BD Multitest™ IMK Kit

50 Tests—Catalog No. 662965

50 Tests with BD Trucount™ Tubes—Catalog No. 662966

23-19814(04)
2023-08
English

℞ Only IVD

1. INTENDED USE

BD Multitest™ IMK Kit 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 ($CD3^+$)
- B lymphocytes ($CD19^+$)
- Natural killer (NK) lymphocytes ($CD3^-CD16^+$ and/or $CD56^+$)
- 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 NK lymphocytes.

The percentages or absolute counts of T lymphocytes, B lymphocytes, helper/inducer T lymphocytes, and suppressor/cytotoxic T lymphocytes are used to characterize and monitor some forms of immunodeficiency[1-3] and autoimmune diseases.[4,5]

Helper/inducer lymphocytes are a subset of T lymphocytes ($CD3^+$) that are $CD4^+$. Determining percentages or counts of helper/inducer T lymphocytes can be useful in monitoring human immunodeficiency virus (HIV)-infected individuals.[6] Individuals with HIV typically exhibit a steady decrease of helper/inducer T lymphocyte counts as the infection progresses.[7]

The Centers for Disease Control (CDC) recommends using reagent combinations containing CD3 antibodies for determining the percentage of T-lymphocyte subsets in HIV-infected subjects.[8] The BD Multitest™ IMK Kit allows helper/inducer T lymphocytes to be identified and enumerated separately from contaminating $CD3^-CD4^+$ monocytes.[9-11]

Suppressor/cytotoxic lymphocytes are a subset of T lymphocytes ($CD3^+$) that are $CD8^+$. The percentage of suppressor/cytotoxic lymphocytes lies outside the normal reference interval in some autoimmune diseases.[12] 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]

NK lymphocytes identified as $CD3^-$ and $CD16^+$ and/or $CD56^+$ have been shown to mediate cytotoxicity against certain tumors and virus-infected cells.[13] NK-mediated cytotoxicity does not require class I or class II major histocompatibility complex (MHC) molecules to be present on the target cell.[14]

3. PRINCIPLES OF THE PROCEDURE

When whole blood is added to the reagent, the fluorochrome-labeled antibodies in the reagent bind specifically to leukocyte surface antigens. The stained samples are treated to lyse erythrocytes. 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[9-11] 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 (see Table 1, Instruments section) is used, absolute counts are determined by the software. If manually performing data analysis, 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. REAGENTS

Reagent Composition

The BD Multitest™ IMK Kit is supplied as one vial each of the following reagents:

- BD Multitest™ CD3/CD8/CD45/CD4 contains FITC-labeled CD3, clone SK7;[15-17] PE-labeled CD8, clone SK1;[18,19] PerCP-labeled CD45, clone 2D1 (HLe-1);[20] and APC-labeled CD4, clone SK3.[18,19,21]
- BD Multitest™ CD3/CD16+CD56/CD45/CD19 contains FITC-labeled CD3, clone SK7; PE-labeled CD16, clone B73.1,[22-24] and PE-labeled CD56, clone NCAM 16.2;[25] PerCP-labeled CD45, clone 2D1 (HLe-1); and APC-labeled CD19, clone SJ25C1.[26]

CD3 reacts with the epsilon chain of the CD3 antigen/T-cell antigen receptor (TCR) complex.[27] The CD3 antigen is present on 61% to 85% of normal peripheral blood lymphocytes.[28]

The CD4[19,29] antigen, molecular weight 55 kilodaltons (kDa),[30] is present on a T-lymphocyte subset[31,32] ($CD3^+CD4^+$) that comprises 28% to 58%[28] of normal peripheral blood lymphocytes.[19,30] The CD4 antigen is present in low density on the cell surface of monocytes and in the cytoplasm of monocytes.

The CD8 antigen is expressed on the 32-kDa α subunit of a disulfide-linked bimolecular complex.[33,34] The CD8 antigen is present on a T-lymphocyte subset,[19,30-32,35,36] as well as on a subset of NK lymphocytes.[37] The CD8 antigen is expressed on 19% to 48% of normal peripheral blood lymphocytes[28] and 60% to 85% of normal thymocytes.[19,30]

CD16 and CD56 together facilitate identification of the NK lymphocyte population.[10,13] CD16 recognizes a 50- to 70-kDa human NK lymphocyte antigen that is an Fc receptor for IgG.[22,23,38] The CD16 antigen reacts variably with granulocytes.[23] CD56 recognizes an extracellular immunoglobulin-like domain common to three molecular weight forms (120, 140, and 180 kDa) of the neural cell adhesion molecule (NCAM).[39-41]

CD19 recognizes a 90-kDa antigen that is present on human B lymphocytes.[26,42] The CD19 antigen is present on approximately 7% to 23% of human peripheral blood lymphocytes[28] and on splenocytes.[43] The CD19 antigen is present on human B lymphocytes at all stages of maturation.[44] CD19 does not react with resting or activated T lymphocytes, granulocytes, or monocytes.[45]

CD45 recognizes human leukocyte antigens, 180 to 220 kDa, that are members of the T200 family.[46] The CD45 antigen is present on all human leukocytes in peripheral blood, including lymphocytes, monocytes, granulocytes, eosinophils, and basophils, and has a role in signal transduction, modifying signals from other surface molecules.[46] The CD45 antibody has been reported to react weakly with mature circulating erythrocytes and platelets.[46,47]

CD3, CD4, CD8, CD16, CD19, and CD45 antibodies are composed of mouse IgG_1 heavy chains and kappa light chains.

The CD56 antibody is composed of mouse IgG_{2b} 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
CD16 PE + CD56 PE	2.75
CD45 PerCP	7.5
CD4 APC	0.92
CD19 APC	2.3

Cross-Reactivity

The CD8 antibody reacts with NK lymphocytes,[37] and with suppressor/cytotoxic T lymphocytes. The CD4 antibody reacts with monocytes, and with helper/inducer T lymphocytes.[21] The CD16 antigen is expressed on neutrophils.[14] The CD56 antigen is present on approximately 5% of $CD3^+$ peripheral blood lymphocytes.[14]

Precautions

- Do not use the reagents if you observe any change in appearance. Precipitation or discoloration indicates instability or deterioration.
- 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. 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.
- The antibody reagents contain sodium azide as a preservative. However, take care to avoid microbial contamination, which can cause erroneous results.

- BD Multitest™ IMK Kit Lysing Solution contains 31.34% 2,2´-oxybisethanol (diethylene glycol), CAS number 111-46-6; 9.77% formaldehyde, CAS number 50-00-0; and 3.43% methanol, CAS number 67-56-1. The lysing solution is classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).
- Go to regdocs.bd.com/regdocs/sdsSearch to download the Safety Data Sheet.

