1. INTENDED USE
BD CD4 fluorescein isothiocyanate (FITC) reagent is a single-color direct immunofluorescence reagent for enumerating percentages of mature human helper/inducer (CD4+) lymphocytes in erythrocyte-lysed whole blood (LWB) or peripheral blood mononuclear cell (PBMC) suspensions. 

2. SUMMARY AND EXPLANATION
Human lymphocytes may 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. T lymphocytes participate in antigen-specific cell-mediated immunity and regulate the secretion of immunoglobulin by B lymphocytes. T lymphocytes may also be classified based on their functional properties as helper/inducer or suppressor/cytotoxic.

Clinical Applications*
The CD41,2 antibody recognizes an antigen expressed primarily on helper/inducer T lymphocytes,3,4 although this antigen is also present in low density on monocytes.5 Helper/inducer lymphocytes are a subset of T lymphocytes (CD3+) that are CD4+. The relative percentage of the CD4+ helper/inducer subset is depressed in many patients with congenital or acquired immune deficiencies such as severe combined immunodeficiency (SCID)6 and acquired immunodeficiency syndrome (AIDS).7† The CD4 antigen is the receptor for the human immunodeficiency virus (HIV).8 Some CD4 antibodies, including the BD CD4

---

* Not all studies in this section employed BD reagents.
antibody, inhibit HIV binding to CD4+ cells. Subjects infected with HIV have been found to exhibit a continuous loss of CD4+ lymphocytes. An increased likelihood of clinical complications in patients with HIV is associated with a decrease in CD4+ T lymphocytes.

3. PRINCIPLES OF THE PROCEDURE

When monoclonal antibody reagents are added to human whole blood, the fluorochrome-labeled antibodies bind specifically to antigens on the surface of leucocytes. Monoclonal antibodies may be used to identify lymphocyte subpopulations.

An aliquot of the stained patient sample is introduced into the flow cytometer and passed in a narrow stream through the path of a laser beam. The stained cells fluoresce when excited by the laser beam and the emitted light is collected and processed by the flow cytometer.

4. REAGENT

Reagent Provided, Sufficient for 100 Tests

BD CD4 FITC reagent is a murine monoclonal antibody provided in buffered saline with gelatin and 0.1% sodium azide, and is available for 100 tests. It contains FITC-labeled CD4, clone SK3, for identification of the CD4+ helper/inducer lymphocyte subset. The fluorescein-to-protein ratio (F:P) for BDIS IgG monoclonal antibody reagents is 2 to 5. The F:P ratio for the CD4 FITC reagent has been optimized for its intended use.

The CD4 antibody is composed of mouse IgG1 heavy chains and kappa light chains. The CD4 antibody recognizes the CD4 antigen, 59 kilodaltons (kDa), which interacts with the class II molecules of the major histocompatibility complex (MHC) and is the primary receptor for the human immunodeficiency virus (HIV).

Precautions

- For in vitro diagnostic use.
- When stored at 2°C–8°C, the antibody reagent is stable until the expiration date shown on the label. Do not use after the expiration date.
- The antibody reagent should not be frozen or exposed to direct light during storage or during incubation with cells. Keep the reagent vial dry.
- Alteration in the appearance of the reagent, such as precipitation or discoloration, indicates instability or deterioration. In such cases, the reagent should not be used.
- The antibody reagents contain sodium azide as a preservative; however, care should be taken to avoid microbial contamination, which may cause erroneous results.

WARNING

All biological specimens and materials coming into contact with them are considered biohazards. Handle as if capable of transmitting infection 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.

† The Centers for Disease Control (CDC) recommends using a two-color reagent combination containing CD3 and CD4 antibodies for determining the percentage of CD4+ lymphocytes in human immunodeficiency virus (HIV)-infected subjects.
Concentration values 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>3.0</td>
</tr>
</tbody>
</table>

5. INSTRUMENT

CD4 FITC reagent is designed for use on a BD flow cytometer equipped with appropriate computer hardware, software, and gating electronics. The flow cytometer must be equipped to detect two-color fluorescence, forward scatter (FSC), and side scatter (SSC).

All performance characteristics were obtained using the BD FACScan™ flow cytometer. Other systems may have different characteristics and should be verified by the user.

