BD Multitest™ CD3/CD8/CD45/CD4

50 Tests per kit—Catalog No. 340499
50 Tests per kit with BD Trucount™ Tubes—Catalog No. 340491

IVD

℞ Only

BD

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CONTENTS

1. INTENDED USE

BD Multitest™ CD3/CD8/CD45/CD4 with optional BD Trucount™ tubes is intended for use with BD FACSLyric™, BD FACSCanto™ II, BD FACSCanto™, and BD FACSCalibur™ flow cytometers to determine the percentages and absolute counts of the following mature human lymphocyte subsets in peripheral whole blood for immunophenotyping:

- T lymphocytes ($CD3^+$)
- Helper/inducer T lymphocytes ($CD3^+CD4^+$)
- Suppressor/cytotoxic T lymphocytes ($CD3^+CD8^+$)

This reagent is indicated for use in the immunological assessment of normal individuals, and patients having, or suspected of having, immune deficiency.

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.

Suppressor/cytotoxic T lymphocytes are a subset of T lymphocytes ($CD3^+$) that are $CD8^+$. Helper/inducer T lymphocytes are a subset of T lymphocytes ($CD3^+$) that are $CD4^+$. $CD3^+CD8^+$ and $CD3^+CD4^+$ percentages or counts are used to characterize and monitor some forms of immunodeficiency[1–3] 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 percentage of suppressor/cytotoxic T lymphocytes lies outside the normal reference range in some autoimmune diseases.[8] The relative percentage of the $CD8^+$ subset is elevated in many patients with congenital or acquired immune deficiencies such as severe combined immunodeficiency (SCID)[1] or acquired immune deficiency syndrome (AIDS).[6]

The Centers for Disease Control (CDC) recommends using reagent combinations containing CD3 antibodies for determining the percentage of T-lymphocyte subsets in HIV-infected subjects.[9] BD Multitest CD3/CD8/CD45/CD4 allows helper/inducer T lymphocytes to be identified and enumerated separately from contaminating $CD3^-CD4^+$ monocytes.[10–12]

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 Multitest reagents employ fluorescence triggering, allowing direct fluorescence gating of the lymphocyte population[10–12] to reduce contamination of unlysed or nucleated red blood cells in the gate.

When BD Trucount tubes are used, a known 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, Instruments section), absolute counts are determined by the software.

If manually performing data analysis using software such as BD CellQuest™ Pro, simply divide the number of positive cellular events by the number of bead events, then multiply by the number of BD Trucount™ beads per pellet divided by the sample volume in µL.

4. REAGENT

Reagent Composition

BD Multitest CD3/CD8/CD45/CD4 contains FITC-labeled CD3, clone SK7;[13–15] PE-labeled CD8, clone SK1;[16,17] PerCP-labeled CD45, clone 2D1 (HLe-1);[18] and APC-labeled CD4, clone SK3.[16,17,19]

CD3 identifies T lymphocytes and recognizes the epsilon chain of the CD3 antigen/T-cell antigen receptor (TCR) complex.[20] This complex is composed of at least six proteins that range in molecular weight from 20 to 30 kilodaltons (kDa).[21] The antigen recognized by CD3 antibodies is noncovalently associated with either α/β or γ/δ TCR (70 to 90 kDa).[22]

CD8 identifies suppressor/cytotoxic T lymphocytes and recognizes the 32-kDa α subunit of a disulfide-linked bimolecular complex.[23] The cytoplasmic domain of the α subunit of the CD8 antigen is associated with the protein tyrosine kinase p56lck.[24] The CD8 molecule interacts with class I major histocompatibility complex (MHC) molecules, resulting in increased adhesion between the $CD8^+$ T lymphocytes and the target cells.[25–27] Binding of the CD8 molecule to class I MHC molecules enhances the activation of resting T lymphocytes.[25–27]

CD45 identifies leucocytes and recognizes a 180- to 220-kDa human leucocyte antigen that is a member of the leucocyte common antigen (LCA) family.[28]

CD4 identifies helper/inducer T lymphocytes and recognizes the CD4 antigen, with a molecular weight of 59 kDa,[29] which interacts with class II MHC molecules and is the primary receptor for HIV.[30,31] The cytoplasmic portion of the antigen is associated with the protein tyrosine kinase p56lck.[24]

CD3, CD8, CD45, and CD4 antibodies are composed of mouse IgG_1 heavy chains and kappa light chains.

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

Reagent	Concentration (µg/mL)
CD3 FITC	2.3
CD8 PE	1.75
CD45 PerCP	7.50
CD4 APC	0.92

Cross-Reactivity

The CD8 antibody reacts with NK lymphocytes[42] and with suppressor/cytotoxic T lymphocytes. The CD4 antibody reacts with monocytes and with helper/inducer T lymphocytes.[19]

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. We recommend performing the reverse pipetting technique according to the pipet manufacturer's instructions.

- Bead count varies by lot of BD Trucount tubes. It is critical to use the bead count shown on the current lot of BD Trucount tubes when entering this value in the software or when manually calculating absolute counts. Do not mix multiple lots of 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.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[32,33] 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.[43]

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. Examine the desiccant each time you open the pouch. If the desiccant has turned from blue to lavender, discard the remaining tubes. Use tubes within 1 hour after removal from the foil pouch. Once the pouch has been opened, the tubes are stable for 1 month. Do not use beyond the expiration date indicated on the packaging.