	Danger
	H302+H312+H332: Harmful if swallowed, in contact with skin or if inhaled. H314: Causes severe skin burns and eye damage. H317: May cause an allergic skin reaction. H335: May cause respiratory irritation. H341: Suspected of causing genetic defects. H350: May cause cancer. H370: Causes damage to organs. H373: May cause damage to organs through prolonged or repeated exposure. H402: Harmful to aquatic life.
Prevention	P201: Obtain special instructions before use. P202: Do not handle until all safety precautions have been read and understood. P260: Do not breathe dust/fume/gas/mist/vapors/spray. P264: Wash thoroughly after handling. P270: Do not eat, drink or smoke when using this product. P271: Use only outdoors or in a well-ventilated area. P272: Contaminated work clothing should not be allowed out of the workplace. P273: Avoid release to the environment. P280: Wear protective gloves/protective clothing/eye protection/face protection.
Response	P301+P330+P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. P312: Call a POISON CENTER/doctor if you feel unwell. P303+P361+P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower]. P363: Wash contaminated clothing before reuse. P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P304+P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing. P310: Immediately call a POISON CENTER/doctor. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P307+P311: IF exposed: Call a POISON CENTER or doctor/physician. P308+P313: If exposed or concerned: Get medical advice/attention.

Storage	P405: Store locked up.
Disposal	P501: Dispose of contents/container to an approved facility in accordance with local, regional, national and international regulations.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[48,49] 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.[50]

Storage and Handling

- Store the reagent at 2–8 °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.
- The reagent is stable if kept in the BD FACSDuet™ instrument for 8 hours per day for 5 days. Do not store the reagent overnight in the instrument. Use of any reagent remaining after being kept in the BD FACSDuet™ instrument for 5 days must be validated by the user.
- Store BD Trucount™ Tubes in their original foil pouch at 2–25 °C. To avoid potential condensation, open the pouch only after it has reached room temperature and carefully reseal the pouch immediately after removing a tube. 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 Multitest™ IMK Kit 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, and perform daily quality control. BD has also developed software that automatically calculates absolute counts when BD Trucount™ Tubes are used. However, 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 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 cytometer must be able to threshold or discriminate using the >650-nm channel. Users of flow cytometers manufactured by companies other than BD should see 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. See the IFU for the cytometer used with your Loader for more information.

The BD FACSDuet™ Sample Preparation System can be used with this product. See the *BD FACSLyric™ Flow Cytometer with the Integrated BD FACSDuet™ Sample Preparation System Instructions for Use* for more information.

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 application v1.1 or later	BD FACSuite™ Clinical application v1.1 or later
BD FACSVia™	BD® CS&T beads	BD FACSVia™ clinical software	BD FACSVia™ 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 Kit and BD Calibrite™ APC Beads	BD FACSComp™ software v4.0 or later	BD Multiset™ software

6. SPECIMEN COLLECTION AND PREPARATION

Collect blood aseptically by venipuncture, using BD Vacutainer® EDTA blood collection tubes.[51] BD Multitest™ IMK Kit reagents and BD Trucount™ Tubes have been validated with both liquid and dry formulations of ethylenediamine-tetraacetic acid (EDTA).

A minimum of 200 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 (20–25 °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 might give aberrant results. Samples obtained from patients taking immunosuppressive drugs can yield poor resolution.[52] Blast cells can interfere with test results. Hemolyzed samples should be rejected.

7. REAGENTS AND MATERIALS

Reagents Provided

- BD Multitest™ CD3/CD8/CD45/CD4
- BD Multitest™ CD3/CD16+CD56/CD45/CD19

Each vial contains reagent in 1 mL of buffered saline with 0.1% sodium azide, sufficient for 50 tests when used as directed.

- BD Multitest™ IMK Kit Lysing Solution, provided as a 10X concentrate

  If calculating absolute counts, order BD Multitest™ IMK Kit with BD Trucount™ Tubes. The reagent comes with two boxes of BD Trucount™ Tubes, each containing two pouches. Each pouch contains 25 tubes, sufficient for 25 tests. BD Trucount™ Tubes contain a freeze-dried pellet of fluorescent beads in a single-use tube.

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 No. 340486 and 340487)
- 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 for dispensing 450 µL of 1X BD Multitest™ IMK Kit Lysing Solution
- Lysable whole blood process control, for example:
- BD Multi-Check™ Control (Catalog No. 349700, 349701, or 349702)
- BD Multi-Check™ CD4 Low Control (Catalog No. 349703, 349704, or 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.

8. INSTRUCTIONS FOR USE

Diluting BD Multitest™ IMK Kit Lysing Solution

Dilute the 10X concentrate 1:10 with room temperature (20–25 °C) deionized water. The prepared solution is stable for 1 month when stored in a glass or high density polyethylene (HDPE) container at room temperature.

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 when patient testing is performed.[53]

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 the BD Multitest™ IMK reagents 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 in the Assay Values sheet.

Staining the Cells

1. For each patient sample, label two 12 × 75-mm tubes with the sample identification number.

   Use letters such as *A* and *B* to differentiate the two tubes.

   For absolute counts, label two BD Trucount™ Tubes in place of the 12 × 75-mm tubes.

   NOTE Before use, verify that the BD Trucount™ bead pellet is under the metal retainer at the bottom of the tube. If this is not the case, discard the BD Trucount™ Tube and replace it with another. Do not transfer beads to another tube.

2. Pipette 20 µL of BD Multitest™ CD3/CD8/CD45/CD4 reagent into the bottom of each tube labeled *A*.

3. Pipette 20 µL of BD Multitest™ CD3/CD16+CD56/CD45/CD19 reagent into the bottom of each tube labeled *B*.

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

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

   NOTE If using BD Trucount™ Tubes, 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.

5. Cap the tubes and vortex gently to mix.
6. Incubate for 15–30 minutes in the dark at room temperature (20–25 °C).
7. Add 450 µL of 1X BD Multitest™ IMK Kit Lysing Solution to each tube.

   Use care to protect the tubes from direct light. Perform the procedure at room temperature (20–25 °C). See Precautions on page 9 and Interfering Conditions on page 14.

8. Cap the tubes and vortex gently to mix.
9. Incubate for 15–30 minutes in the dark at room temperature (20–25 °C).

   Samples are 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 (20–25 °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.[54]

   NOTE If you are using one of the Loaders, 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 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 cytometer-specific BD software is used, absolute counts will be determined by the software.