For information on use of this reagent for research purposes with other BD FACS™ brand instruments, contact the BD Customer Support Center.

6. SPECIMEN AND COLLECTION PREPARATION

Blood samples may be prepared for flow cytometric analysis by using either LWB or PBMC preparation procedures. However, LWB is the recommended procedure since PBMC preparations yield more technique-dependent results.

Collect blood aseptically by venipuncture into a sterile BD Vacutainer® EDTA blood collection tube. A minimum of 1 mL of whole blood is required for LWB cell preparation and a minimum of 2 mL of whole blood is required for PBMC preparation. Blood should be stained within 6 hours of venipuncture for optimal results.

Anti-coagulated blood may be stored at room temperature (20°C–25°C) for up to 6 hours until ready for staining. Blood samples refrigerated prior to staining may give aberrant results.

A white blood cell (WBC) count and a differential white-cell count should be obtained from the same sample of whole blood before staining. Samples with counts greater than 9.8 x 10³ WBC/µL may be diluted with 1X phosphate-buffered saline (PBS) containing 0.1% sodium azide. For samples with counts less than 3.5 x 10³ WBC/µL, more blood may be needed and a separation procedure may be required to concentrate the cells.

Interfering Conditions

Previously fixed and stored patient specimens should not be used. Whole blood samples refrigerated prior to staining may give aberrant results. For optimal results, blood samples should be stained within 6 hours of venipuncture. Samples obtained from patients taking immunosuppressive drugs may yield poor resolution. The presence of blast cells may interfere with test results. In LWB preparations, the presence of unlysed or nucleated red blood cells (RBCs) may interfere with test results. Hemolyzed samples should be rejected. Follow the collection tube manufacturer’s guidelines for the minimum volume of blood to be collected.

CAUTION Use standard precautions when obtaining, handling, and disposing human blood samples and potentially carcinogenic reagents.

7. PROCEDURE

Reagent Provided
See Reagent Provided, Sufficient for 100 Tests, and Precautions in Section 4, Reagent.

Reagents and Materials Required But Not Provided

- BD Simultest™ Leucogate™ (CD45 FITC/CD14 PE) reagent, 1 mL, (Catalog No. 340040). For determining a lymphocyte analysis gate, refer to the BD CellQuest Software Reference Manual, the BD Simultest Leucogate Instructions for Use (IFU), and the BD Monoclonal Antibodies Source Book, Section 5.2. Store at 2°C–8°C.
- BD Calibrite™ beads (Catalog No. 349502). For detailed information on use, refer to the BD Calibrite Beads IFU.
- BD Vacutainer EDTA blood collection tubes or equivalent.
- Falcon® disposable 12 x 75-mm polystyrene test tubes or equivalent.
- Vortex mixer.
- Low-speed centrifuge (minimum speed 200g) with swinging-bucket rotor and 12 x 75-mm tube carriers.
- Vacuum aspirator with trap.
- Micropipettor with tips.
- Phosphate-buffered saline (PBS) (1X) (Dulbecco’s modified, pH 7.2 ±0.2; 0.01 mol/L PO4; and 0.15 mol/L NaCl). This reagent does not contain calcium, magnesium, phenol red, or sodium azide. Filter PBS through a 0.2-µm filter before use. Store at 2°C–8°C.
- PBS with 0.1% sodium azide.
- BD FACSFlow™ sheath fluid (Catalog No. 342003) or equivalent. CAUTION Use only BD FACSFlow sheath fluid diluent to dilute BD Calibrite beads.
- Paraformaldehyde (1%) for cell fixation. Dissolve 1 g of paraformaldehyde in 100 mL of freshly prepared PBS by carefully heating at temperatures up to 56°C in a chemical fume hood. Adjust to pH 7.4 ±0.2 with either 0.1 mol/L NaOH or 0.1 mol/L HCl. Filter with a 0.45-µm filter. Store in glass at 2°C–8°C.

Materials for LWB Cell Preparation

- BD FACS™ lysing solution (10X), 100 mL, (Catalog No. 349202). Store at 2°C–25°C. For use, dilute 1:10 with room temperature (20°C–25°C) reagent-grade water. Store in a glass container at room temperature. The prepared solution is stable for 1 month at room temperature. Refer to the BD FACS Lysing Solution IFU.
- Reagent-grade (both distilled and deionized) water.