5. INSTRUMENTS

BD Multitest CD3/CD8/CD45/CD4 and BD Trucount tubes are designed for use on a flow cytometer equipped with appropriate computer hardware and software. BD has developed cytometer-specific software that can set photomultiplier tube (PMT) voltages and fluorescence compensation, check instrument sensitivity and performance, or perform daily quality control. BD has also developed software that automatically calculates absolute counts when BD Trucount tubes are used. However, other software packages manufactured by companies other than BD, can be used for data acquisition and analysis and absolute counts can be calculated manually. We recommend the BD systems listed in Table 1 for cytometer setup, acquisition, and analysis. See the corresponding reagent, cytometer, or software instructions for use (IFU) for details.

Results can be achieved using other platforms. The flow cytometer must be equipped with 635-nm and 488-nm lasers and must be capable of detecting light scatter (forward and side) and four-color fluorescence with emission detectable in four ranges:

- 515–545 nm
- 562–607 nm
- >650 nm
- 652–668 nm

The flow cytometer must be able to threshold or discriminate using the >650-nm channel. Users of flow cytometers manufactured by companies other than BD should refer to the manufacturer's instructions for setting up four-color immunophenotyping.

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

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

Table 1 Recommended BD systems

Flow cytometer	Setup beads	Setup software	Analysis software
BD FACSLyric™	BD™ CS&T beads BD™ FC beads 7-color kit	BD FACSuite™ Clinical software	BD FACSuite Clinical software
BD FACSCanto™ BD FACSCanto™ II	BD FACS™ 7-color setup beads	BD FACSCanto™ clinical software	BD FACSCanto clinical software
BD FACSCalibur™	BD Calibrite™ 3-color beads BD Calibrite™ APC beads	BD FACSComp™ software v4.0 or later	BD Multiset™ software

6. SPECIMEN COLLECTION AND PREPARATION

Collect blood aseptically by venipuncture[34,35] into a sterile BD Vacutainer® EDTA (ethylenediamine-tetraacetic acid) blood collection tube, or equivalent. BD Multitest CD3/CD8/CD45/CD4 and BD Trucount tubes have been validated with both liquid and dry formulations of EDTA.

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 dilution, especially when determining absolute counts with BD Trucount beads.

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 48 hours of draw and then 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.[36] Blast cells can interfere with test results. Hemolyzed samples should be rejected.

7. REAGENTS AND MATERIALS

Reagent Provided

The reagent, sufficient for 50 tests when used as directed, is provided in 1 mL of buffered saline with 0.1% sodium azide.

- BD Multitest CD3/CD8/CD45/CD4 (Catalog No. 340499), or
- BD Multitest CD3/CD8/CD45/CD4 with BD Trucount tubes (Catalog No. 340491)

  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. Two pouches of BD Trucount tubes are provided in the kit.

Reagents and Materials Required But Not Provided

- For BD FACSLyric flow cytometers:

  BD CS&T beads (Catalog Nos. 662413, 662414)

  BD FC beads 7-color kit (Catalog No. 662961)

- For BD FACSCanto and BD FACSCanto II flow cytometers:

  BD FACS 7-color setup beads (Catalog No. 335775)

- For BD FACSCalibur flow cytometers:

  BD Calibrite 3-color kit and BD Calibrite APC beads (Catalog Nos. 340486 and 340487, respectively)

- BD FACS™ lysing solution (Catalog No. 349202)

  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, BD Calibrite APC 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 process control, for example,
  - BD™ Multi-Check control (Catalog Nos. 349700, 349701, 349702)
  - BD™ Multi-Check CD4 Low Control (Catalog Nos. 349703, 349704, 349705)

NOTE We recommend running BD Trucount™ controls (Catalog No. 340335) to verify pipetting technique. The controls are supported on the BD FACSCalibur system. Do not use BD Trucount controls with BD FACSCanto clinical software. BD Trucount control beads can interfere with absolute count results.

* Falcon is a registered trademark of Corning Incorporated.

8. INSTRUCTIONS FOR USE

Diluting 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.

Performing Reverse Pipetting

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

Performing Quality Control

In accordance with the College of American Pathologists (CAP) guidelines, we recommend running two levels of liquid control material (process control). Controls should be run at least once each day that patient testing is performed.[38]

Use commercial controls providing established values for percent positive and absolute counts with each run to assess system performance. BD offers BD Multi-Check control and BD Multi-Check CD4 Low control to use as process controls.

To perform quality control:

1. Thoroughly mix the appropriate BD Multi-Check control, or equivalent process control.

   See the IFU for the control for detailed instructions.

2. Stain the control sample using BD Multitest CD3/CD8/CD45/CD4 as described in the following section.

The control sample should be processed like patient samples to monitor the ongoing performance of the entire analytic process.

3. Acquire the stained control sample on the flow cytometer.
4. 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.
5. Verify that the results are within the values reported on the Assay Values sheet.

Staining the Cells

Use care to protect the tubes from direct light. Perform the procedure at room temperature. See Precautions and Interfering Conditions.

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 use, 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.
2. Pipette 20 µL of BD Multitest CD3/CD8/CD45/CD4 into the bottom of the tube.