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

A = X/Y × 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

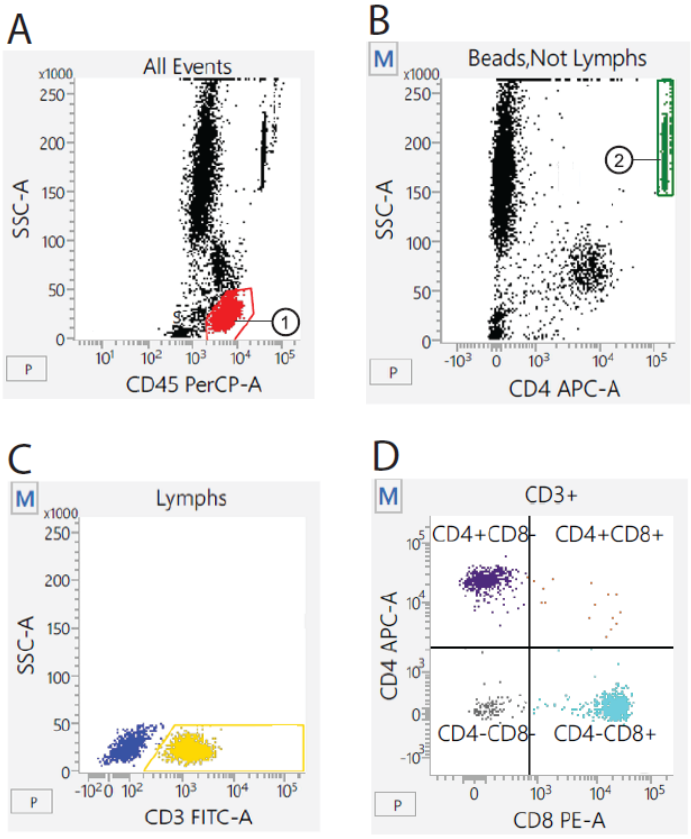
V is the sample volume (50 µL)

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 application 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.

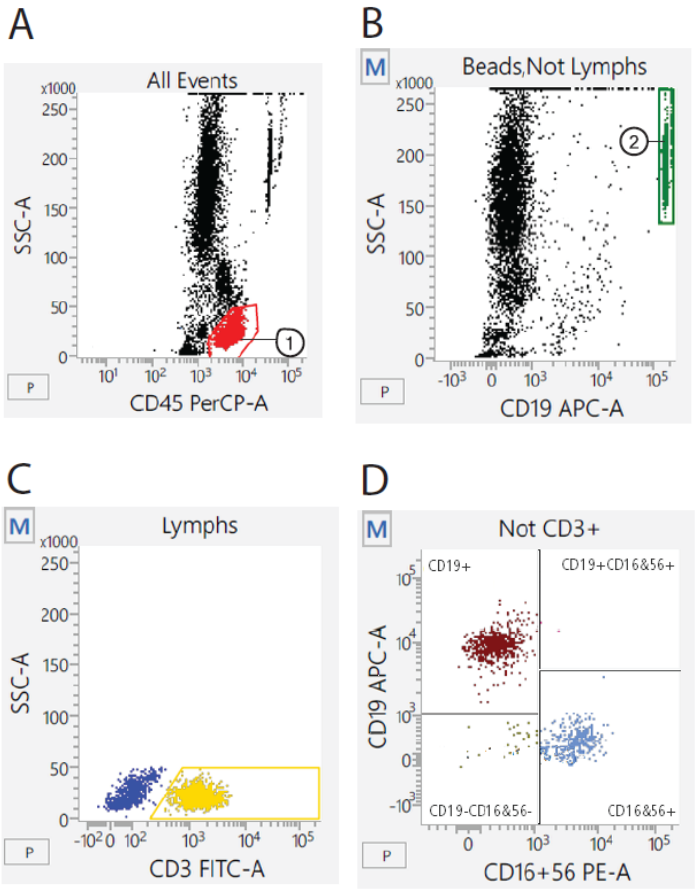
Figure 1 Representative data from a hematologically normal adult sample stained with BD Multitest™ CD3/CD8/CD45/CD4 in a BD Trucount™ Tube (BD FACSLyric™)



A hematologically normal adult sample stained with BD Multitest™ CD3/CD16+CD56/CD45/CD19 in a BD Trucount™ Tube was acquired with BD FACSuite™ Clinical application using a BD FACSLyric™ flow cytometer. See Figure 2. 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 CD19 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 B lymphocytes ($CD19^+$) and NK lymphocytes ($CD16\&CD56^+$) in the CD16+56 PE-A vs CD19 APC-A dot plot.

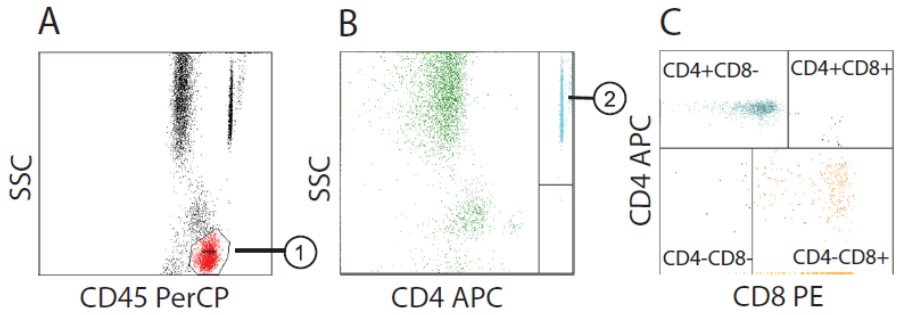
Figure 2 Representative data from a hematologically normal adult sample stained with BD Multitest™ CD3/CD16+CD56/CD45/CD19 in a BD Trucount™ Tube (BD FACSLyric™)



BD FACSCanto™ II flow cytometer

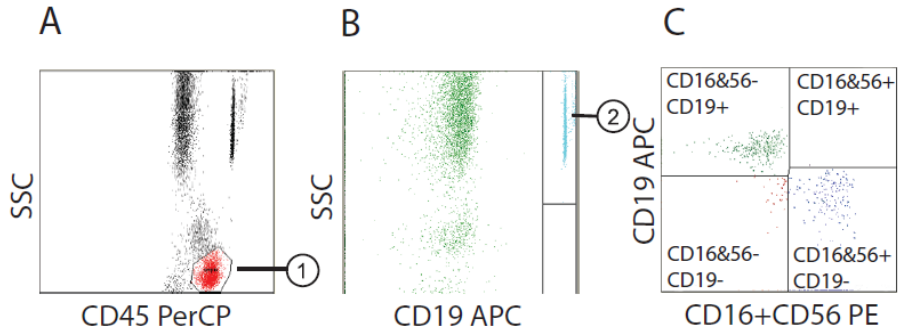
A hematologically normal adult sample stained with BD Multitest™ CD3/CD8/CD45/CD4 in a BD Trucount™ Tube was acquired using a BD FACSCanto™ II cytometer. See Figure 3. Panel A shows $CD45^+$ lymphocytes (1) identified in the CD45 PerCP vs SSC dot plot. Panel B shows BD Trucount™ absolute count bead events (2) in the 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 3 Representative data from a hematologically normal adult sample stained with BD Multitest™ CD3/CD8/CD45/CD4 in a BD Trucount™ Tube (BD FACSCanto™ II)



A hematologically normal adult sample stained with BD Multitest™ CD3/CD16+CD56/CD45/CD19 in a BD Trucount™ Tube was acquired on a BD FACSCanto™ II cytometer. See Figure 4. 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 CD19 APC vs SSC dot plot. Panel C shows B lymphocytes ($CD16\&56^-CD19^+$) and NK lymphocytes ($CD16\&56^+CD19^-$) in the CD16+CD56 PE vs CD19 APC dot plot.