Materials for PBMC Preparation

- Ficoll-Paque® separation medium. § Refer to the Ficoll-Paque IFU for materials and reagents required.
- Cell viability testing reagents and materials for the ethidium bromide and acridine orange method (refer to Viability Staining Using Ethidium

---

§ Ficoll-Paque is a registered trademark of GE Healthcare.
Bromide and Acridine Orange, Section 2.3, in the BD Monoclonal Antibodies Source Book) or the trypan blue exclusion method.\(^{22}\)

**Staining and Fixing the Cells**

The staining procedures for LWB and PBMCs are described below. In either procedure, use care to protect the tubes from direct light. Both procedures should be performed at room temperature (20°C–25°C). For LWB, use room temperature reagents. For PBMCs, use reagents at 2°C–25°C. See Precautions in Section 4, Reagent.

**LWB Cell Preparation**

Whole blood samples are first stained with BD Simultest Leucogate reagent (Tube A), Mouse IgG\(_1\) FITC Control reagent (Tube B), and the CD4 FITC reagent (Tube C). Diluted (1X) BD FACS Lysing Solution is then used to lyse RBCs following staining. See Interfering Conditions in Section 6, Specimen and Collection Preparation.

1. For each patient sample, label three 12 x 75-mm tubes A, B, and C. Also label each tube with the sample identification number.

2. Place 20 µL of BD Simultest Leucogate reagent into Tube A, 20 µL of Mouse IgG\(_1\) FITC Control reagent into Tube B, and 20 µL of the CD4 FITC reagent into Tube C.

3. For each sample tube, use a fresh micropipettor tip and carefully add 100 µL of well-mixed, anticoagulated whole blood into the bottom of each of the three labeled tubes. The recommended WBC concentration is 3.5 x 10\(^3\) to 9.8 x 10\(^3\) WBC/µL. Vortex thoroughly at low speed for 3 seconds and incubate for 15–30 minutes at room temperature (20°C–25°C).

**NOTE** Protect samples from direct light during this incubation procedure and exercise care to prevent blood from running down the side of the tube. If whole blood remains on the side of the tube, it will not be stained with the reagent.

4. Dilute 10X BD FACS lysing solution to 1X following the instructions in under Materials for LWB Cell Preparation in Section 7, Procedure. Add 2 mL of room temperature (20°C–25°C) 1X BD FACS lysing solution to each tube. Immediately vortex thoroughly at low speed for 3 seconds and incubate for 10–12 minutes at room temperature (20°C–25°C) in the dark. Do not exceed 12 minutes.

**NOTE** Avoid prolonged exposure of the cells to lytic reagents, which may cause white cell destruction.

5. Immediately after incubation, centrifuge tubes at 300 g for 5 minutes at room temperature (20°C–25°C).

6. Aspirate the supernatant leaving approximately 50 µL of residual fluid in the tube to avoid disturbing the pellet.

7. Vortex thoroughly at low speed to resuspend the cell pellet in the residual fluid and then add 2 mL of PBS with 0.1% sodium azide to each tube. Vortex thoroughly at low speed for 3 seconds. Centrifuge at 200g for 5 minutes at room temperature (20°C–25°C).

8. Aspirate the supernatant leaving approximately 50 µL of residual fluid
in the tube to avoid disturbing the pellet.

9. Vortex thoroughly at low speed to resuspend the cell pellet in the residual fluid and then add 0.5 mL of 1% paraformaldehyde to each tube. Vortex thoroughly at low speed for 3 seconds. Make sure the cells are well mixed with the fixing solution.

10. The cells are now ready to be analyzed on the flow cytometer. Cap or cover the prepared tubes and store at 2°C–8°C in the dark until flow cytometric analysis. Analyze the fixed cells within 24 hours after staining. Vortex the cells thoroughly (at low speed) to reduce aggregation before running them through the flow cytometer.

PBMC Preparation
Peripheral blood samples are first separated using Ficoll-Paque. The PBMC suspension is then stained with BD Simultest Leucogate reagent (Tube A), Mouse IgG1 FITC Control reagent (Tube B), and the CD4 FITC reagent (Tube C). See Interfering Conditions in Section 6, Specimen and Collection Preparation.

