WHO Prequalification of Diagnostics Programme
PUBLIC REPORT

Product: BD FACSCount™ Instrument System with FACSCount™ Control Kit and BD FACSCount™ Reagent Kit
Number: PQDx 0124-045-00

Abstract

The BD FACSCount™ Instrument System with FACSCount™ Control Kit and BD FACSCount™ Reagent Kit with product codes 337858, 340167 and 340166 manufactured by Becton, Dickinson and Company, BD Biosciences, FDA cleared regulatory version, was accepted for the WHO list of prequalified diagnostics and was listed on 12 November 2012.

The BD FACSCount™ system includes the BD FACSCount™ instrument, software, a workstation, reagents, and controls. The BD FACSCount™ instrument is a compact cell counter with a built-in computer. Reagent tubes are introduced to the instrument via the sample holder that lifts the tubes to the sample injection probe. The sheath tank and waste tank, which are equipped with liquid level detectors to indicate empty and full conditions, are easily accessible through a hinged door at the front of the instrument. A laser beam intersects the sample stream within a flow cell. The screen displays control and sample results, prompts, and messages that assist the user with operation or inform the errors. Results print automatically on thermal paper after samples are run.

The BD FACSCount™ software which is contained in a floppy disk is required to start up and run the instrument. The disk also stores the last entered reagent lot ID and control bead lot ID information, control run results, the last values entered in the Setup screen, the number of tubes run since the last daily clean, the date of the last long clean run, and the Results file. During operation, the software monitors the sheath fluid supply, waste level, and laser power. BD FACSCount™ software enables automated analysis without any operator intervention. Patients’ results are summarized on a printed sample report. Quality controls in the software ensure that reported results are accurate by detecting and flagging error conditions and suppressing results when control limits are exceeded.

The BD FACSCount™ workstation provides a place to hold blood specimens, reagent tubes, controls, fixative solution, caps, and cleaning tubes when preparing and running samples.

The BD FACSCount™ reagent kit is intended for in vitro diagnostic use in enumerating the absolute counts of CD4⁺, CD8⁺, and CD3⁺ T lymphocytes in unlysed whole blood, using the BD FACSCount™ instrument. A single test requires one ready-to-use reagent tube pair consisting of: the CD4/CD3 tube to determine the absolute number of helper/inducer T lymphocytes. The second pair is the CD8/CD3 tube to determine the absolute number of suppressor/cytotoxic T lymphocytes. Both tubes measure the absolute number of total T lymphocytes (CD3).
The BD FACSCount™ control kits consist of paired control bead sets, containing beads at four levels: zero, low, medium, and high. BD FACSCount control beads can be added to samples prepared with normal blood to validate laboratory practices and methodology and system linearity. The control run generates a printed report summarizing system performance. The result of the last control run is reported on each subsequent sample printout, to provide confidence in the result.

When whole blood is added to the reagents, fluorochrome-labeled antibodies in the reagents bind specifically to lymphocyte surface antigens followed by fixation stage. For enumerating absolute counts of CD4⁺, CD8⁺, and CD3⁺ T lymphocytes in unlysed whole blood sample is run on the instrument where the cells come in contact with the laser light, which causes the fluorochrome labelled cells to fluoresce. This fluorescent light provides the information necessary for the instrument to count the cells. In addition to containing the antibody reagent, the reagent tubes also contain a known number of fluorochrome-integrated reference beads. These beads function as a fluorescence standard for locating the lymphocytes and also as a quantitation standard for enumerating the cells. The FACSCount™ software identifies the T-lymphocyte populations and calculates the absolute counts of CD4⁺ cells (helper/inducer T lymphocytes), CD8⁺ cells (suppressor/cytotoxic T lymphocytes), CD3⁺ cells (total T lymphocytes) and calculates the CD4/CD8 ratio (helper/suppressor T-lymphocyte ratio).