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

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

   NOTE If using a BD Trucount tube, we recommend using the reverse pipetting technique to pipette the sample onto the side of the tube just above the metal retainer. 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.
5. Incubate for 15 minutes in the dark at room temperature (20°C–25°C).
6. Add 450 µL of 1X BD FACS lysing solution to the tube.
7. Cap the tube and vortex gently to mix.
8. Incubate for 15 minutes in the dark at room temperature (20°C–25°C).

   The sample is now ready to be analyzed on the flow cytometer. If samples will not be analyzed immediately after staining, store them in the dark at room temperature (20°C–25°C).

Acquiring the Samples

1. Vortex the cells thoroughly at low speed.

   It is important to reduce aggregation before running samples on the flow cytometer.[37]

   NOTE If you are using a Loader, vortex tubes immediately before placing them into the Loader racks.

2. Install the tube on the cytometer and acquire the sample.

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

3. Analyze the data using the appropriate cytometer-specific software. See the cytometer's IFU for more information.

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 FACSuite Clinical, BD FACSCanto clinical, or BD Multiset software is used, absolute counts will be determined by the software.

For manual data analysis using BD CellQuest™ Pro or other software, the absolute count of the cell population (A) can be calculated using the following equation:

$A = X/Y \times N/V$, where:

X is the number of positive cell events

Y is the number of bead events

N is the number of 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)

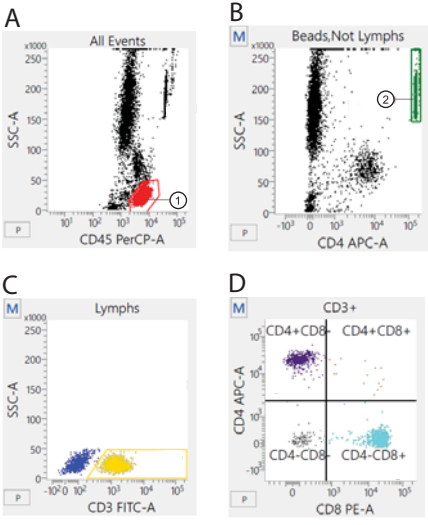
Representative Data

BD FACSLyric flow cytometer

A hematologically normal adult sample stained with BD Multitest CD3/CD8/CD45/CD4 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 CD4 APC-A vs SSC-A dot plot.

Panel C shows CD3+ T lymphocytes in the CD3 FITC-A vs SSC-A dot plot. Panel D shows suppressor/cytotoxic (CD4−CD8+) and helper/inducer (CD4+CD8−) T lymphocytes in the CD8 PE-A vs CD4 APC-A dot plot.

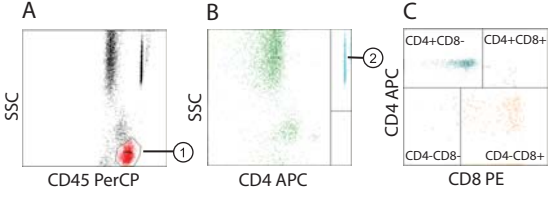
Figure 1 Representative data from a hematologically normal adult sample stained with BD Multitest CD3/CD8/CD45/CD4 in a BD Trucount tube (BD FACSLyric)



BD FACSCanto II flow cytometer

A hematologically normal adult sample stained with BD Multitest CD3/CD8/CD45/CD4 in a BD Trucount tube was acquired using a BD FACSCanto II cytometer. See Figure 2. 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 CD4 APC vs SSC dot plot. Panel C shows suppressor/cytotoxic ($CD4^-CD8^+$) and helper/inducer ($CD4^+CD8^-$) T lymphocytes in the CD8 PE vs CD4 APC dot plot.

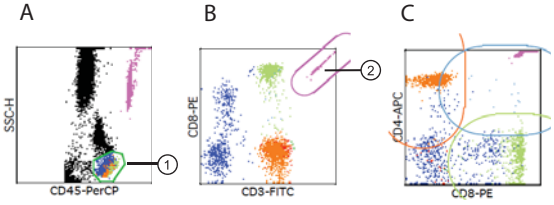
Figure 2 Representative data from a hematologically normal adult sample stained with BD Multitest CD3/CD8/CD45/CD4 in a BD Trucount tube (BD FACSCanto II)



BD FACSCalibur flow cytometer

A hematologically normal adult sample stained with BD Multitest CD3/CD8/CD45/CD4 in a BD Trucount tube was acquired using a BD FACSCalibur 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 CD8 PE dot plot. Panel C shows suppressor/cytotoxic ($CD4^-CD8^+$) and helper/inducer ($CD4^+CD8^-$) T lymphocytes in the CD8 PE vs CD4 APC dot plot.

Figure 3 Representative data from a hematologically normal adult sample stained with BD Multitest CD3/CD8/CD45/CD4 in a BD Trucount tube (BD FACSCalibur)



10. LIMITATIONS

- Laboratories must establish their own normal reference intervals for the BD Multitest CD3/CD8/CD45/CD4 parameters that can be affected by gender of patient, age of patient, and preparative technique. Race of patient[39] and individual variations of epitope expression[40] 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.[41] Reference intervals provided are for information only.
- BD Multitest CD3/CD8/CD45/CD4 has not been validated for use with heparin or acid citrate dextrose (ACD) liquid anticoagulants in determining absolute counts with BD Trucount tubes.
- BD Multitest CD3/CD8/CD45/CD4 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 manufacturers' equipment.

