BD Tritest™
CD4/CD8/CD3

50 Tests per kit—Catalog No. 342414
50 Tests per kit with
BD Trucount™ Tubes—Catalog No. 342445
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1. INTENDED USE

BD Tritest™ CD4 fluorescein isothiocyanate (FITC)/CD8 phycoerythrin (PE)/CD3 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+), helper/inducer (CD3+CD4+) T lymphocytes, and suppressor/cytotoxic (CD3+CD8+) T lymphocytes in erythrocyte-lysed whole blood. When used with BD Trucount™ tubes, absolute counts of these populations can be enumerated from a single tube.

2. SUMMARY AND EXPLANATION

Human lymphocytes can be divided into three major subset 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 immunodeficiency and autoimmune diseases. Determining percentages or counts of helper/inducer T lymphocytes can be useful in monitoring human immunodeficiency virus (HIV)-infected individuals. Individuals with HIV typically exhibit a steady decrease of helper/inducer T-lymphocyte counts as the infection progresses.

Suppressor/cytotoxic lymphocytes are a subset of T lymphocytes (CD3+) that are CD8+. CD3+CD8+ percentages or counts are used to characterize and monitor some forms of immunodeficiency and autoimmune diseases. Suppressor/cytotoxic lymphocyte values lie outside the normal reference range in some autoimmune diseases and in certain immune reactions such as acute graft-versus-host disease (GVHD) and transplant rejection. The CD8+ subset is elevated in...
many patients with either congenital or acquired immune deficiencies, such as severe combined immunodeficiency (SCID) or acquired immune deficiency syndrome (AIDS). The CD8+ cell population is often decreased in active systemic lupus erythematosus (SLE), but can also be increased in SLE patients undergoing steroid therapy.

The Centers for Disease Control (CDC) recommend using reagent combinations containing CD3 antibodies for determining T-lymphocyte subsets in HIV-infected subjects. The BD Tritest CD4/CD8/CD3 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 CD4/CD8/CD3 reagent is provided in 1 mL of buffered saline with 0.1% sodium azide. It contains FITC-labeled CD4, clone SK3; PE-labeled CD8, clone SK1; and PerCP-labeled CD3, clone SK7.

CD4 identifies helper/inducer T lymphocytes and recognizes the CD4 antigen, 55 kilodaltons (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. CD4, CD8, and CD3 antibodies are composed of mouse γ heavy chains and kappa light chains.

CD8 identifies suppressor/cytotoxic T lymphocytes and recognizes an antigen expressed as a disulfide-linked bimolecular complex with a 32-kDa α subunit. The cytoplasmic domain of the α subunit of the CD8 antigen is associated with the protein tyrosine kinase p56lck. CD8 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, CD8, and CD3 antibodies are composed of mouse γ 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>CD4 FITC</td>
<td>1.0</td>
</tr>
<tr>
<td>CD8 PE</td>
<td>1.5</td>
</tr>
<tr>
<td>CD3 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 2.5 tests.

Cross-Reactivity

The CD4 antibody reacts with monocytes as well as with helper/inducer T lymphocytes.\(^{18}\) The CD8 antibody reacts with NK lymphocytes\(^{43}\) as well as with suppressor/cytotoxic T lymphocytes.

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 14). 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 lyse/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).
• Some samples may 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.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection33,34 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.35

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 CD4/CD8/CD3 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 (IFU) for details.

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

Ensure that the instrument is properly set up and passes daily quality control before use.

<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;T beads</td>
<td>BD FACSuite™ Clinical software</td>
<td>BD FACSuite Clinical software</td>
</tr>
<tr>
<td></td>
<td>BD™ FC beads, 7-color kit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BD FACSVia™</td>
<td>BD CS&amp;T beads</td>
<td>BD FACSVia™ clinical software</td>
<td>BD FACSVia clinical software</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BD FACSCalibur™</td>
<td>BD Caliber™ 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 T-lymphocyte subset percentages and absolute counts
- 12 × 75-mm polystyrene tubes to automatically calculate T-lymphocyte subset percentages only

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\textsuperscript{36,37} into a sterile BD Vacutainer\textregistered{} EDTA (ethylenediaminetetraacetic acid) blood collection tube or equivalent. The BD Tritest CD4/CD8/CD3 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.

Anticoagulated blood stored at room temperature (20°C–25°C) must be stained within 48 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 CD4/CD8/CD3 (Catalog No. 342414), or
- BD Tritest CD4/CD8/CD3 with BD Trucount tubes (BD Catalog No. 342445)

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 × 75-mm Falcon® 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
- 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.