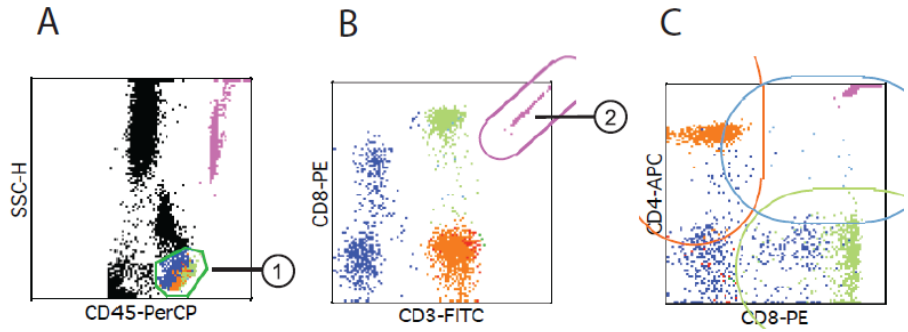
Figure 4 Representative data from a hematologically normal adult sample stained with BD Multitest™ CD3/CD16+CD56/CD45/CD19 in a BD Trucount™ Tube (BD FACSCanto™ II)



BD FACSCalibur™ flow cytometer

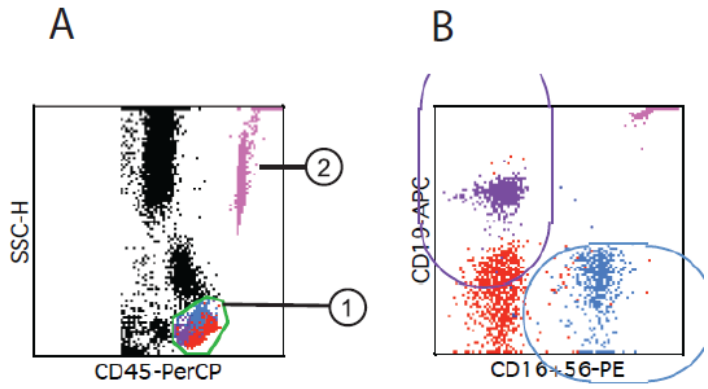
A hematologically normal adult sample stained with BD Multitest™ CD3/CD8/CD45/CD4 in a BD Trucount™ Tube was acquired using a BD FACSCalibur™ cytometer. See Figure 5. Panel A shows $CD45^+$ lymphocytes (1) identified in the CD45 PerCP vs SSC dot plot. Panel B shows BD Trucount™ absolute count bead events (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 5 Representative data from a hematologically normal adult sample stained with BD Multitest™ CD3/CD8/CD45/CD4 in a BD Trucount™ Tube (BD FACSCalibur™)



A hematologically normal adult sample stained with BD Multitest™ CD3/CD16+CD56/CD45/CD19 in a BD Trucount™ Tube was acquired on a BD FACSCalibur™ cytometer. See Figure 6. Panel A shows $CD45^+$ lymphocytes (1) and BD Trucount™ absolute count bead events (2) in the CD45 PerCP vs SSC dot plot. Panel B shows B lymphocytes ($CD19^+$) and NK lymphocytes ($CD16^+$, $CD56^+$, or both) identified in the CD16+CD56 PE vs CD19 APC dot plot.

Figure 6 Representative data from a hematologically normal adult sample stained with BD Multitest™ CD3/CD16+CD56/CD45/CD19 in a BD Trucount™ Tube (BD FACSCalibur™)



10. LIMITATIONS

- Laboratories must establish their own normal reference intervals for the BD Multitest™ IMK Kit parameters that can be affected by gender of patient, age of patient, and preparative technique. Race of patient[55] and individual variations of epitope expression[56] 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.[57] Reference intervals provided are for information only.
- The BD Multitest™ IMK Kit has not been validated by BD Biosciences for use with heparin or acid citrate dextrose (ACD) liquid anticoagulants in determining absolute counts with BD Trucount™ Tubes.
- The BD Multitest™ IMK Kit 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 the BD Multitest™ IMK Kit with and without BD Trucount™ Tubes were determined in a study using the BD FACSLyric™ flow cytometer.[57] Subjects were hematologically normal adults between the ages of 19 and 80 years. Similar studies carried out at different times using other BD flow cytometers used samples from different populations, which can contribute to differences in the reference intervals between studies.[58-60] See the first limitation (in the preceding section) for more information about reference intervals.

Table 2 Representative reference intervals for the BD Multitest™ IMK Kit

Lymphocyte Subset	N[a]	Units	Mean	95% Range
Average $CD3^+$	130	%	71.88	56.86-82.5
		cells/µL	1,555.87	827-2,547
$CD3^+CD4^+$	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
$CD3^-CD19^+$	130	%	13.69	5.14-22.96
		cells/µL	292.73	60-551
$CD3^-(CD16+CD56)^+$	130	%	13.25	5.42-29.65
		cells/µL	281.04	102-617

a. N = number of samples

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™ IMK (CD3/CD8/CD45/CD4 and CD3/CD16+CD56/CD45/CD19) reagents in BD Trucount™ Tubes and analyzed on the BD FACSLyric™ flow cytometer using BD FACSuite™ Clinical application. The results were compared with results from the reagents analyzed on the BD FACSCanto™ II flow cytometer using BD FACSCanto™ Clinical Software.

Whole blood samples were collected at random at five clinical study sites. Method comparison statistics are reported for all cell subsets.[61] See Table 3.

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

Lymphocyte Subset	N	Units	R^2	Slope	Intercept	Range
Average $CD3^+$	336	%	0.99	1.00	0.47	1.34–95.78
		cells/µL	0.99	1.04	1.43	6–6,499
$CD3^+CD4^+$	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
$CD3^-CD19^+$	336	%	1.00	1.02	–0.24	0.00–92.43
		cells/µL	1.00	1.02	–0.16	0–2,770
$CD3^-(CD16+CD56)^+$	336	%	0.99	1.00	–0.85	1.51–87.67
		cells/µL	0.99	0.96	–3.95	14–1,502

Method comparison (BD FACSLyric™ flow cytometer with BD FACSDuet™ system)

Whole blood specimens were collected at three clinical study sites. An aliquot of each specimen was stained with each of the BD Multitest™ IMK (CD3/CD8/CD45/CD4 and CD3/CD16+CD56/CD45/CD19) reagents in a BD Trucount™ Tube using the BD FACSDuet™ system. Stained samples were automatically transferred to an integrated BD FACSLyric™ flow cytometer and acquired using a BD FACS™ Universal Loader and BD FACSuite™ Clinical application. A second aliquot of each specimen was stained manually with the reagents in a BD Trucount™ Tube. Stained samples were acquired on a standalone BD FACSLyric™ flow cytometer using a BD FACS™ Universal Loader and BD FACSuite™ Clinical application.

Results were compared between samples prepared using the BD FACSDuet™ system and samples prepared manually.[57] See Table 4.