11. EXPECTED VALUES

Reference intervals for BD Multitest CD3/CD8/CD45/CD4 with and without BD Trucount tubes were determined.[41] Subjects were hematologically normal adults between the ages of 19 and 80† years in the study to determine reference intervals for the BD FACSLyric flow cytometer. The studies for the other instruments were carried out at different times using samples from different populations, which can contribute to differences in the reference intervals between instruments. See the first limitation in the preceding section for more information about reference intervals.

BD FACSLyric Flow Cytometer

Table 2 Representative reference intervals for BD Multitest CD3/CD8/CD45/CD4

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

a. N = number of samples

† The subjects were between the ages of 18 and 65 years in the studies used to determine reference intervals for the BD FACSCanto II, BD FACSCanto, and BD FACSCalibur flow cytometers.

BD FACSCanto II, BD FACSCanto, and BD FACSCalibur Flow Cytometers

Table 3 Representative reference intervals for BD Multitest CD3/CD8/CD45/CD4

Lymphocyte Subset	N	Units	Mean	95% Range
$CD3^+$	164	%	72	56–86
		cells/µL	1,513	773–2,737
$CD3^+CD4^+$	164	%	45	33–58
		cells/µL	941	404–1,612
$CD3^+CD8^+$	164	%	24	13–39
		cells/µL	511	220–1,129

12. PERFORMANCE CHARACTERISTICS

BD FACSLyric Flow Cytometer

Method comparison (BD FACSLyric flow cytometer)

Lymphocyte subset percentages and absolute counts were enumerated with the BD Multitest CD3/CD8/CD45/CD4 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 FACSCanto II flow cytometer using BD FACSCanto clinical software version 2.4 or later.

Whole blood samples were collected at random at five clinical study sites. Method comparison statistics are reported for all cell subsets.[44] See Table 4.

Table 4 Method comparison statistics for lymphocyte subsets (BD FACSLyric flow cytometer)

Lymphocyte Subset	N	Units	R^2	Slope	Intercept	Range
$CD3^+$	336	%	0.99	1.00	0.50	1.29–94.83
		cells/µL	0.99	1.03	3.96	6–6,553

Table 4 Method comparison statistics for lymphocyte subsets (BD FACSLyric flow cytometer)

Lymphocyte Subset	N	Units	R^2	Slope	Intercept	Range
$CD3^+CD4^+$	336	%	1.00	1.01	–0.26	0.12–84.65
		cells/µL	0.99	1.02	–0.04	1–3,194
$CD3^+CD8^+$	336	%	0.99	1.00	–0.08	0.26–82.93
		cells/µL	0.99	1.02	–0.59	1–5,774

Within-site precision (BD FACSLyric flow cytometer)

A 21-day study was conducted at one site, BD Biosciences, to assess within-site precision.[45] 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 using four lots of BD Multitest CD3/CD8/CD45/CD4. Two separate runs were analyzed during each of the 21 tested days for a total of 42 runs.

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

‡ CD-Chex Plus is a registered trademark of Streck, Inc.

Table 5 Within-site precision of lymphocyte subset percentages in low analyte concentration (CDL[a]) (BD FACSLyric flow cytometer)

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

a. CDL = CD-Chex Plus CD4 Low control

Table 6 Within-site precision of lymphocyte subset percentages in normal analyte concentration (CDN[a]) (BD FACSLyric flow cytometer)

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

a. CDN = CD-Chex Plus control

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

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

Table 8 Within-site precision of lymphocyte subset absolute counts in normal analyte concentration (CDN) (BD FACSLyric flow cytometer)

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

Inter-laboratory reproducibility (BD FACSLyric flow cytometer)

A study was conducted to assess inter-laboratory reproducibility. A single lot of each process control, CD-Chex Plus CD4 Low control and CD-Chex Plus control, was provided to each of four clinical laboratories. The control samples were stained using the BD Multitest CD3/CD8/CD45/CD4 reagent. Two separate runs were analyzed during each of five non-consecutive tested days for a total of ten runs.

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

Table 9 Inter-laboratory reproducibility of lymphocyte subset percentages in low analyte concentration (CDL) (BD FACSLyric flow cytometer)

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

Table 10 Inter-laboratory reproducibility of lymphocyte subset percentages in normal analyte concentration (CDN) (BD FACSLyric flow cytometer)

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

Table 11 Inter-laboratory reproducibility of lymphocyte subset absolute counts in low analyte concentration (CDL) (BD FACSLyric flow cytometer)

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

Table 12 Inter-laboratory reproducibility of lymphocyte subset absolute counts in normal analyte concentration (CDN) (BD FACSLyric flow cytometer)

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

Whole blood repeatability (BD FACSLyric flow cytometer)

A whole blood repeatability study was performed to demonstrate system repeatability using 53 donor samples. Each donor sample was stained in duplicate using the BD Multitest CD3/CD8/CD45/CD4 reagent in BD Trucount tubes and run on 12 instruments for a total of 24 runs per sample.