In order to perform the assay, the following are required:

**Instrumentation:**
- BD FACSCount™ Instrument System (337858)
- Accessories supplied with the instrument include a workstation and a coring station,

**Software:**
- BD FACSCount™ Users Guide and Software (344619)

**Reagents:**
- BD FACSCount™ Reagent Kit (340167) (50 pairs of CD3 PE-Cy™5 / CD4 PE and CD3 PE-Cy5 / CD8 PE reagents, fixative (two 5-ml vials of 5% formaldehyde solution) and tube caps (50 Tests))
- BD FACSCount™ Control Kit (340166) (25 Tests)
- BD FACSFlow Sheath Fluid (342003) or equivalent

**Accessories:**
- Cleaning Tubes (343685)
- Caps for Cleaning Tubes (343514)
- Pipette Tips in Bulk (340293)
- Thermal Paper Roll (332839)

**Reagents or materials required but not provided:**
- Vacutainer K₂ or K₃ EDTA blood collection tubes or equivalent
- Disposable pipette tips (340292) or equivalent
- Vortex mixer
• Barcode reader
• BD FACSCount™ pipette or equivalent

Storage:
The BD FACSCount™ Reagent Kit and BD FACSCount™ Control Kit should be stored at 2 to 8 °C.

Shelf-life:
BD FACSCount™ Reagent kit: 23 months.
BD FACSCount™ Control kit: 24 months.

### Summary of prequalification status for BD FACSCount™ Instrument System with FACSCount™ Control Kit and BD FACSCount™ Reagent Kit

<table>
<thead>
<tr>
<th>Status on PQ list</th>
<th>Date</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dossier assessment</td>
<td>20 April 2012</td>
<td>MR</td>
</tr>
<tr>
<td>Inspection status</td>
<td>08 October 2012</td>
<td>MR</td>
</tr>
<tr>
<td>Laboratory evaluation</td>
<td>01 October 2012</td>
<td>MR</td>
</tr>
</tbody>
</table>

MR: Meets Requirements
NA: Not Applicable
FT: Fast-tracked

BD FACSCount™ Instrument System with FACSCount™ Control Kit and BD FACSCount™ Reagent Kit was accepted for the WHO list of prequalified diagnostics on the basis dossier assessment, manufacturing site inspection and laboratory evaluation.

### Background information

BD Biosciences submitted an application for prequalification of BD FACSCount™ System PQDx 0124-045-00. Based on the established WHO prioritization criteria, BD FACSCount™ Instrument System with FACSCount™ Control Kit and BD FACSCount™ Reagent Kit was given priority for prequalification.

### Product dossier assessment

BD Biosciences submitted a product dossier for BD FACSCount™ System as per the “Instructions for compilation of a product dossier” (PQDx_018 v1). The information submitted in the product dossier was reviewed by WHO staff and external experts (assessors) appointed by WHO in accordance with the internal procedure on the screening and assessment of a product dossier (PQDx_009 v2). Based on the product dossier screening
and assessment findings, a recommendation was made to accept the product dossier for BD FACSCount™ Instrument System with FACSCount™ Control Kit and BD FACSCount™ Reagent Kit.

Commitments for prequalification:

The manufacturer committed to amend and submit additional documentation on the following issue:

1. an updated version of the risk analysis and control summary.

Manufacturing site inspection

An inspection was conducted at the site of manufacture of the BD FACSCount™ System (in 2350 Qume Drive, San Jose, 95131 CA, USA in accordance with the procedure described in ‘Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics (PQDx_014 v1)’.

The inspection found that the manufacturer had a well-established quality management system and manufacturing practices in place that would ensure the manufacture of a product of consistent quality. The manufacturer’s final responses to the observations and minor nonconformities and an action plan for outstanding issues were accepted on 08/10/2012.

Commitments for prequalification:

1. Studies, including stability, in use and precision studies that take into account typical end user conditions in resource limited settings, will form part of clinical study protocols. References such as EP25-A, Vol ,29 No.20; Evaluation of Stability of In Vitro Diagnostic reagents will be considered in relevant protocols.
2. Becton Dickinson will inform WHO of changes made subsequent to the site inspection, such as change in location of site of manufacture of major components of the test, or other changes to the manufacturing process that may affect the quality of the product.

Laboratory evaluation

BD FACSCount™ System using the FACSCount™ Reagent Kit were evaluated in two WHO collaborating laboratories namely Institute of Tropical Medicine, Belgium and Muhimbili University of Health and Allied Science, Tanzania between April and September 2012. The evaluation was conducted using the WHO evaluation protocol which was also approved by in-country ethical review boards in Belgium and Tanzania.