Table 4 Method comparison statistics for lymphocyte subsets (BD FACSLyric™ with BD FACSDuet™)

Lymphocyte Subset	N	Units	R^2	Slope	Intercept	Range
Average $CD3^+$	373	%	0.99	0.99	0.41	45.31–99.14
		cells/µL	0.99	1.00	0.85	89–11,376
$CD3^+CD4^+$	373	%	0.99	1.00	–0.02	0.37–91.86
		cells/µL	0.99	1.00	–0.15	4–7,911
$CD3^+CD8^+$	373	%	0.99	0.99	–0.01	2.52–86.68
		cells/µL	0.98	0.99	3.16	52–5,796
$CD3^-CD19^+$	373	%	0.97	1.00	–0.03	0.17–31.8
		cells/µL	0.98	0.99	–0.08	8–2,236

Lymphocyte Subset	N	Units	R^2	Slope	Intercept	Range
$CD3^-(CD16+CD56)^+$	373	%	0.98	1.00	0.23	0.52–44.27
		cells/µL	0.98	1.02	0.28	9–2,188

Within-site precision (BD FACSLyric™ flow cytometer)

A 21-day study was conducted at one site, BD Biosciences, to assess within-site precision.[62] 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 the BD Multitest™ IMK (CD3/CD8/CD45/CD4 and CD3/CD16+CD56/CD45/CD19) reagents. Two separate runs were analyzed during each of the 21 tested days for a total of 42 runs.

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

Table 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)
Average $CD3^+$	57.25	0.83	0.90
$CD3^+CD4^+$	11.66	0.62	0.64
$CD3^+CD8^+$	40.36	1.04	1.06
$CD3^-CD19^+$	21.70	0.79	0.82
$CD3^-(CD16+CD56)^+$	19.40	0.84	0.85

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)
Average $CD3^+$	76.77	0.59	0.62
$CD3^+CD4^+$	50.74	1.01	1.02
$CD3^+CD8^+$	22.22	0.80	0.80
$CD3^-CD19^+$	12.09	0.55	0.55
$CD3^-(CD16+CD56)^+$	10.34	0.59	0.59

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)
Average $CD3^+$	870.51	3.15	3.29
$CD3^+CD4^+$	176.91	6.59	6.67
$CD3^+CD8^+$	612.12	4.55	4.65
$CD3^-CD19^+$	330.87	5.22	5.35
$CD3^-(CD16+CD56)^+$	295.88	6.03	6.21

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)
Average $CD3^+$	1,733.81	3.05	3.21
$CD3^+CD4^+$	1,142.52	4.04	4.18
$CD3^+CD8^+$	500.42	5.56	5.67
$CD3^-CD19^+$	273.84	6.02	6.16
$CD3^-(CD16+CD56)^+$	234.38	7.41	7.52

Within-site precision (BD FACSLyric™ flow cytometer with BD FACSDuet™ system)

A 21-day study was conducted at one site, BD Biosciences, to assess within-site precision.[50] Estimates of precision for the enumeration of lymphocyte subset percentages and absolute counts were determined across three BD FACSDuet™ systems, each integrated with a BD FACSLyric™ flow cytometer, and at least three operators by acquiring two concentrations of analyte, CD-Chex Plus CD4 Low control and CD-Chex Plus control, stained in duplicate using three lots of the BD Multitest™ IMK (CD3/CD8/CD45/CD4 and CD3/CD16+CD56/CD45/CD19) reagents. Two separate runs were analyzed during each of the 21 tested days for a total of 42 runs.

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

Table 9 Within-site precision of lymphocyte subset percentages in low analyte concentration (CDL^a) (BD FACSLyric™ with BD FACSDuet™)

Lymphocyte Subset	Mean (%)	SD (Repeatability)	SD (Within-site precision)
Average $CD3^+$	63.67	0.81	0.82
$CD3^+CD4^+$	14.94	0.68	0.70

Lymphocyte Subset	Mean (%)	SD (Repeatability)	SD (Within-site precision)
$CD3^+CD8^+$	44.04	1.11	1.12
$CD3^-CD19^+$	18.24	0.71	0.72
$CD3^-(CD16+CD56)^+$	16.87	0.72	0.74

a. CDL = CD-Chex Plus CD4 Low control

Table 10 Within-site precision of lymphocyte subset percentages in normal analyte concentration (CDN[a]) (BD FACSLyric™ with BD FACSDuet™)

Lymphocyte Subset	Mean (%)	SD (Repeatability)	SD (Within-site precision)
Average $CD3^+$	77.45	0.64	0.64
$CD3^+CD4^+$	48.79	0.96	0.96
$CD3^+CD8^+$	26.77	0.91	0.91
$CD3^-CD19^+$	11.93	0.64	0.64
$CD3^-(CD16+CD56)^+$	9.98	0.55	0.55

a. CDN = CD-Chex Plus control

Table 11 Within-site precision of lymphocyte subset absolute counts in low analyte concentration (CDL) (BD FACSLyric™ with BD FACSDuet™)

Lymphocyte Subset	Mean (cells/µL)	%CV (Repeatability)	%CV (Within-site precision)
Average $CD3^+$	737.07	3.10	4.20
$CD3^+CD4^+$	173.18	5.97	6.57
$CD3^+CD8^+$	510.44	4.40	5.28
$CD3^-CD19^+$	210.89	5.69	6.37
$CD3^-(CD16+CD56)^+$	195.06	5.40	6.21

Table 12 Within-site precision of lymphocyte subset absolute counts in normal analyte concentration (CDN) (BD FACSLyric™ with BD FACSDuet™)

Lymphocyte Subset	Mean (cells/µL)	%CV (Repeatability)	%CV (Within-site precision)
Average $CD3^+$	1,751.12	3.99	6.27
$CD3^+CD4^+$	1,103.49	5.23	6.88
$CD3^+CD8^+$	605.62	6.23	7.70

Lymphocyte Subset	Mean (cells/µL)	%CV (Repeatability)	%CV (Within-site precision)
$CD3^{-}CD19^{+}$	269.47	6.93	8.53
$CD3^{-}(CD16+CD56)^{+}$	225.59	6.95	8.45

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™ IMK reagents. Two separate runs were analyzed during each of five non-consecutive tested days for a total of 10 runs.