Table 13 Whole blood repeatability of lymphocyte subset percentages (BD FACSLyric flow cytometer)

Lymphocyte subset	Mean (%)	Within run repeatability (SD)	Total repeatability (SD)
$CD3^+$	73.54	0.96	0.96
$CD3^+CD4^+$	33.46	0.83	0.83
$CD3^+CD8^+$	37.93	0.93	0.93

Table 14 Whole blood repeatability of lymphocyte subset absolute counts (BD FACSLyric flow cytometer)

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

Stability (BD FACSLyric flow cytometer)

The stability of the BD Multitest CD3/CD8/CD45/CD4 reagent in BD Trucount tubes was assessed by studying:

- 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 up to 51 hours post draw and stained samples were tested up to 26 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 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 15.

Table 15 Linear ranges of lymphocyte subsets (BD FACSLyric flow cytometer)

Lymphocyte Subset	Range (cells/µL)
$CD3^+$	3–5,148
$CD3^+CD4^+$	5–2,931
$CD3^+CD8^+$	7–3,480

Analytical measurement range (BD FACSLyric flow cytometer)

The analytical measurement range (AMR) for BD Multitest CD3/CD8/CD45/CD4 on the BD FACSLyric flow cytometer was determined. The lower end of the AMR was determined based on results from a limit of quantitation (LoQ) study and the upper end of the AMR was determined based on results from the method comparison study.

Table 16 AMR of lymphocyte subsets (BD FACSLyric flow cytometer)

Lymphocyte Subset	AMR (cells/µL)
$CD3^+$	15–5,000
$CD3^+CD4^+$	10–3,000
$CD3^+CD8^+$	11–3,000

BD FACSCanto II Flow Cytometer

Method comparison (BD FACSCanto II flow cytometer)

Lymphocyte subset percentages and absolute counts were enumerated with BD Multitest CD3/CD8/CD45/CD4 in BD Trucount tubes and analyzed on a BD FACSCanto II flow cytometer using BD FACSCanto clinical software version 2.1. The results were compared with results from the reagent analyzed on the BD FACSCanto flow cytometer using BD FACSCanto clinical software version 2.0.

Whole blood samples were collected at random at one clinical laboratory. Regression statistics are reported in Table 17.

Table 17 Regression analysis for subset percentages and absolute counts (BD FACSCanto II flow cytometer)

Subset	N	Units	R^2	Slope	Intercept	Range
$CD3^+$	104	%	0.984	0.97	2.72	52–92
		cells/µL	0.991	0.97	27.59	221–3,873
$CD3^+CD4^+$	104	%	0.994	1.01	0.20	2–57
		cells/µL	0.986	0.95	18.25	11–1,905
$CD3^+CD8^+$	104	%	0.993	1.00	0.34	11–81
		cells/µL	0.988	0.95	28.36	68–3,577

Precision (BD FACSCanto II flow cytometer)

Estimates of precision were determined at one site, BD Biosciences, using two specimens run in duplicate at two different levels of analyte concentration. Samples were run on three different instruments with three different operators (one operator and one instrument per day). Two separate runs were analyzed during each of the 21 tested days for a total of 42 runs. Calibration with BD FACS 7-color setup beads was performed before each run for a total of 42 runs. One reagent lot and one calibrator lot were used for the duration of the study.

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

Table 18 Precision of lymphocyte subset percentages in low analyte concentration (CDL[a]) (BD FACSCanto II flow cytometer)

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

a. CDL = CD-Chex Plus CD4 Low control

Table 19 Precision of lymphocyte subset percentages in normal analyte concentration (CDC[a]) (BD FACSCanto II flow cytometer)

Lymphocyte Subset	Mean (%)	SD (Repeatability)	SD (Within-device precision)
$CD3^+$	73.0	0.63	0.67
$CD3^+CD4^+$	46.8	0.81	0.82

Table 19 Precision of lymphocyte subset percentages in normal analyte concentration (CDC[a]) (BD FACSCanto II flow cytometer)

Lymphocyte Subset	Mean (%)	SD (Repeatability)	SD (Within-device precision)
$CD3^+CD8^+$	25.4	0.78	0.80

a. CDC = CD-Chex Plus control

Table 20 Precision of absolute counts in low analyte concentration (CDL) (BD FACSCanto II flow cytometer)

Lymphocyte Subset	Mean (cells/µL)	%CV (Repeatability)	%CV (Within-device precision)
$CD3^+$	1,086.0	3.5	3.6
$CD3^+CD4^+$	205.6	5.9	5.9
$CD3^+CD8^+$	866.0	3.8	3.9

Table 21 Precision of absolute counts in normal analyte concentration (CDC) (BD FACSCanto II flow cytometer)

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

Stability (BD FACSCanto II flow cytometer)

The stability of BD Multitest CD3/CD8/CD45/CD4 reagent in BD Trucount tubes was assessed by studying:

- 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 up to 48 hours post draw and stained samples were 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 48 hours of draw and analyzing samples within 24 hours of staining.

Linearity (BD FACSCanto II flow cytometer)

Linearity was assessed for the BD FACSCanto II system within a WBC concentration range of 0 to 3.3 x 10^4 WBC/µL. Results were observed to be linear across the following ranges.