The BD FACSCount™ system is an automated dedicated instrument. It utilizes BD FACSCount™ reagents in ready-to-use reagent tube format to enumerate CD3+, CD4+ counts and CD8+ T lymphocyte absolute counts after 30 minutes incubation. 50 µl of well mixed unlysed whole blood and BD FACSCount™ reagents are required to perform the assays. Fluorescence
reference beads included in the reagent tubes, ensure accurate enumeration of lymphocyte subsets of interest.

A total of 479 fresh blood samples were used to study failure rates, reproducibility (intra-laboratory variation, intra-assay variation, inter-assay variation and instrument precision), carry over and agreement with the FACSCalibur™ as the reference method. Lastly, ease to use was assessed.

The acceptance criteria for reproducibility studies was that the assay should have a percentage coefficient of variation (%CV) of less than 15% for CD4⁺ T counts less than or equal to 200/µL and less than 10% for CD4 counts more than 200 cells/µL, while the carry-over constant (k) should be less than 2%. Consecutive routine blood samples collected in EDTA vacutainer tubes with at least 3.0 ml of blood brought to the laboratories were used to compare BD FACSCount™ CD4 reagents and BD FACSCount™ reagents against FACSCalibur™ as the reference method. Agreement between the dedicated and the reference method was assessed using the regression analysis, Bland Altman plots and/or percentage similarity methods.

In laboratory 1, a total of 7/240 (1.7%) samples failed to run in the instrument while in laboratory 2, a total of 13/200 (6.5%) samples failed. Intra-laboratory variation studies showed mean %CV of 5.2%, and 6.1%, in laboratory 1 and laboratory 2, respectively. The mean inter-assay variability for CD4 less than 200/µL was 4.4%, and 5.5%, in laboratory 1 and laboratory 2, respectively. The mean instrument precision was 3.6%, and 6.6%, laboratory 1 and laboratory 2, respectively. The carryover was less than 2% in both laboratories. Regarding agreement with the reference method, the correlation coefficients were high with minimal bias in both laboratories.
1. Labels

1.1 Component specification sheet
BD Biosciences

1. Material: 3M 7323 Bright Silver Polyester, 0.8 mil 300 high strength acrylic adhesive. Compuplan laminate (typewriter printable), with permanent clear adhesive.

2. Text, borders and graphics: Black

3. Font: Frutiger 65 Bold and Frutiger 55 Roman, see drawing for sizes.

4. 3" height x 4" wide

5. Corner Radius: 1/8

6. Artwork: 343813 Rev A

Unless otherwise specified,

1. Ink to be UL/CSA recognized
2. Label material and adhesive to conform to UL/CSA specifications.

CONFIDENTIAL

MODEL BD FACSCCount™ Instrument

Cat No. 337858

SN

VAC: 100 - 240V -50-60Hz

Power 100 W

Fuel (A): 2.5 A - 210 V

(13570) rev 7/5

BD Biosciences

.125" Radius

LABEL PERIPHERY
Border prints black

2 - of - 2
1.2 BD FACCountTM Controls

1.3 BD FACCountTM System – Fixative Solution

1.4 BD FACCountTM Caps

1.5 BD FACCountTM Reagents
2. Instructions for use

1. INTENDED USE

The BD FACSCount™ reagent kit is intended for in vitro diagnostic use in enumerating the absolute counts of CD4, CD8, and CD3 T lymphocytes in unlysed whole blood, using the BD FACSCount™ instrument.

Applications

Absolute CD4, CD8, and CD3 T-lymphocyte counts have been used to evaluate the immune status of patients with, or suspected of developing, immune deficiencies such as acquired immune deficiency syndrome (AIDS).1,2

The CD4 antigen is the receptor for the human immunodeficiency virus (HIV).3 The absolute number of CD4 T lymphocytes is the cellular parameter most closely associated with HIV disease progression and patient prognosis.4

The CD4/CD8 T-lymphocyte ratio is known as the helper-suppressor ratio. The relative percentage of CD4 T lymphocytes declines and the relative percentage of CD8 T lymphocytes increases in HIV infection, resulting in a decline in the helper/suppressor ratio.5

2. PRINCIPLES OF THE PROCEDURE

A single test requires one convenient, ready-to-use reagent tube pair. The CD4/CD3 tube determines the absolute number of helper/inducer T lymphocytes. The CD8/CD3 tube determines the absolute number of suppressor/cytotoxic T lymphocytes. Both tubes measure the absolute number of total T lymphocytes (CD3).