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

Table 13 Inter-laboratory reproducibility of lymphocyte subset percentages in low analyte concentration (CDL) (BD FACSLyric™ flow cytometer)

Lymphocyte subset	Mean (%)	SD
Average $CD3^{+}$	57.12	1.00
$CD3^{+}CD4^{+}$	12.12	0.61
$CD3^{+}CD8^{+}$	40.74	1.12
$CD3^{-}CD19^{+}$	21.74	0.83
$CD3^{-}(CD16+CD56)^{+}$	19.44	1.06

Table 14 Inter-laboratory reproducibility of lymphocyte subset percentages in normal analyte concentration (CDN) (BD FACSLyric™ flow cytometer)

Lymphocyte subset	Mean (%)	SD
Average $CD3^{+}$	76.74	0.77
$CD3^{+}CD4^{+}$	51.67	1.58
$CD3^{+}CD8^{+}$	23.23	0.85
$CD3^{-}CD19^{+}$	12.02	0.66
$CD3^{-}(CD16+CD56)^{+}$	10.30	0.62

Table 15 Inter-laboratory reproducibility of lymphocyte subset absolute counts in low analyte concentration (CDL) (BD FACSLyric™ flow cytometer)

Lymphocyte subset	Mean (cells/µL)	%CV
Average $CD3^+$	883.06	4.18
$CD3^+CD4^+$	187.01	7.30
$CD3^+CD8^+$	628.51	5.23
$CD3^-CD19^+$	336.79	5.71
$CD3^-(CD16+CD56)^+$	301.25	7.40

Table 16 Inter-laboratory reproducibility of lymphocyte subset absolute counts in normal analyte concentration (CDN) (BD FACSLyric™ flow cytometer)

Lymphocyte subset	Mean (cells/µL)	%CV
Average $CD3^+$	1,747.70	3.98
$CD3^+CD4^+$	1,177.59	5.17
$CD3^+CD8^+$	529.63	6.05
$CD3^-CD19^+$	273.58	7.23
$CD3^-(CD16+CD56)^+$	234.28	7.50

Inter-laboratory reproducibility (BD FACSLyric™ flow cytometer with BD FACSDuet™ system)

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 three clinical sites. The control samples were stained using three lots of the BD Multitest™ IMK (CD3/CD8/CD45/CD4 and CD3/CD16+CD56/CD45/CD19) reagents with one lot of BD Trucount™ Tubes using the BD FACSDuet™ system and automatically transferred to an integrated BD FACSLyric™ flow cytometer and acquired using the BD FACS™ Universal Loader. Two separate runs were performed each day. Results obtained over 15 non-consecutive test days were analyzed.

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

Table 17 Inter-laboratory reproducibility of lymphocyte subset percentages in low analyte concentration (CDL) (BD FACSLyric™ with BD FACSDuet™)

Lymphocyte subset	Mean (%)	SD
Average $CD3^+$	57.11	0.77
$CD3^+CD4^+$	9.97	0.61
$CD3^+CD8^+$	42.94	1.05

Lymphocyte subset	Mean (%)	SD
$CD3^{-}CD19^{+}$	21.46	0.84
$CD3^{-}(CD16+CD56)^{+}$	20.16	0.85

Table 18 Inter-laboratory reproducibility of lymphocyte subset percentages in normal analyte concentration (CDN) (BD FACSLyric™ with BD FACSDuet™)

Lymphocyte subset	Mean (%)	SD
Average $CD3^{+}$	75.97	0.60
$CD3^{+}CD4^{+}$	49.83	0.91
$CD3^{+}CD8^{+}$	24.60	0.73
$CD3^{-}CD19^{+}$	12.19	0.57
$CD3^{-}(CD16+CD56)^{+}$	11.17	0.58

Table 19 Inter-laboratory reproducibility of lymphocyte subset absolute counts in low analyte concentration (CDL) (BD FACSLyric™ with BD FACSDuet™)

Lymphocyte subset	Mean (cells/µL)	%CV
Average $CD3^{+}$	958.18	5.68
$CD3^{+}CD4^{+}$	167.16	8.47
$CD3^{+}CD8^{+}$	719.77	6.68
$CD3^{-}CD19^{+}$	360.46	7.21
$CD3^{-}(CD16+CD56)^{+}$	338.44	7.53

Table 20 Inter-laboratory reproducibility of lymphocyte subset absolute counts in normal analyte concentration (CDN) (BD FACSLyric™ with BD FACSDuet™)

Lymphocyte subset	Mean (cells/µL)	%CV
Average $CD3^{+}$	1,980.54	5.64
$CD3^{+}CD4^{+}$	1,303.23	6.62
$CD3^{+}CD8^{+}$	643.31	7.28
$CD3^{-}CD19^{+}$	317.00	8.36
$CD3^{-}(CD16+CD56)^{+}$	290.39	8.15

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™ IMK (CD3/CD8/CD45/CD4

and CD3/CD16+CD56/CD45/CD19) reagents in BD Trucount™ Tubes and run on 12 instruments for a total of 24 runs per sample for each reagent.

Table 21 Whole blood repeatability of lymphocyte subset percentages (BD FACSLyric™ flow cytometer)

Lymphocyte subset	Mean (%)	Within run repeatability (SD)	Total repeatability (SD)
Average $CD3^+$	73.59	0.69	0.69
$CD3^+CD4^+$	33.46	0.83	0.83
$CD3^+CD8^+$	37.93	0.93	0.93
$CD3^-CD19^+$	13.02	0.67	0.67
$CD3^-(CD16+CD56)^+$	12.37	0.71	0.71

Table 22 Whole blood repeatability of lymphocyte subset absolute counts (BD FACSLyric™ flow cytometer)

Lymphocyte subset	Mean (cells/µL)	Within run repeatability (%CV)	Total repeatability (%CV)
Average $CD3^+$	1,398.45	3.21	3.35
$CD3^+CD4^+$	633.59	5.32	5.40
$CD3^+CD8^+$	726.59	5.42	5.53
$CD3^-CD19^+$	229.21	7.32	7.47
$CD3^-(CD16+CD56)^+$	215.01	7.84	7.94

Whole blood repeatability (BD FACSLyric™ flow cytometer with BD FACSDuet™ system)

A whole blood repeatability study was performed to demonstrate system repeatability using 27 donor specimens. Each donor specimen was stained in duplicate using three lots of the BD Multitest™ IMK (CD3/CD8/CD45/CD4 and CD3/CD16+CD56/CD45/CD19) reagents in BD Trucount™ Tubes and run on three BD FACSDuet™ instruments, each integrated with a BD FACSLyric™ flow cytometer, for a total of 18 runs per sample.

Table 23 Whole blood repeatability of lymphocyte subset percentages (BD FACSLyric™ with BD FACSDuet™)

Lymphocyte subset	Mean (%)	Within run repeatability (SD)	Total repeatability (SD)
Average $CD3^+$	76.67	0.74	0.75
$CD3^+CD4^+$	31.18	0.89	0.89
$CD3^+CD8^+$	44.04	1.01	1.05
$CD3^-CD19^+$	11.97	0.67	0.67
$CD3^-(CD16+CD56)^+$	10.66	0.81	0.83

Table 24 Whole blood repeatability of lymphocyte subset absolute counts (BD FACSLyric™ with BD FACSDuet™)

Lymphocyte subset	Mean (cells/µL)	Within run repeatability (%CV)	Total repeatability (%CV)
Average $CD3^+$	1,575.57	3.45	3.85
$CD3^+CD4^+$	636.55	5.12	5.38
$CD3^+CD8^+$	905.37	5.17	5.65
$CD3^-CD19^+$	246.37	7.55	7.80
$CD3^-(CD16+CD56)^+$	217.20	10.46	11.02

Stability (BD FACSLyric™ flow cytometer with and without BD FACSDuet™ system)

A study was conducted to assess blood sample stability and stained sample stability using the BD Multitest™ IMK (CD3/CD8/CD45/CD4 and CD3/CD16+CD56/CD45/CD19) reagents in BD Trucount™ Tubes. 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 up to 51 hours post draw and stained samples were tested up to 26 hours post stain. All samples were maintained at room temperature (20–25 °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. The specimen stability and stained sample stability apply to use on a BD FACSLyric™ flow cytometer with and without integration with a BD FACSDuet™ system.