1. Refer to the Ficoll-Paque IFU for instructions on performing mononuclear cell separation.

2. After the cells have been separated, determine the viability of the cells by staining with ethidium bromide and acridine orange (refer to Viability Staining Using Ethidium Bromide and Acridine Orange, Section 2.3, in the BD Monoclonal Antibodies Source Book) or by using trypan blue exclusion. Greater than 90% of the cells should be viable for the sample to be acceptable for use.

3. The cell suspension must be adjusted to a concentration of 1.5 to $2.5 \times 10^7$ cells/mL with PBS containing 0.1% sodium azide. Count cells using a microscope and hemacytometer or a hematology cell counter.

4. For each patient sample, label three 12 x 75-mm tubes A, B, and C. Also label each tube with the sample identification number.

5. Place 20 µL of BD Simultest Leucogate reagent into Tube A, 20 µL of Mouse IgG1 FITC Control reagent into Tube B, and 20 µL of the CD4 FITC reagent into Tube C.

6. For each sample tube, use a fresh micropipettor tip and carefully add 50 µL of mononuclear cell suspension into the bottom of each of the three labeled tubes. Vortex thoroughly at low speed for 3 seconds and incubate for 15–30 minutes at room temperature (20°C–25°C).

7. Add 2 mL of PBS with 0.1% sodium azide to each tube, and vortex thoroughly at low speed for 3 seconds. Centrifuge tubes at 300 g for 5 minutes at room temperature (20°C–25°C).

8. Aspirate the supernatant leaving approximately 50 µL of residual fluid in the tube to avoid disturbing the pellet.

9. Vortex thoroughly at low speed to resuspend the cell pellet in the residual fluid.
fluid and then add 0.5 mL of 1% paraformaldehyde to each tube. Vortex thoroughly at low speed for 3 seconds. Make sure that the cells are well mixed with the fixing solution.

10. The cells are now ready to be analyzed on the flow cytometer. Cap or cover the prepared tubes and store at 2°C–8°C in the dark until flow cytometric analysis. Analyze the fixed cells within 24 hours after staining. Vortex the cells thoroughly (at low speed) to reduce aggregation before running them through the flow cytometer.21

Flow Cytometry

Follow the BD instructions for two-color flow cytometric analysis (for BD Simultest Leucogate reagent). See Figure 1, Figure 2, and Figure 3 for representative data.

Figure 1 shows the Leucogate lymphocyte analysis gate (Tube A), Figure 2 shows the fluorescence markers set on the Control Tube B, and Figure 3 and Figure 4 show the CD4+ helper/inducer lymphocyte population present in Tube C.

NOTE For the CD4 FITC reagent (Tube C), the markers set with the Control reagent (Tube B) may be observed to be inappropriate, since monocytes express low-density CD4 antigen. If there are dim-staining CD4+ monocytes in the sample, the number of CD4+ lymphocytes may be overestimated if the marker that was set on the negative control is retained (see Figure 3). In this case, it may be necessary to move the marker on the x-axis to the right to delineate bright-staining CD4+ lymphocytes from dim-staining CD4+ monocytes.18,23 This may be done by moving the M1 marker for Tube C to the point that marks the ascent of the steep CD4+ lymphocyte peak (see Figure 4).

Figure 2 BD FACScan histogram (Tube B) of LWB sample, from same donor as in Figure 1, stained with Mouse IgG1 FITC Control reagent. The figure displays fluorescence 1 (FL1) versus number of events (NoE), and the fluorescence marker (M1 marker) separates dimly stained events (interval [M0,M1]) from brightly stained events (interval [M1,M2]) in the lymphocyte gate. The marker is set at the lowest point where the
mouse IgG1 FITC negative control peak returns to baseline.

Figure 3  BD FACScan LWB histogram, from the same donor as in Figure 1, displaying FL1 versus number of events (NoE) for the CD4 FITC reagent (Tube C). The markers were set with the Control reagent (Tube B). Note the values for the percent of gated events (1) for CD4+ ([M1,M2] = 54.80%) and gated events (2) for CD4– ([M0,M1] = 45.20%). Also note the CD4+ monocytes (3).