When whole blood is added to the reagents, fluorochrome-labeled antibodies in the reagents bind specifically to lymphocyte surface antigens. After a fixative solution is added to the reagent tubes, the
sample is run on the instrument. Here, the cells come in contact with the laser light, which causes the fluorochrome-labeled cells to fluoresce. This fluorescent light provides the information necessary for the instrument to count the cells.

In addition to containing the antibody reagent, the reagent tubes also contain a known number of fluorochrome-integrated reference beads. These beads function as a fluorescence standard for locating the lymphocytes and also as a quantitation standard for enumerating the cells.

Analysis is automatic. The software identifies the T-lymphocyte populations and calculates the absolute counts. Results include:

- Absolute counts of CD4+ cells (helper/inducer T lymphocytes)
- Absolute counts of CD8+ cells (suppressor/cytotoxic T lymphocytes)
- Absolute counts of CD3+ cells (total T lymphocytes)
- CD4/CD8 ratio (helper/suppressor T-lymphocyte ratio)

### 3. COMPOSITION

CD4, clone SK3, is derived from the hybridization of mouse NS-1 myeloma cells with spleen cells from BALB/c mice immunized with human peripheral blood T lymphocytes.

The CD4 antigen is present on T-helper/inducer lymphocytes and monocytes.

CD8, clone SK1, is derived from the hybridization of mouse NS-1 myeloma cells with spleen cells from BALB/c mice immunized with human peripheral blood T lymphocytes.

The CD8 antigen is expressed on the 32-kilodalton (kDa) α subunit of a disulfide-linked bimolecular complex. The majority of peripheral blood CD8+ T lymphocytes expresses an α/β heterodimer (Mr 32, 30 kDa), while CD8+CD16+ natural killer (NK) lymphocytes and CD8+ T-cell receptor (TCR)-γδ+ T lymphocytes express an α/α homodimer (Mr 30 kDa). CD8+ TCR-α/β T lymphocytes can express either an α/α homodimer or an α/β heterodimer.

CD3, clone SK7, is derived from the hybridization of mouse NS-1 myeloma cells with spleen cells from BALB/c mice immunized with human thymocytes.

CD3 reacts with the epsilon chain of the CD3 antigen/T-cell antigen receptor (TCR) complex. The antigen recognized by CD3 antibodies is noncovalently associated with either α/β or γδ TCR (70 to 90 kDa).

CD4, CD8, and CD3 are each composed of mouse IgG1 heavy chains and kappa light chains.

Each reagent is supplied in phosphate-buffered saline (PBS) containing 0.1% BSA and 0.1% sodium azide.

**WARNING** Sodium azide is harmful if swallowed (R22). Keep away from foodstuffs, beverages, and feed. Keep container tightly closed (S7). Do not breathe gas, fumes, vapor, or spray (S23). Wear suitable protective clothing, gloves, and eye/face protection (S36/37/39). In case of contact with eyes, rinse immediately with plenty of water and seek medical advice (S26). Take off immediately all contaminated clothing (S27). Contact with acids liberates very toxic gas (R32). This material and its container must be disposed of as
hazardous waste (S60). Contact with acidic solutions and metal compounds over time may form potentially explosive metal azides. Should any of this material be introduced into a sanitary sewer system, flush with copious amounts of water.

4. STORAGE AND HANDLING

Each antibody reagent is stable until the expiration date shown on the label when stored at 2°C to 8°C. Do not use after the expiration date. Do not freeze the reagent or expose it to direct light during storage or incubation with cells. Keep the outside of the reagent vial dry.

Do not use the reagent if you observe any change in appearance. Precipitation or discoloration indicates instability or deterioration.