* Falcon is a registered trademark of Corning Incorporated.
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

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.

2. Pipette 20 µL of BD Tritest CD4/CD8/CD3 reagent into the bottom of the 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 14. 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 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.39
- 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.37

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

Visually inspect the CD3 vs SSC dot plot. The T-lymphocyte population should appear as a compact, CD3+ cluster with low SSC. Do not proceed with analysis if the T-lymphocyte population is diffuse and if there is little or no separation between it and other clusters.
Representative Data

BD FACSlyric flow cytometer

A hematologically normal adult sample stained with BD Tritest CD4/CD8/CD3 in a BD Trucount tube was acquired with BD FACSuite Clinical software using a BD FACSlyric flow cytometer. See Figure 1. Panel A shows CD3+ lymphocytes (1) identified in the CD3 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 suppressor/cytotoxic (CD4–CD8+) and helper/inducer (CD4+CD8–) T lymphocytes in the CD4 FITC-A vs CD8 PE-A dot plot.

Figure 1 Representative data from a hematologically normal adult sample stained with BD Tritest CD4/CD8/CD3 in a BD Trucount tube (BD FACSlyric)

BD FACS Via flow cytometer

A hematologically normal adult sample stained with BD Tritest CD4/CD8/CD3 in a BD Trucount tube was acquired with BD FACS Via clinical software using a BD FACS Via flow cytometer. See Figure 2. Panel A shows forward and side scatter for the sample. Panel B shows CD3+ T cells identified in the CD3 PerCP vs SSC dot plot. Panel C shows BD Trucount absolute count bead events in the APC vs SSC dot plot. Panel D shows suppressor/cytotoxic (4–8+) and helper/inducer (4+8–) T cells in the CD4 FITC vs CD8 PE dot plot.
A hematologically normal adult sample stained with BD Tritest CD4/CD8/CD3 in a BD Trucount tube was acquired using a BD FACSCalibur flow cytometer. See Figure 3. Panel A shows CD3+ lymphocytes (1) identified in the CD3 PerCP vs SSC dot plot. Panel B shows BD Trucount absolute count bead events (2) in the CD4 FITC vs CD8 PE dot plot. Panel C shows suppressor/cytotoxic (CD4–CD8+) and helper/inducer (CD4+CD8+) T lymphocytes in the CD4 FITC vs CD8 PE dot plot.
9. RESULTS

Results are reported as the percentage of positive cells per T-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 CD4/CD8/CD3 reagent parameters that can be affected by gender of patient, age of patient, and preparative technique. Race of patient and individual variations of epitope expression 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. Reference intervals provided are for information only.

- The BD Tritest CD4/CD8/CD3 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 CD4/CD8/CD3 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 T-lymphocyte subsets for BD Tritest CD4/CD8/CD3 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.
12. PERFORMANCE CHARACTERISTICS

BD FACSLyric Flow Cytometer

Method comparison (BD FACSLyric flow cytometer)

T-lymphocyte subset percentages and absolute counts were enumerated with the BD Tritest CD4/CD8/CD3 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.

Table 2 Representative reference intervals for BD Tritest CD4/CD8/CD3

<table>
<thead>
<tr>
<th>T-lymphocyte Subset (cells/µL)</th>
<th>N</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>880</td>
<td>410</td>
<td>1,590</td>
</tr>
<tr>
<td>Suppressor/cytotoxic T lymphocytes</td>
<td>523</td>
<td>490</td>
<td>190</td>
<td>1,140</td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>516</td>
<td>1,410</td>
<td>690</td>
<td>2,540</td>
</tr>
</tbody>
</table>

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

Table 3 Method comparison statistics for T-lymphocyte subsets (BD FACSLyric flow cytometer)

<table>
<thead>
<tr>
<th>T-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>121</td>
<td>%</td>
<td>0.99</td>
<td>0.993</td>
<td>0.142</td>
<td>11.16–75.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cells/µL</td>
<td>0.98</td>
<td>1.043</td>
<td>3.832</td>
<td>111–1,556</td>
</tr>
</tbody>
</table>
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 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® CD4 Low control and CD-Chex Plus® control, stained in duplicate with four lots of BD Tritest CD4/CD8/CD3. 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 T-lymphocyte subset percentages and absolute counts, respectively.