Figure 4  BD FACScan LWB histogram, from the same donor, displaying FL1 versus number of events (NoE) for the CD4 FITC reagent (Tube C). The FL1 marker (M1 marker) was reset on the positive population to distinguish dim-staining CD4+ monocytes (3) from bright-staining CD4+ lymphocytes. Note the values after the marker has been moved for the percent of gated events (1) for CD4+ ([M1,M2] = 53.39%) and gated events (2) for CD4– ([M0,M1] = 46.61%).

Quality Control

For optimal results, we recommend using BD Calibrite beads and BD FACSComp™ software for setting the photomultiplier tube (PMT) voltages, setting the fluorescence compensation, and checking instrument sensitivity prior to use of BD CellQuest software and the CD4 FITC reagent on the BD FACScan flow cytometer. Refer to the BD FACScan User’s Guide and the BD CellQuest Software Reference Manual for information on optimizing the flow cytometer prior to analyzing patient samples.

We recommend that a control sample from a normal adult subject be run daily to optimize instrument settings and as a quality control check of the system. Correct results for a hematologically normal adult are illustrated in Figure 3 and Figure 4.

Mouse IgG1 Control reagent is run with each patient sample to set fluorescence markers between unstained and positively stained lymphocyte clusters and to detect the presence of nonspecific staining that would indicate erroneous patient results.
Visual inspection of the histogram obtained for Tube C is necessary to ensure that fluorescence markers are correctly set and that there is minimal nonspecific staining. If poor separation between unstained and positive populations is seen on visual inspection of the histogram for the normal control, nonspecific staining or incomplete staining can be inferred and the run should be rejected.

Use the following criteria for inspection of the dot plots, contour plots, or histograms obtained for each sample to evaluate the quality of the data obtained.

1. The operator should reject the results if one or more of the following conditions is observed for the normal control: no separation between cellular populations; too few lymphocytes (less than 500); excessive RBC or nucleated RBC contamination and debris (greater than 10%); excessive monocyte (greater than 3%) or granulocyte (greater than 6%) contamination of the lymphocyte gate; or the number of lymphocytes within the gate is less than 95% of total lymphocytes in the sample.

2. If there is no obvious reason for the normal control to fail, a sample from another normal control should be stained and run and the entire staining procedure repeated on all subsequent samples.

3. In LWB preparations, samples with nucleated RBCs may contain too much debris because of incomplete lysis of nucleated erythrocytes with BD FACS lysing solution. Too much debris may also occur when assaying blood samples from patients with certain hematologic disorders where red cells are difficult to lyse, as in myelofibrosis and spherocytosis. Nucleated erythrocytes will be counted as debris and, if debris exceeds 10%, the sample results should be rejected.

4. Under certain conditions, CD4-stained lymphocytes may form cell aggregates. As a result, these aggregated cells (escapees) may not be included within the lymphocyte gate and a lower percentage of CD4+ lymphocyte events will be obtained (refer to The Escapee Phenomenon, Section 8.2, in the BD Monoclonal Antibodies Source Book). For cell suspensions prepared by Ficoll-Paque separation, we recommend that a protein-containing medium be used (refer to The Escapee Phenomenon, Section 8.2, in the BD Monoclonal Antibodies Source Book).

NOTE Determinations of percentages of CD4+ lymphocytes made on LWB should not be compared to determinations made on PBMCs. Each laboratory should establish its own reference range appropriate to its standard technique.

8. RESULTS

Percent Lymphocyte Conversion

When the Percent Lymphocyte Conversion computation is performed, the CD4+ lymphocyte subset is reported as a percentage of lymphocytes in the lymphocyte analysis gate. If the computation is not performed, results will be reported as a percentage of the gated events.

Absolute Counts

An absolute cell count can be computed if a WBC count and the lymphocyte
percentage from an independent differential white-cell count are obtained using standard laboratory procedures.