5. REAGENTS

Reagent Provided, Sufficient for 50 Tests

The BD FACSCount reagent kit contains the following:

- CD4 PE/CD3 PE-Cy™5 tubes (green top)
- CD8 PE/CD3 PE-Cy5 tubes (clear top)
- 220 reagent tube caps

**NOTE** Use the caps to prevent spillage of patient samples and controls while vortexing, during incubation, and before and after running samples on the instrument.

- Two 5-mL vials of 5% formaldehyde in PBS, used as fixative solution

**WARNING** Formaldehyde is toxic by inhalation, in contact with skin, and if swallowed (R23/24/25). It is allergenic and is known to the state of California to cause cancer through inhalation of the gas or vapor. Do not breathe gas, fumes, vapor, or spray (S23). Formaldehyde may cause sensitization by skin contact (R43). Wear suitable protective clothing, gloves, and eye/face protection. Keep away from foodstuffs, beverages, and feed. This material and its container must be disposed of as hazardous waste (S60).

**Reagents or Materials Required but Not Provided**

- BD Vacutainer® K₂ or K₃ EDTA blood collection tubes, or equivalent
- Disposable pipet tips
- Vortex mixer
- BD FACSCount system

6. PROCEDURE

Collecting and Preparing Patient Samples

**WARNING** All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing and gloves.

**WARNING** Patient blood samples must be collected in BD Vacutainer K₂ or K₃ EDTA blood collection tubes (or equivalent), and stored no longer than 48 hours at room temperature (20°C to 25°C, 68°F to 77°F). Results obtained
from samples that do not meet these criteria might be inaccurate.

The following procedure explains how to prepare patient samples by adding blood, then fixative solution, to the CD4 and CD8 reagent tubes.

To begin, place the patient blood in the BD FACSCount workstaton. Remove one reagent tube pair for each patient. Reseal the foil bag and return the unused reagent pairs to the refrigerator.

1. Label the tab of one reagent tube pair with the patient accession number or number that identifies the tube of blood.

See the **BD FACSCount System User's Guide** for instructions on entering accession numbers for each sample.

2. Set the vortex mixer to a mid-range speed and vortex the pair upside down for 5 seconds, then upright for 5 seconds.

3. Open the reagent tubes with the coring station.

See the **BD FACSCount System User's Guide** for directions on using the coring station.

4. Make sure that the whole blood is adequately mixed. If necessary, mix by inversion.

5. Changing tips between tubes, pipette 50 μL of the whole blood into each of the two reagent tubes.

Discard tips in an appropriate biohazard container.

For information on pipetting, see the **BD FACSCount System User's Guide**.

6. Cap the tubes and vortex upright for 5 seconds.

7. Place the reagent pair in the workstation and close the cover to protect the reagents from light.

8. Incubate the tubes for 60 to 120 minutes at room temperature (20°C to 25°C, 68°F to 77°F).

9. Uncap the tubes and discard the caps in an appropriate biohazard container.

10. Changing tips between tubes, pipette 50 μL of fixative solution into each reagent tube.

Discard tips in an appropriate biohazard container.

11. Recap the reagent tubes with new caps and vortex upright for 5 seconds.

Run the tubes on the BD FACSCount instrument within 48 hours of preparation. Store reagent tubes in the workstation until you are ready to run them on the instrument.

**Running Patient Samples**

See the **BD FACSCount System User's Guide** for detailed information on running patient samples. Make sure you enter the patient accession number before you begin.

1. Vortex the reagent pair upright for 5 seconds.

2. Uncap the CD4 tube (green top) and set the cap aside.

3. Place the reagent pair in the sample holder so the CD4 tube is in the run position and press RUN.

A software message will tell you when the analysis is complete.

4. When analysis of the CD4 tube is complete, remove the reagent pair and recap the CD4 tube.
5. Uncap the CD8 tube (clear top) and set the cap aside.
6. Replace the pair so the CD8 tube is now in the run position and press RUN.
7. When the sample tube lowers, remove the reagent pair and recap the CD8 tube.

Discard the reagent pair in an appropriate biohazard container.

Repeat steps 1 through 7 until all samples have been run.