Table 3 Method comparison statistics for T-lymphocyte subsets (BD FACSLyric flow cytometer)

<table>
<thead>
<tr>
<th>T-lymphocyte Subset (%)</th>
<th>Mean</th>
<th>SD (Repeatability)</th>
<th>SD (Within-site precision)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+CD8+</td>
<td>22.35</td>
<td>1.11</td>
<td>1.17</td>
</tr>
<tr>
<td>CD3+</td>
<td>72.76</td>
<td>1.23</td>
<td>1.31</td>
</tr>
</tbody>
</table>

Table 4 Within-site precision of T-lymphocyte subset percentages in low analyte concentration (CDLa) (BD FACSLyric flow cytometer)

<table>
<thead>
<tr>
<th>T-lymphocyte Subset (%)</th>
<th>Mean</th>
<th>SD (Repeatability)</th>
<th>SD (Within-site precision)</th>
</tr>
</thead>
<tbody>
<tr>
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The following tables present standard deviations (SDs) and coefficients of variation (CVs) for within-site precision and repeatability of T-lymphocyte subset percentages and absolute counts, respectively.

Table 3 Method comparison statistics for T-lymphocyte subsets (BD FACSLyric flow cytometer)

<table>
<thead>
<tr>
<th>T-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>22.35</td>
<td>1.11</td>
<td>1.17</td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td>72.76</td>
<td>1.23</td>
<td>1.31</td>
</tr>
</tbody>
</table>

Table 4 Within-site precision of T-lymphocyte subset percentages in low analyte concentration (CDLa) (BD FACSLyric flow cytometer)

<table>
<thead>
<tr>
<th>T-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>22.35</td>
<td>1.11</td>
<td>1.17</td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td>72.76</td>
<td>1.23</td>
<td>1.31</td>
</tr>
</tbody>
</table>

* CD-Chex Plus is a registered trademark of Streck, Inc.
Stability (BD FACSLyric flow cytometer)

A study was conducted to assess blood sample stability and stained sample stability using the BD Tritest CD4/CD8/CD3 reagent. The study measured:

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

<table>
<thead>
<tr>
<th>T-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>65.13</td>
<td>1.19</td>
<td>1.20</td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td>32.98</td>
<td>1.18</td>
<td>1.21</td>
</tr>
</tbody>
</table>

a. CDC = CD-Chex Plus control

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

<table>
<thead>
<tr>
<th>T-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>186.70</td>
<td>6.20</td>
<td>6.92</td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td>607.80</td>
<td>4.73</td>
<td>5.20</td>
</tr>
<tr>
<td>CD3+</td>
<td>835.30</td>
<td>4.29</td>
<td>4.81</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>T-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>1,052.39</td>
<td>6.62</td>
<td>6.88</td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td>532.87</td>
<td>7.47</td>
<td>7.67</td>
</tr>
<tr>
<td>CD3+</td>
<td>1,615.83</td>
<td>6.26</td>
<td>6.56</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 CD4/CD8/CD3 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 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°C–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.

Linearity (BD FACSLyric flow cytometer)

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

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

<table>
<thead>
<tr>
<th>T-lymphocyte Subset</th>
<th>Range (cells/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+CD4+</td>
<td>5–6,810</td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td>5–5,869</td>
</tr>
<tr>
<td>CD3+</td>
<td>5–11,452</td>
</tr>
</tbody>
</table>
BD FACSVia Flow Cytometer

Method comparison (BD FACSVia flow cytometer)

T-lymphocyte subset percentages and absolute counts were enumerated with the BD Tritest CD4/CD8/CD3 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 FACS Calibur 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 9 Method comparison statistics for T-lymphocyte subsets (BD FACSVia flow cytometer)

<table>
<thead>
<tr>
<th>T-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>1.00</td>
<td>-0.48</td>
<td>1.7-86.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cells/µL</td>
<td>0.99</td>
<td>0.97</td>
<td>-2.28</td>
<td>16-3,041</td>
</tr>
<tr>
<td>CD3⁺CD8⁺</td>
<td>165</td>
<td>%</td>
<td>0.99</td>
<td>1.01</td>
<td>-0.10</td>
<td>3.7-94.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cells/µL</td>
<td>0.99</td>
<td>0.99</td>
<td>-5.84</td>
<td>20-3,466</td>
</tr>
<tr>
<td>CD3⁺</td>
<td>165</td>
<td>cells/µL</td>
<td>0.99</td>
<td>0.98</td>
<td>-0.19</td>
<td>167-4,434</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 T-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 three lots of BD Tristr CD4/CD8/CD3. 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 T-lymphocyte subset percentages and absolute counts, respectively.