9. LIMITATIONS

- Laboratories must establish their own normal reference ranges for the CD4 FITC reagent assay parameters, which may be affected by sex of patient, age of patient, and preparative technique. Race of patient may also have an effect, although sufficient data is not available to establish this. Age, sex, clinical status, and race of subjects should be known when a reference range is determined.
- If the results are to be expressed in absolute counts, an independent differential white-cell count and a WBC must also be run on the same sample of blood. The precision of the absolute count result obtained will depend on the precision for determining percent of CD4+ lymphocytes and the precision for the WBC and the differential white count assays. Absolute count values of lymphocyte subsets may not be comparable across laboratories because of variations in methods for determining white blood cell counts and/or white blood cell differential values.
- The CD4+ lymphocyte value derived from the CD4 FITC reagent may be different from the value determined with the Simultest CD3/CD4 reagent, which reports helper/inducer T lymphocytes that are positive for both CD3 and CD4, thereby eliminating CD3–CD4+ monocytes.
- Abnormal states of health are not always represented by abnormal percentages of CD4+ helper/inducer lymphocytes. That is, an individual who may be in an abnormal state of health may exhibit the same CD4+ lymphocyte percentages as a healthy individual. Results from the use of the CD4 FITC reagent must be used in conjunction with other information available from the clinical evaluation and from additional independent diagnostic procedures, and interpreted by a medically qualified diagnostician to establish presence or absence of specific disease states.
- The CD4 FITC reagent is not intended for screening samples for the presence of leukemic cells or for use in phenotyping samples from leukemia patients. The presence of blast cells may not allow the operator to set an adequate lymphocyte analysis gate using BD Simultest Leucogate reagent.
- A normal subject has been reported to have no reaction with CD4. However, this lack of reactivity has not been observed in studies of over 300 subjects.
- PBMC specimens should have viability greater than 90%. Dead cells may stain nonspecifically. Prolonged contact of mononuclear cells with some lymphocyte separation media may reduce cell viability. Refer to the manufacturer’s instructions for information.

10. EXPECTED VALUES

Leucocyte Subsets
BD has investigated the normal reference ranges for the CD4 FITC reagent in normal male and female subjects using the BD FACScan flow cytometer at six clinical sites (five European sites and one US site). The formulation tested used CD4 FITC with CD8 (Leu-2a) PE reagent. Values of CD4+ lymphocytes obtained with the
CD4 FITC reagent were equivalent to those obtained with BD Simultest CD4/CD8 reagent. The expected normal reference ranges of CD4+ helper/inducer lymphocytes are shown in Table 1. Ranges are presented as percentages of lymphocytes in the analysis gate using the BD Simultest Leucogate reagent tube and the Quadrant Correction/Percent Lymphocyte Conversion option in the BD Simultest IMK Plus software, one of the BD Simulset™ family of software.

Table 1 Representative reference ranges (LWB)a
CD4 FITC reagent assay parameters in hematologically normal adults as percentages of total gated lymphocytes (converted) (data pooled from six clinical sites)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sex</th>
<th>Age</th>
<th>n</th>
<th>Mean</th>
<th>95% Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helper/inducer lymphocytes</td>
<td>Male</td>
<td>18–40</td>
<td>77</td>
<td>39.9</td>
<td>27.3–52.5</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>41–70</td>
<td>88</td>
<td>43.9</td>
<td>28.5–59.2</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>18–40</td>
<td>85</td>
<td>44.0</td>
<td>31.4–56.7</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>41–70</td>
<td>54</td>
<td>48.9</td>
<td>34.0–63.8</td>
</tr>
</tbody>
</table>

a. The data was collected on a BD FACScan flow cytometer using BD Simultest IMK Plus reagents which included Leucogate, Control γ/δTCR and CD4/CD8 reagents. BD Simultest IMK Plus software was used in this study.

Adult reference ranges should not be used with pediatric blood samples.

Race may also be a variable in the establishment of normal reference ranges, although insufficient data was collected by BD to determine this.

NOTE Expected normal values may vary depending upon age, sex, race of patient, or cell preparation method. Because of these differences, each laboratory should establish its own normal reference range for each parameter.

Absolute Counts
Absolute counts will exhibit significant interlaboratory variation depending upon the procedure employed for obtaining the WBC and differential white count.

11. PERFORMANCE CHARACTERISTICS
Performance of the CD4 FITC reagent was established by testing at either one US clinical site or at BD laboratories in San Jose, California, or both.

Within-Sample Reproducibility
Table 2 and Table 3 show the average within-sample reproducibility obtained for both LWB and PBMC samples.