Subset	Range (cells/µL)
$CD3^+$	6–5,998
$CD3^+CD4^+$	1–3,669
$CD3^+CD8^+$	2–2,324

BD FACSCanto Flow Cytometer

Method comparison (BD FACSCanto flow cytometer)

Lymphocyte subset percentages and absolute counts were enumerated with BD Multitest CD3/CD8/CD45/CD4 in BD Trucount tubes and analyzed on the BD FACSCanto flow cytometer using BD FACSCanto 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.

Whole blood samples were collected at random at one clinical laboratory. Regression statistics are reported in Table 22.

Table 22 Regression analysis for subset percentages and absolute counts (BD FACSCanto flow cytometer)

Subset	N	Units	R	Slope	Intercept	Range
$CD3^+$	108	%	0.993	1.00	–0.17	40–93
		cells/µL	0.987	0.99	–6.27	75–5,257
$CD3^+CD4^+$	108	%	0.998	0.99	0.26	1–70
		cells/µL	0.991	0.97	10.80	3–3,211
$CD3^+CD8^+$	108	%	0.996	1.00	0.27	10–81
		cells/µL	0.983	0.96	24.60	68–2,754

Precision (BD FACSCanto flow cytometer)

Estimates of precision were determined at one site, BD Biosciences, using two specimens run in duplicate at two different levels of analyte concentration. Samples were run on three different instruments with three different operators (one operator and one instrument per day). Two separate runs were analyzed during each of the 20 tested days for a total of 40 runs. Calibration with BD FACS 7-color setup beads was performed before each run for a total of 40 runs. One reagent lot and one calibrator lot were used for the duration of the study.

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

Table 23 Precision of lymphocyte subset percentages in low analyte concentration (MCL[a]) (BD FACSCanto flow cytometer)

Lymphocyte Subset	Mean (%)	SD (Repeatability)	SD (Within-device precision)
$CD3^+$	57.5	1.16	1.22
$CD3^+CD4^+$	17.6	0.73	0.77
$CD3^+CD8^+$	39.1	0.97	1.17

a. MCL = BD Multi-Check CD4 Low control

Table 24 Precision of lymphocyte subset percentages in normal analyte concentration (MCN[a]) (BD FACSCanto flow cytometer)

Lymphocyte Subset	Mean (%)	SD (Repeatability)	SD (Within-device precision)
$CD3^+$	69.9	1.15	1.21
$CD3^+CD4^+$	50.3	1.04	1.18
$CD3^+CD8^+$	19.8	1.03	1.15

a. MCN = BD Multi-Check control

Table 25 Precision of absolute counts in low analyte concentration (MCL) (BD FACSCanto flow cytometer)

Lymphocyte Subset	Mean (cells/µL)	%CV (Repeatability)	%CV (Within-device precision)
$CD3^+$	307.4	3.2	4.1
$CD3^+CD4^+$	94.9	5.8	6.5
$CD3^+CD8^+$	210.7	5.1	5.8

Table 26 Precision of absolute counts in normal analyte concentration (MCN) (BD FACSCanto flow cytometer)

Lymphocyte Subset	Mean (cells/µL)	%CV (Repeatability)	%CV (Within-device precision)
$CD3^+$	743.9	3.9	4.8
$CD3^+CD4^+$	539.4	5.7	5.9
$CD3^+CD8^+$	212.8	6.4	7.1

Stability (BD FACSCanto flow cytometer)

The stability of BD Multitest CD3/CD8/CD45/CD4 reagent in BD Trucount tubes was assessed by studying:

- 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 up to 48 hours post draw and stained samples were 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 48 hours of draw and analyzing samples within 24 hours of staining.

Linearity (BD FACSCanto flow cytometer)

Linearity was assessed for the BD FACSCanto system within a WBC concentration range of 0 to 3.0 x 10^4 WBC/µL. Results were observed to be linear across the following ranges.

Subset	Range (cells/µL)
$CD3^+$	48–9,627

Subset	Range (cells/µL)
$CD3^+CD4^+$	29–5,827
$CD3^+CD8^+$	22–4,076

BD FACSCalibur Flow Cytometer

Method comparison (BD FACSCalibur flow cytometer)

Lymphocyte subset percentages and absolute counts enumerated with BD Multitest CD3/CD8/CD45/CD4 in BD Trucount tubes were compared with results from BD Tritest™ CD3/CD4/CD45, or CD3/CD8/CD45 in BD Trucount tubes.

Whole blood samples from normal and abnormal donors were collected at random at two clinical laboratories and evaluated in both systems. Regression statistics reported in Table 27 indicate that the results are substantially equivalent.

Table 27 Regression analysis for subset percentages and absolute counts (BD FACSCalibur flow cytometer)

Subset	N	Units	R	Slope	Intercept	Range
$CD3^+$	124	%	1.0	1.002	0.254	22–90
		cells/µL	0.98	1.028	–20.451	189–2,987
$CD3^+CD4^+$	124	%	1.0	0.996	–0.434	1–62
		cells/µL	0.98	1.015	–7.692	93–1,904
$CD3^+CD8^+$	124	%	1.0	1.018	–0.383	13–78
		cells/µL	0.99	1.001	2.494	132–2,229

Within-specimen reproducibility (BD FACSCalibur flow cytometer)

Estimates of within-specimen reproducibility were determined at three clinical laboratories from five replicates of each sample collected from normal and abnormal donors. Means, SDs, and/or CVs are provided for subset percentages and absolute counts greater than 100 cells/µL in Table 28 and Table 29.