Linearity (BD FACSLyric™ flow cytometer with and without BD FACSDuet™ system)

Linearity of the BD Multitest™ IMK Kit was assessed for the BD FACSLyric™ flow cytometer, with and without an integrated BD FACSDuet™ system, using triplicate measurements of 11 equally spaced

concentrations of WBCs. Lymphocyte subsets were observed to be linear across the following ranges. See Table 25.

Table 25 Linear ranges of lymphocyte subsets (BD FACSLyric™ flow cytometer with and without BD FACSDuet™)

Lymphocyte Subset	Range (cells/µL)	
	BD FACSLyric™	BD FACSLyric™ with BD FACSDuet™
Average $CD3^+$	5–5,181	4–5,310
$CD3^+CD4^+$	1–3,184	3–3,016
$CD3^+CD8^+$	7–3,480	2–3,130
$CD3^-CD19^+$	0–2,061	0–2,237
$CD3^-(CD16+CD56)^+$	2–1,396	1–1,419

Analytical measurement range (BD FACSLyric™ flow cytometer with and without BD FACSDuet™ system)

The analytical measurement range (AMR) for the BD Multitest™ IMK Kit 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 26 AMR of lymphocyte subsets (BD FACSLyric™ flow cytometer with and without BD FACSDuet™ system)

Lymphocyte Subset	Range (cells/µL)
Average $CD3^+$	14–5,000
$CD3^+CD4^+$	10–3,000
$CD3^+CD8^+$	11–3,000
$CD3^-CD19^+$	14–2,000
$CD3^-(CD16+CD56)^+$	10–1,200

BD FACSCanto™ II Flow Cytometer

Method comparison (BD FACSCanto™ II flow cytometer)

Lymphocyte subset percentage and absolute counts were enumerated with the BD Multitest™ IMK (CD3/CD8/CD45/CD4 and CD3/CD16+CD56/CD45/CD19) reagents in BD Trucount™ Tubes and analyzed on the BD FACSCanto™ II flow cytometer using BD FACSCanto™ Clinical Software version 2.1. The results were compared with results from the reagents 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 27.

Table 27 Regression analysis for subset absolute counts and percentages (BD FACSCanto™ II flow cytometer)

Lymphocyte Subset	N	Units	R^2	Slope	Intercept	Range
Average $CD3^+$	104	cells/µL	0.991	0.97	27.59	221–3,873
		%	0.984	0.97	2.72	52–92
$CD3^+CD4^+$	104	cells/µL	0.986	0.95	18.25	11–1,905
		%	0.994	1.01	0.20	2–57
$CD3^+CD8^+$	104	cells/µL	0.988	0.95	28.36	68–3,577
		%	0.993	1.00	0.34	11–81
$CD3^-CD19^+$	104	cells/µL	0.979	0.97	2.37	0–834
		%	0.986	0.97	0.32	0–38
$CD3^-$ $(CD16+CD56)^+$	104	cells/µL	0.961	0.88	10.56	20–606
		%	0.957	0.93	0.19	2–32

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 present SDs and CVs for within-device precision and repeatability of lymphocyte subset percentages and absolute counts, respectively.

Table 28 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)
Average $CD3^+$	54.1	0.96	0.98
$CD3^+CD4^+$	10.3	0.53	0.53
$CD3^+CD8^+$	43.2	1.33	1.34
$CD3^-CD19^+$	26.1	0.86	0.86
$CD3^-$ $(CD16+CD56)^+$	18.2	0.87	0.87

a. CDL = CD-Chex Plus CD4 Low control

Table 29 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)
Average $CD3^+$	73.0	0.63	0.67
$CD3^+CD4^+$	46.8	0.81	0.82
$CD3^+CD8^+$	25.4	0.78	0.80
$CD3^-CD19^+$	15.4	0.54	0.56
$CD3^-$ $(CD16+CD56)^+$	10.6	0.51	0.52

a. CDC = CD-Chex Plus control

Table 30 Precision of absolute counts in low analyte concentration (CDL) (BD FACSCanto™ II flow cytometer)

Lymphocyte Subset (cells/µL)	Mean	%CV (Repeatability)	%CV (Within-device precision)
Average $CD3^+$	1,086.0	3.5	3.6
$CD3^+CD4^+$	205.6	5.9	5.9
$CD3^+CD8^+$	866.0	3.8	3.9
$CD3^-CD19^+$	526.1	6.2	6.4
$CD3^-$ $(CD16+CD56)^+$	367.1	5.9	6.1

Table 31 Precision of absolute counts in normal analyte concentration (CDC) (BD FACSCanto™ II flow cytometer)

Lymphocyte Subset (cells/µL)	Mean	%CV (Repeatability)	%CV (Within-device precision)
Average $CD3^+$	2,105.4	2.7	2.9
$CD3^+CD4^+$	1,347.1	3.6	3.8
$CD3^+CD8^+$	731.4	4.7	4.7
$CD3^-CD19^+$	443.5	5.5	5.6
$CD3^-$ $(CD16+CD56)^+$	306.3	6.0	6.0

Stability (BD FACSCanto™ II flow cytometer)

The stability of the BD Multitest™ IMK (CD3/CD8/CD45/CD4 and CD3/CD16+CD56/CD45/CD19) reagents 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 (20–25 °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 of the BD Multitest™ IMK Kit was assessed for the BD FACSCanto™ II system within a WBC 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
CD4	1–3,669
CD8	2–2,324
CD19	1–857
CD16+CD56	1–447

BD FACSCanto™ Flow Cytometer

Method comparison (BD FACSCanto™ flow cytometer)

Lymphocyte subset percentage and absolute counts were enumerated with the BD Multitest™ IMK (CD3/CD8/CD45/CD4 and CD3/CD16+CD56/CD45/CD19) reagents 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. See Table 32.

Table 32 Regression analysis for subset absolute counts and percentages (BD FACSCanto™ flow cytometer)

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

Lymphocyte Subset	N	Unit	R	Slope	Intercept	Range
$CD3^{-}CD19^{+}$	108	cells/µL	0.990	1.02	–7.93	0–2,527
		%	0.994	0.99	–0.05	0–42
$CD3^{-}$ $(CD16+CD56)^{+}$	108	cells/µL	0.981	0.95	10.80	11–1,374
		%	0.989	1.00	0.39	2–45

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 present SDs and CVs for within-device precision and repeatability of lymphocyte subset percentages and absolute counts, respectively.