7. EXPECTED RESULTS

BD Biosciences has investigated the normal reference ranges for the BD FACSCount reagents parameters in 151 normal male and female subjects using the BD FACSCount system at three sites (one US clinical site, one European clinical site, and BD Biosciences in San Jose, California). The expected normal reference ranges from these sites are shown in Table 1. All results presented in this product insert were obtained through studies conducted by BD Biosciences investigators.

The ranges obtained were tested for differences by clinical site, sex of subject, and age of subject. For each cell population, comparisons were made between values obtained at each study site, values obtained for males and females, and values obtained for different age groups. When comparison indicated a significant difference, data from the groups was not pooled, and separate reference ranges are given. The normal reference range for absolute counts is calculated from a fitted distribution. The ability to pool reference ranges across sites for the BD FACSCount reagent parameters is an indication of good between-laboratory reproducibility.

There are significant differences between the age groups for CD8+ suppressor/cytotoxic T lymphocytes. We present more than one upper and lower limit for CD8+ suppressor/cytotoxic T lymphocyte and CD4/CD8 ratio ranges because of age differences that have been observed. We also observed differences between males and females for CD4+ helper/inducer T lymphocytes. This group was separated because of the differences observed (see Table 1).

Table 1. Representative reference ranges (absolute counts from three sites) of BD FACSCount reagent parameters in hematologically normal adults (ages 18–65)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sex</th>
<th>Age</th>
<th>n</th>
<th>Mean (cells/µL)</th>
<th>95% Range*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+CD3+</td>
<td>Female</td>
<td>18–65</td>
<td>57</td>
<td>798</td>
<td>470–1,298</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>18–65</td>
<td>92</td>
<td>702</td>
<td>355–1,213</td>
</tr>
<tr>
<td>CD8+CD3-</td>
<td>Both</td>
<td>18–60</td>
<td>92</td>
<td>433</td>
<td>208–796</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>41–65</td>
<td>58</td>
<td>346</td>
<td>144–699</td>
</tr>
<tr>
<td>CD3+</td>
<td>Both</td>
<td>18–65</td>
<td>151</td>
<td>1,206</td>
<td>688–1,955</td>
</tr>
<tr>
<td>CD4/CD8 Ratio</td>
<td>Both</td>
<td>18–60</td>
<td>92</td>
<td>1.87</td>
<td>0.92–3.41</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>41–65</td>
<td>58</td>
<td>2.49</td>
<td>0.83–6.10</td>
</tr>
</tbody>
</table>

a. The 95% range is between the 2.5 and 97.5 percentiles from a distribution fit to the raw data.
b. Two subjects did not have a gender classification.
c. One subject had a missing value for CD8. Cells/µL unit is not applicable to the CD4/CD8 ratio mean.
Adult reference ranges should not be used with pediatric blood samples.

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

8. PERFORMANCE CHARACTERISTICS

Performance of the BD FACSCount reagents was established by testing at four US sites, one European site, and BD Biosciences in San Jose, California.

**Within-Sample Reproducibility**

Blood samples from each of five normal and nine abnormal subjects were obtained, aliquoted (30 times for normals and 9 times for abnormals), stained with the BD FACSCount reagents, and fixed within 12 hours of sample collection. Analysis was performed within 24 hours using three BD FACSCount systems in the same laboratory. Ten of the 30 aliquots were run on each system. Table 2 shows the within-sample reproducibility obtained for normal subjects and Table 3 shows the within-sample reproducibility obtained for abnormal subjects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean* (cells/μL)</th>
<th>n</th>
<th>SD as an Estimate of Within-Sample Reproducibilityb</th>
<th>dfe</th>
<th>CVd</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+CD3+</td>
<td>720</td>
<td>150</td>
<td>26</td>
<td>135</td>
<td>3.56</td>
</tr>
<tr>
<td>CD8+CD3+</td>
<td>473</td>
<td>150</td>
<td>17</td>
<td>135</td>
<td>3.61</td>
</tr>
<tr>
<td>CD3+</td>
<td>1,261</td>
<td>150</td>
<td>28</td>
<td>135</td>
<td>2.21</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td>1.76</td>
<td>150</td>
<td>0.99</td>
<td>135</td>
<td>5.06</td>
</tr>
</tbody>
</table>

a. Mean is the pooled mean. For example, Y = the mean of the individual means. Cells/μL unit is not applicable to the CD4/CD8 ratio mean.
b. SD = standard deviation (the pooled standard deviation)

\[
SD = \sqrt{\frac{s_1^2 + s_2^2 + \cdots + s_k^2}{n_1 + n_2 + \cdots + n_k}}
\]