Table 28 Within-specimen reproducibility of subset percentages (BD FACSCalibur flow cytometer)

Subset	N	Mean (%)	SD
$CD3^+$	46	72.0	1.06
$CD3^+CD4^+$	46	26.1	0.85
$CD3^+CD8^+$	46	42.0	0.93

Table 29 Within-specimen reproducibility of absolute counts (BD FACSCalibur flow cytometer)

Subset	N	Mean (cells/µL)	%CV
$CD3^+$	46	1,219.9	6.34
$CD3^+CD4^+$	38	565.2	7.02
$CD3^+CD8^+$	46	687.8	6.79

Stability (BD FACSCalibur flow cytometer)

The stability of BD Multitest CD3/CD8/CD45/CD4 reagent in BD Trucount tubes was assessed by studying:

- 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 up to 48 hours post draw and stained samples were 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 48 hours of draw and analyzing samples within 24 hours of staining.

Linearity (BD FACSCalibur flow cytometer)

Linearity was assessed within a WBC concentration of 0.2 x 10^3 to 29.7 x 10^3 WBC/µL and a lymphocyte concentration of 0.1 x 10^3 to 9.0 x 10^3 lymphocytes/µL. Results were observed to be linear within the $CD3^+$ range, the $CD3^+CD4^+$ range, and the $CD3^+CD8^+$ range.

13. TROUBLESHOOTING

Problem	Possible Cause	Solution
The resolution between debris and cells is poor.	Specimen is of poor quality.	Check viability.
	Specimen is too old.	Obtain a new specimen and stain it promptly.
Staining is dim or fading.	Cell concentration was too high at the staining step.	Check the cell concentration and adjust as needed.
	Stained cells were stored too long before acquiring them.	Repeat staining with a fresh specimen and acquire it promptly.
Few or no cells are recorded.	Cell concentration was too low.	Resuspend fresh specimen at a higher concentration. Repeat staining and acquisition.
	Cytometer is malfunctioning.	Troubleshoot the instrument. See the cytometer instructions for use for more information.

WARRANTY

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

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REFERENCES

1 Schmidt RE. Monoclonal antibodies for diagnosis of immunodeficiencies. *Blut*. 1989;59:200-206.

2 Nicholson JKA. Use of flow cytometry in the evaluation and diagnosis of primary and secondary immunodeficiency diseases. *Arch Pathol Lab Med*. 1989;113:598-605.

3 Foucar K, Goeken JA. Clinical application of immunologic techniques to the diagnosis of lymphoproliferative and immunodeficiency disorders. *Lab Med*. 1982;13:403-413.

4 Cohen SB, Weetman AP. Activated interstitial and intraepithelial thyroid lymphocytes in autoimmune thyroid disease. *Acta Endocrinol*. 1988;119:161-166.

5 Smolen JS, Chused TM, Leiserson WM, Reeves JP, Alling D, Steinberg AD. Heterogeneity of immunoregulatory T-cell subsets in systemic lupus erythematosus: correlation with clinical features. *Am J Med*. 1982;72:783-790.

6 Giorgi JV, Hultin LE. Lymphocyte subset alterations and immunophenotyping by flow cytometry in HIV disease. *Clin Immunol Newslett*. 1990;10:55-61.

7 Landay A, Ohlsson-Wilhelm B, Giorgi JV. Application of flow cytometry to the study of HIV infection. *AIDS*. 1990;4:479-497.

8 Antel J, Bania M, Noronha A, Neely S. Defective suppressor cell function mediated by $T8^+$ cell lines from patients with progressive multiple sclerosis. *J Immunol*. 1986;137:3436-3439.

9 Centers for Disease Control. 1997 Revised guidelines for performing $CD4^+$ T-cell determinations in persons infected with human immunodeficiency virus (HIV). *MMWR*. 1997;46:1-29.

10 Nicholson JKA, Jones BM, Hubbard M. CD4 T-lymphocyte determinations on whole blood specimens using a single-tube three-color assay. *Cytometry*. 1993;14:685-689.

11 Nicholson J, Kidd P, Mandy F, Livnat D, Kagan J. Three-color supplement to the NIAID DAIDS guideline for flow cytometric immunophenotyping. *Cytometry*. 1996;26:227-230.

12 Nicholson JKA, Hubbard M, Jones BM. Use of CD45 fluorescence and side-scatter characteristics for gating lymphocytes when using the whole blood lysis procedure and flow cytometry. *Cytometry*. 1996;26: 16-21.

13 Haynes BF. Summary of T-cell studies performed during the Second International Workshop and Conference on Human Leukocyte Differentiation Antigens. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID, eds. *Leukocyte Typing II: Human T Lymphocytes*. New York, NY: Springer-Verlag; 1986:3-30.

14 Kan EAR, Wang CY, Wang LC, Evans RL. Noncovalently bonded subunits of 22 and 28 kd are rapidly internalized by T cells reacted with Anti–Leu-4 antibody. *J Immunol*. 1983;131:536-539.

15 Knowles RW. Immunochemical analysis of the T-cell–specific antigens. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID, eds. *Leukocyte Typing II: Human T Lymphocytes*. New York, NY: Springer-Verlag; 1986;259-288.

