte Subset</th>
<th>Range (cells/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+CD4+</td>
<td>5–6,500</td>
</tr>
<tr>
<td>CD3+</td>
<td>5–10,000</td>
</tr>
</tbody>
</table>

**BD FACSCalibur Flow Cytometer**

**Method comparison (BD FACSCalibur flow cytometer)**

Lymphocyte subset percentage enumerations with BD Tritest CD3/CD4/CD45 reagent were compared with results from BD Simultest™ CD3/CD4 reagent. Absolute counts were compared to results from the BD FACSCount™ instrument.

Aliquots of the same blood sample from normal and abnormal donors were analyzed. Method comparison statistics reported in Table 15 indicate that the results are substantially equivalent.
Within-Specimen Reproducibility (BD FACSCalibur flow cytometer)

Ten aliquots of specimens from three samples representing high, medium, and low CD4 counts were assessed. The percent-positive results were as follows:

- %CD3: mean = 70, pooled SD = 0.7
- %CD4: mean = 47, pooled SD = 0.9

The results for absolute counts are shown in Table 16.

### Table 15 Method comparison statistics

<table>
<thead>
<tr>
<th>Subset</th>
<th>Unit</th>
<th>N</th>
<th>Slope</th>
<th>Intercept</th>
<th>R</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helper/inducer T lymphocytes</td>
<td>%</td>
<td>168</td>
<td>0.98</td>
<td>0.6</td>
<td>0.99</td>
<td>1–78</td>
</tr>
<tr>
<td></td>
<td>(cells/µL)</td>
<td>199</td>
<td>1.04</td>
<td>1</td>
<td>0.99</td>
<td>0–1,880³</td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>%</td>
<td>168</td>
<td>0.92</td>
<td>5.7</td>
<td>0.96</td>
<td>24–95</td>
</tr>
<tr>
<td></td>
<td>(cells/µL)</td>
<td>197</td>
<td>1.03</td>
<td>-7</td>
<td>0.99</td>
<td>120–2,860³</td>
</tr>
</tbody>
</table>

a. Absolute counts are rounded to the nearest 10 cells/µL.

### Table 16 Within-specimen reproducibility for BD Tritest CD3/CD4/CD45 reagent

<table>
<thead>
<tr>
<th>Subset (cells/µL)</th>
<th>Level</th>
<th>Mean</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helper/inducer T lymphocytes</td>
<td>High</td>
<td>2,034</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>Med</td>
<td>1,352</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>371</td>
<td>7.1</td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>High</td>
<td>2,716</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>Med</td>
<td>1,897</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>704</td>
<td>7.0</td>
</tr>
</tbody>
</table>
Stability (BD FACScalibur flow cytometer)

A study was conducted to assess blood sample stability and stained sample stability using the BD Tritest CD3/CD4/CD45 reagent. The study measured:

- Changes associated with the storage of whole blood before staining
- Changes as a result of time between staining and data acquisition
- The combined effect of the two

Whole blood samples were tested either up to 72 hours post draw with stained samples tested up to 6 hours post stain, or up to 24 hours post draw with stained samples tested up to 24 hours post stain. All samples were maintained at room temperature (20°C–25°C) before staining or acquisition.

Based on the results of this study, we recommend staining samples within 72 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.

Linearity (BD FACScalibur flow cytometer)

Linearity was assessed by testing within a WBC concentration of 2.5 x 10^3 to 31.0 x 10^3 WBC/µL and a lymphocyte concentration of 2.0 x 10^2 to 16.7 x 10^3 lymphocytes/µL. Results were observed to be linear within the CD3+CD4+ range (68 to 7.2 x 10^3 cells/µL) and the CD3+ range (123 to 1.1 x 10^4 cells/µL).

WARRANTY

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REFERENCES