\(s^2 = \text{variance of the ith sample for } 1 \leq i \leq k\)

\(k = \text{number of samples}\)

\(n_i = \text{number of observations}\)

c. df = degrees of freedom: the number of subjects (5), times the number of instruments (3), times the number of tubes – 1) (9) = 135

d. CV = coefficient of variation

\[
CV = \frac{SD}{Y} \times 100
\]
Table 3. Within-sample reproducibility for BD FACSCount reagent parameters (nine abnormal subjects) as absolute counts

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (cells/μL)</th>
<th>Subjects</th>
<th>n</th>
<th>SD as an Estimate of Within-Sample Reproducibility</th>
<th>df</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+CD3+</td>
<td>484</td>
<td>9</td>
<td>81</td>
<td>19</td>
<td>54</td>
<td>3.91</td>
</tr>
<tr>
<td>CD8+CD3+</td>
<td>1,067</td>
<td>9</td>
<td>81</td>
<td>27</td>
<td>54</td>
<td>2.57</td>
</tr>
<tr>
<td>CD3+</td>
<td>1,657</td>
<td>9</td>
<td>81</td>
<td>48</td>
<td>54</td>
<td>2.91</td>
</tr>
<tr>
<td>CD4/CD8 Ratio</td>
<td>0.53</td>
<td>9</td>
<td>81</td>
<td>0.03</td>
<td>54</td>
<td>6.03</td>
</tr>
</tbody>
</table>

a. Mean is the pooled mean. For example, $\bar{Y}$ = the mean of the individual means. Cells/μL unit is not applicable to the CD4/CD8 ratio mean.
b. SD = standard deviation (the pooled standard deviation)
c. df = degrees of freedom: the number of subjects (9), times the number of instruments (3), times (the number of tubes −1) (2) = 54
d. CV = coefficient of variation

= $SD \times 100$

Between-Instrument Reproducibility

Using the same data obtained from the previous Within-Sample Reproducibility protocol, samples of whole blood from 5 normal subjects were divided into 30 aliquots. Ten aliquots were run on each of three different instruments. The samples were stained with BD FACSCount reagents and fixed within 12 hours of sample collection. Results are shown in Table 4.

Table 4. Between-instrument reproducibility for BD FACSCount reagent parameters (five normal subjects and three instruments) as absolute counts (continued)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Instrument</th>
<th>Mean (cells/μL)</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+CD3+</td>
<td>1</td>
<td>729</td>
<td>28</td>
<td>3.82</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>715</td>
<td>22</td>
<td>3.05</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>715</td>
<td>27</td>
<td>3.75</td>
</tr>
<tr>
<td></td>
<td>instrument variation</td>
<td>720</td>
<td>0.04</td>
<td>0.00</td>
</tr>
<tr>
<td>CD3+</td>
<td>1</td>
<td>1,268</td>
<td>27</td>
<td>2.16</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1,272</td>
<td>20</td>
<td>1.57</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1,242</td>
<td>34</td>
<td>2.77</td>
</tr>
<tr>
<td></td>
<td>instrument variation</td>
<td>1,261</td>
<td>0.04</td>
<td>0.00</td>
</tr>
<tr>
<td>CD4/CD8 Ratio</td>
<td>1</td>
<td>1.78</td>
<td>0.09</td>
<td>5.00</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.74</td>
<td>0.09</td>
<td>5.18</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.76</td>
<td>0.09</td>
<td>4.98</td>
</tr>
</tbody>
</table>

a. Mean is the pooled mean, for example, $\bar{Y}$ = the mean of the individual means. Cells/μL unit is not applicable to the CD4/CD8 ratio mean.
b. SD = standard deviation, the pooled standard deviation
c. CV = coefficient of variation
d. Estimated from an analysis of the variance model
Between-Laboratory Reproducibility

To estimate the amount of variability in BD FACSCount parameters introduced by the laboratory, blood samples from 60 normal and 72 abnormal subjects were stained and analyzed at two clinical sites. Whole blood samples were stained and fixed within 12 hours of collection, then analyzed within 24 hours on the BD FACSCount instrument at each site. Results are presented in Table 5 and Table 6.