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

<table>
<thead>
<tr>
<th>T-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>19.7</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td>70.7</td>
<td>0.9</td>
<td>1.1</td>
</tr>
</tbody>
</table>

\(^a\) MCL = BD Multi-Check CD4 Low control

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

<table>
<thead>
<tr>
<th>T-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>63.9</td>
<td>0.9</td>
<td>1</td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td>31.3</td>
<td>0.8</td>
<td>0.9</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 CD4/CD8/CD3 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 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°C–25°C) before staining or acquisition.

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

<table>
<thead>
<tr>
<th>T-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>148.9</td>
<td>5.8</td>
<td>6.6</td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td>533.5</td>
<td>5.1</td>
<td>5.5</td>
</tr>
<tr>
<td>CD3+</td>
<td>754.4</td>
<td>5</td>
<td>5.5</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>T-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>754.6</td>
<td>4.5</td>
<td>5.2</td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td>369.0</td>
<td>4.6</td>
<td>5.3</td>
</tr>
<tr>
<td>CD3+</td>
<td>1,180.2</td>
<td>4.1</td>
<td>5.1</td>
</tr>
</tbody>
</table>
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.

Linearity (BD FACSVia flow cytometer)

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

<table>
<thead>
<tr>
<th>T-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+CD8+</td>
<td>5–3,500</td>
</tr>
<tr>
<td>CD3+</td>
<td>5–10,000</td>
</tr>
</tbody>
</table>

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

BD FACSCalibur Flow Cytometer

Method comparison (BD FACSCalibur flow cytometer)

Absolute counts for CD3+CD4+ and CD3+CD8+ lymphocytes were compared to results from BD FACSCount. 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.

<table>
<thead>
<tr>
<th>Subset (cells/µL)</th>
<th>N</th>
<th>Slope</th>
<th>Intercept</th>
<th>R</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helper/inducer</td>
<td>201</td>
<td>1.02</td>
<td>4</td>
<td>0.99</td>
<td>0–1,880a</td>
</tr>
<tr>
<td>T lymphocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Within-Specimen Reproducibility (BD FACSCalibur flow cytometer)

Ten aliquots of specimens from three samples representing high, medium, and low CD4 counts were assessed. The results for absolute counts are shown in Table 16.

Table 16 Within-specimen reproducibility for BD Tritest CD4/CD8/CD3 reagent

<table>
<thead>
<tr>
<th>Subset (cells/µL)</th>
<th>Level</th>
<th>Mean</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suppressor/cytotoxic T lymphocytes</td>
<td>High</td>
<td>729</td>
<td>25</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Med</td>
<td>483</td>
<td>19</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>334</td>
<td>12</td>
<td>3.7</td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>High</td>
<td>2,770</td>
<td>100</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>Med</td>
<td>1,869</td>
<td>52</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>711</td>
<td>18</td>
<td>2.6</td>
</tr>
</tbody>
</table>

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

Table 15 Method comparison statistics

<table>
<thead>
<tr>
<th>Subset (cells/µL)</th>
<th>N</th>
<th>Slope</th>
<th>Intercept</th>
<th>R</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suppressor/cytotoxic T lymphocytes</td>
<td>196</td>
<td>1.00</td>
<td>29</td>
<td>0.99</td>
<td>70–1,980a</td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>199</td>
<td>1.02</td>
<td>8</td>
<td>0.99</td>
<td>70–2,860a</td>
</tr>
</tbody>
</table>

Within-Specimen Reproducibility (BD FACSCalibur flow cytometer)

Ten aliquots of specimens from three samples representing high, medium, and low CD4 counts were assessed. The results for absolute counts are shown in Table 16.
Stability (BD FACSCalibur flow cytometer)
A study was conducted to assess blood sample stability and stained sample stability using the BD Tritest CD4/CD8/CD3 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

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.

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), the CD3+CD8+ range (43 to 3.9 x 10^3 cells/µL), and the CD3+ range (124 to 1.1 x 10^4 cells/µL).

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REFERENCES


27 Gallagher PF, Fazekas de St. Groth B, Miller JFAP. CD4 and CD8 molecules can physically associate with the same T-cell receptor. Proc Natl Acad Sci, USA. 1989;86:10044-10048.