Table 2 Within-sample reproducibility for CD4 FITC reagent assay parameters (LWB)a (five normal subjects and five abnormal subjects) as percentages of lymphocytes (converted)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Helper/inducer lymphocytes</td>
<td>46.3</td>
<td>1.5</td>
<td>3.2</td>
<td>15</td>
</tr>
<tr>
<td>Abnormal</td>
<td>Helper/inducer lymphocytes</td>
<td>18.8</td>
<td>2.1</td>
<td>12.2</td>
<td>15</td>
</tr>
</tbody>
</table>

a. The data was collected on a BD FACScan flow cytometer using BD Simultest IMK Plus reagents (Lacogate, Control γ/δTCR, and CD4/CD8 reagents). BD Simultest IMK Plus software was used in this study.

Table 3 Within-sample reproducibility for CD4 FITC reagent assay parameters (PBMCs)a (five normal subjects) as percentages of lymphocytes (converted)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Helper/inducer lymphocytes</td>
<td>50.8</td>
<td>1.9</td>
<td>3.8</td>
<td>15</td>
</tr>
</tbody>
</table>

a. The data was collected on a BD FACScan flow cytometer using CD4 FITC, BD Simultest Leucogate, and Mouse IgG1 FITC Control reagents. CONSORT™ 30 software was used in this study.
Between-Instrument Reproducibility

Table 4 and Table 5 show these results.

Table 4 Between-instrument reproducibility for CD4 FITC reagent assay parameters (LWB)\(^a\) (ten normal subjects and three instruments) as percentages of lymphocytes (converted)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>Helper/inducer lymphocytes</td>
<td>45.8</td>
<td>1.26</td>
<td>2.47</td>
<td>20</td>
</tr>
</tbody>
</table>

\(^a\) The data was collected on a BD FACScan flow cytometer using BD Simultest IMK Plus reagents (Leucogate, Control \(\gamma_1/\gamma_2\) and CD4/CD8 reagents). BD Simultest IMK Plus software was used in this study.

Table 5 Between-instrument reproducibility for CD4 FITC reagent assay parameters (PBMC)\(^a\) (five normal subjects and three instruments) as percentages of lymphocytes (converted)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5</td>
<td>Helper/inducer lymphocytes</td>
<td>50.41</td>
<td>1.44</td>
<td>2.77</td>
<td>25</td>
</tr>
</tbody>
</table>

\(^a\) The data was collected on a BD FACScan flow cytometer using CD4 FITC, BD Simultest Leucogate, and Mouse IgG1 FITC Control reagents. CONSORT 30 software was used in this study.

Between-Laboratory Reproducibility

Between-laboratory reproducibility is indicated by the ability to pool the normal reference ranges for the CD4 FITC reagent parameters (Table 1).

CD4 FITC versus Comparative Method

Table 6 shows a summary of these results.

Table 6 CD4 FITC\(^a\) reagent versus comparative method\(^b\) using LWB and the BD FACScan

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Slope</th>
<th>Intercept</th>
<th>(r)</th>
<th>(R^2)</th>
<th>Range of Data (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helper/inducer lymphocytes</td>
<td>1.16</td>
<td>-1.81</td>
<td>0.96</td>
<td>0.81</td>
<td>10–60</td>
</tr>
</tbody>
</table>

\(^a\) The data was collected on the BD FACScan flow cytometer using BD Simultest IMK Plus reagents (Leucogate, Control \(\gamma_1/\gamma_2\), and CD4/CD8 reagents). BD Simultest IMK Plus software was used in this study.

\(^b\) The data was collected on the BD FACScan flow cytometer using Leucogate reagent, Control \(\gamma_1/\gamma_2\) reagent, and BD Simultest Immune Monitoring Kit CD4 FITC/CD8 PE/CD14 FITC + PE reagents.

Stability of Stained Cell Preparations

We recommend analyzing samples within 24 hours of staining.

Cross-Reactivity

The CD4 antibody reacts with monocytes as well as helper/inducer T lymphocytes.\(^3\)

Linearity-Recovery

For an LWB sample, the normal range is \(3.5 \times 10^3\) to \(9.8 \times 10^3\) WBC/µL. Results are expected to be linear from \(3.5 \times 10^3\) to \(9.8 \times 10^3\) WBC/µL. For PBMCs, optimal performance is assured at \(0.75 \times 10^6\) to \(1.25 \times 10^6\) WBC/sample. PBMC concentration should be adjusted to \(1.5 \times 10^7\) to \(2.5 \times 10^7\) cells/mL.

WARRANTY

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

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

REFERENCES