Table 33 Precision of lymphocyte subset percentages in low analyte concentration (MCL[a]) (BD FACSCanto™ flow cytometer)

Lymphocyte Subset (%)	Mean	SD (Repeatability)	SD (Within-device precision)
Average $CD3^{+}$	57.5	1.16	1.22
$CD3^{+}CD4^{+}$	17.6	0.73	0.77
$CD3^{+}CD8^{+}$	39.1	0.97	1.17
$CD3^{-}CD19^{+}$	20.5	0.70	1.05
$CD3^{-}$ $(CD16+CD56)^{+}$	18.6	1.03	1.14

a. MCL = BD Multi-Check™ CD4 Low Control

Table 34 Precision of lymphocyte subset percentages in normal analyte concentration (MCN[a]) (BD FACSCanto™ flow cytometer)

Lymphocyte Subset (%)	Mean	SD (Repeatability)	SD (Within-device precision)
Average $CD3^{+}$	69.9	1.15	1.21
$CD3^{+}CD4^{+}$	50.3	1.04	1.18
$CD3^{+}CD8^{+}$	19.8	1.03	1.15
$CD3^{-}CD19^{+}$	13.6	0.68	0.77
$CD3^{-}$ $(CD16+CD56)^{+}$	13.7	0.89	1.03

a. MCN = BD Multi-Check™ Control

Table 35 Precision of absolute counts in low analyte concentration (MCL) (BD FACSCanto™ flow cytometer)

Lymphocyte Subset (cells/µL)	Mean	%CV (Repeatability)	%CV (Within-device precision)
Average $CD3^+$	307.4	3.2	4.1
$CD3^+CD4^+$	94.9	5.8	6.5
$CD3^+CD8^+$	210.7	5.1	5.8
$CD3^-CD19^+$	108.5	4.9	7.1
$CD3^-$ $(CD16+CD56)^+$	98.6	6.6	8.2

Table 36 Precision of absolute counts in normal analyte concentration (MCN) (BD FACSCanto™ flow cytometer)

Lymphocyte Subset (cells/µL)	Mean	%CV (Repeatability)	%CV (Within-device precision)
Average $CD3^+$	743.9	3.9	4.8
$CD3^+CD4^+$	539.4	5.7	5.9
$CD3^+CD8^+$	212.8	6.4	7.1
$CD3^-CD19^+$	143.3	7.0	7.8
$CD3^-$ $(CD16+CD56)^+$	145.0	8.0	9.9

Stability (BD FACSCanto™ flow cytometer)

The stability of the BD Multitest™ IMK (CD3/CD8/CD45/CD4 and CD3/CD16+CD56/CD45/CD19) reagents 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 (20–25 °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 of the BD Multitest™ IMK Kit 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
CD4	29–5,827
CD8	22–4,076
CD19	5–1,131
CD16+CD56	4–671

BD FACSCalibur™ Flow Cytometer

Method comparison (BD FACSCalibur™ flow cytometer)

Lymphocyte subset percentage and absolute counts enumerated with the BD Multitest™ CD3/CD8/CD45/CD4 and BD Multitest™ CD3/CD16+CD56/CD45/CD19 reagents in BD Trucount™ Tubes were compared with results from BD Tritest™ CD3/CD4/CD45, BD Tritest™ CD3/CD8/CD45, BD Tritest™ CD3/CD16+CD56/CD45, or BD Tritest™ CD3/CD19/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 indicate that the results are substantially equivalent. See Table 37 and Table 38.

Table 37 Regression analysis for BD Multitest™ CD3/CD8/CD45/CD4 (BD FACSCalibur™ flow cytometer)

Subset	N	Unit	R	Slope	Intercept	Range
Average $CD3^+$	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

Table 38 Regression analysis for BD Multitest™ CD3/CD16+CD56/CD45/CD19 (BD FACSCalibur™ flow cytometer)

Subset	N	Unit	R	Slope	Intercept	Range
Average $CD3^+$	126	%	0.99	0.99	1.74	21.5– 90.0
		cells/µL	0.98	1.01	–10.18	100– 2,883
$CD3^-CD19^+$	126	%	0.99	0.98	–0.25	0–59
		cells/µL	0.98	0.95	1.77	3–877.6
$CD3^-$ $(CD16+CD56)^+$	126	%	0.97	0.97	–0.85	3–40.0
		cells/µL	0.96	0.92	–5.44	37–901

Within-specimen reproducibility (BD FACSCalibur™ flow cytometer)

Estimates of within-sample reproducibility were determined at three clinical laboratories from five replicates of each sample collected from normal and abnormal donors. Means and SDs or CVs (or both) are provided for subset percentages and absolute counts greater than 100 cells/µL in Table 39 and Table 40 (BD Multitest™ CD3/CD8/CD45/CD4) and Table 41 and Table 42 (BD Multitest™ CD3/CD16+CD56/CD45/CD19).

Table 39 Within-specimen reproducibility of BD Multitest™ CD3/CD8/CD45/CD4 subset percentages (BD FACSCalibur™ flow cytometer)

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

Table 40 Within-specimen reproducibility of BD Multitest™ CD3/CD8/CD45/CD4 absolute counts (BD FACSCalibur™ flow cytometer)

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

Table 41 Within-specimen reproducibility of BD Multitest™ CD3/CD16+CD56/CD45/CD19 subset percentages (BD FACSCalibur™ flow cytometer)

Subset	N	Mean (%)	SD
Average $CD3^+$	46	72.1	0.99
$CD3^-CD19^+$	46	15.6	0.71
$CD3^-$ $(CD16+CD56)^+$	46	10.8	0.73

Table 42
Within-specimen reproducibility of BD Multitest™ CD3/CD16+CD56/CD45/CD19 absolute counts (BD FACSCalibur™ flow cytometer)

Subset	N	Mean (cells/µL)	%CV
Average $CD3^+$	46	1,217	4.7
$CD3^-CD19^+$	37	278	5.8
$CD3^-$ $(CD16+CD56)^+$	33	217	8.3

Stability (BD FACSCalibur™ flow cytometer)

The stability of BD Multitest™ reagents 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 (20–25 °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 of the BD Multitest™ IMK Kit was assessed within a WBC concentration of 0.2×10^3 to 29.7×10^3 WBC/µL and a lymphocyte concentration of 0.1×10^3 to 9.0×10^3 lymphocytes/µL. Results were observed to be linear within the $CD3^+$ range, the $CD3^+CD4^+$ range, the $CD3^+CD8^+$ range, the $CD3^-CD16+CD56^+$ range, and the $CD19^+$ 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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PATENTS AND TRADEMARKS

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

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Symbols Glossary

Please refer to product labeling for applicable symbols.

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

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

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

L006715(08) 2023-03

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HISTORY

Revision	Date	Changes made
23-19814(04)	2023-08	Updated legal manufacturer address. Updated symbols glossary. Added History section. Updated Patents and Trademarks section. Updated concentration percentages for BD Multitest™ IMK Kit Lysing Solution. Updated table in Precautions section.