16 Evans RL, Wall DW, Platsoucas CD, et al. Thymus-dependent membrane antigens in man: inhibition of cell-mediated lympholysis by monoclonal antibodies to the T_{H2} antigen. *Proc Natl Acad Sci USA*. 1981;78:544-548.

17 Bernard A, Boumsell L, Hill C. Joint report of the first international workshop on human leucocyte differentiation antigens by the investigators of the participating laboratories: T2 protocol. In: Bernard A, Boumsell L, Dausset J, Milstein C, Schlossman SF, eds. *Leukocyte Typing*. New York, NY: Springer-Verlag; 1984:25-60.

18 Cobbold SP, Hale G, Waldmann H. Non-lineage, LFA-1 family, and leucocyte common antigens: new and previously defined clusters. In: McMichael AJ, ed. *Leucocyte Typing III: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1987:788-803.

19 Wood GS, Warner NL, Warnke RA. Anti-Leu-3/T4 antibodies react with cells of monocyte/macrophage and Langerhans lineage. *J Immunol*. 1983;131:212-216.

20 van Dongen JJM, Krissansen GW, Wolvers-Tettero ILM, et al. Cytoplasmic expression of the CD3 antigen as a diagnostic marker for immature T-cell malignancies. *Blood*. 1988;71:603-612.

21 Brenner MB, McClean J, Dialynas DP, et al. Identification of a putative second T cell receptor. *Nature*. 1986;322:145-149.

22 Clevers H, Alarcón B, Wileman T, Terhorst C. The T cell receptor/CD3 complex: a dynamic protein ensemble. *Annu Rev Immunol*. 1988;6:629-662.

23 Moebius U. Cluster report: CD8. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1989:342-343.

24 Rudd CE, Burgess KE, Barber EK, Schlossman SF. Monoclonal antibodies to the CD4 and CD8 antigens precipitate variable amounts of CD4/CD8-associated $p56^{lck}$ activity. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1989:326-327.

25 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.

26 Anderson P, Blue M-L, Morimoto C, Schlossman SF. Cross-linking of T3 (CD3) with T4 (CD4) enhances the proliferation of resting T lymphocytes. *J Immunol*. 1987;139:678-682.

27 Eichmann K, Jönsson J-I, Falk I, Emmrich F. Effective activation of resting mouse T lymphocytes by cross-linking submitogenic concentrations of the T cell antigen receptor with either Lyt-2 or L3T4. *Eur J Immunol*. 1987;17:643-650.

28 Schwinzer R. Cluster report: CD45/CD45R. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1989:628-634.

29 Organizing Committee of the Fourth International Workshop on Human Leucocyte Differentiation Antigens. Appendix A: CD guide. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens*. New York, NY: Oxford University Press Inc; 1989:1074-1093.

30 Dalgleish AG, Beverley PCL, Clapham PR, Crawford DH, Greaves MF, Weiss RA. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature*. 1984;312:763-767.

31 Maddon PJ, Dalgleish AG, McDougal JS, Clapham PR, Weiss RA, Axel R. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell*. 1986;47:333-348.

32 Centers for Disease Control. Perspectives in disease prevention and health promotion update: universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens in health-care settings. *MMWR*. 1988;37:377-388.

33 *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M29-A4.

34 *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture: Approved Standard—Sixth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2007. CLSI document GP41-A6.

35 *Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline—Second Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2007. CLSI document H42-A2.

36 Giorgi JV. Lymphocyte subset measurements: significance in clinical medicine. In: Rose NR, Friedman H, Fahey JL, eds. *Manual of Clinical Laboratory Immunology*. 3rd ed. Washington, DC: American Society for Microbiology; 1986:236-246.

37 Jackson AL, Warner NL. Preparation, staining, and analysis by flow cytometry of peripheral blood leukocytes. In: Rose NR, Friedman H, Fahey JL, eds. *Manual of Clinical Laboratory Immunology*. 3rd ed. Washington, DC: American Society for Microbiology; 1986:226-235.

38 Flow Cytometry Checklist. http://www.cap.org/apps/docs/laboratory_accreditation/laboratory_accreditation_checklist_order_form_.pdf

39 Prince HE, Hirji K, Waldbeser LS, Plaeger-Marshall S, Kleinman S, Lanier LL. Influence of racial background on the distribution of T-cell subsets and Leu 11-positive lymphocytes in healthy blood donors. *Diagn Immunol*. 1985;3:33-37.

40 Angadi CV. Lack of Leu-3a epitope on T-helper (CD4) lymphocytes. *J Clin Lab Anal*. 1990;4:193-195.

41 *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2010. CLSI document EP28-A3c.

42 Lanier LL, Le AM, Phillips JH, Warner NL, Babcock GF. Subpopulations of human natural killer cells defined by expression of the Leu-7 (HNK-1) and Leu-11 (NK-15) antigens. *J Immunol*. 1983;131:1789-1796.

43 Nicholson JK, Browning SW, Orloff SL, McDougal JS. Inactivation of HIV-infected H9 cells in whole blood preparations by lysing/fixing reagents used in flow cytometry. *J Immunol Methods*. 1993;160:215-218.

44 *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Third Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2013. CLSI document EP09-A3.

45 *Evaluation of Precision of Quantitative Measurements Procedures; Approved Guideline—Third Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document EP05-A3.