Table 5. Between-laboratory reproducibility for BD FACSCount reagent parameters at two clinical sites (normal)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean(^a) (cells/μL)</th>
<th>Variance Component</th>
<th>SD(^b)</th>
<th>%CV(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+CD3+</td>
<td>822 Within site</td>
<td>38.8</td>
<td>4.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>822 Between site</td>
<td></td>
<td>0(^d)</td>
<td>0.00</td>
</tr>
<tr>
<td>CD8+CD3+</td>
<td>540 Within site</td>
<td>23.6</td>
<td>4.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>540 Between site</td>
<td></td>
<td>5.4(^d)</td>
<td>1.01</td>
</tr>
<tr>
<td>CD3+</td>
<td>1,472 Within site</td>
<td>42.8</td>
<td>2.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,472 Between site</td>
<td></td>
<td>15.1(^d)</td>
<td>1.03</td>
</tr>
<tr>
<td>CD4/CD8</td>
<td>1.70 Within site</td>
<td>0.098</td>
<td>5.77</td>
<td></td>
</tr>
<tr>
<td>ratio</td>
<td>1.70 Between site</td>
<td></td>
<td>0(^d)</td>
<td>0.00</td>
</tr>
</tbody>
</table>

\(^a\) Mean is the pooled mean, for example, \(Y\) = the mean of the individual means. Cells/μL unit is not applicable to the CD4/CD8 ratio mean.

\(^b\) SD = standard deviation, the pooled standard deviation

\(^c\) CV = coefficient of variation

\(^d\) Site component of variation estimated from an analysis of the variance model

Table 6. Between-laboratory reproducibility for BD FACSCount reagent parameters at two clinical sites (abnormal)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean(^a) (cells/μL)</th>
<th>Variance Component</th>
<th>SD(^b)</th>
<th>%CV(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+CD3+</td>
<td>328 within site</td>
<td>11.0</td>
<td>3.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>325 between site</td>
<td></td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>CD8+CD3+</td>
<td>752 within site</td>
<td>28.4</td>
<td>3.77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>752 between site</td>
<td></td>
<td>4.6(^d)</td>
<td>0.62</td>
</tr>
<tr>
<td>CD3+</td>
<td>1,080 within site</td>
<td>40.2</td>
<td>3.73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,080 between site</td>
<td></td>
<td>6.0(^d)</td>
<td>0.56</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td>0.337 within site</td>
<td>0.0137</td>
<td>4.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.337 between site</td>
<td></td>
<td>0.0043</td>
<td>1.34</td>
</tr>
</tbody>
</table>

\(^a\) Mean is the pooled mean, for example, \(Y\) = the mean of the individual means. Cells/μL unit is not applicable to the CD4/CD8 ratio mean.

\(^b\) SD = standard deviation, the pooled standard deviation

\(^c\) CV = coefficient of variation

\(^d\) Site component of variation estimated from an analysis of the variance model

BD FACSCount Reagents versus Comparative Method

The same blood samples, from both normal and abnormal subjects, were analyzed with BD Simulset™ IMK-lymphocyte reagents (using BD Simulset™ software) on the BD FACScan flow cytometer, the Sysmex NE8000 hematology counter, and with BD FACSCount reagents on the BD FACSCount system. The BD Simulset IMK-lymphocyte samples were stained, lysed, washed, and fixed. Flow cytometric analysis was performed using the BD FACScan flow cytometer. The BD FACSCount samples were prepared and analyzed using the BD FACSCount system. Results were compared using linear regression. A summary of the results is presented in Table 7. Figure 1 shows the specific fitted distribution plots for the parameters listed in Table 7.
Table 7. BD FACSCount system versus comparative method absolute counts

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Slope</th>
<th>Intercept</th>
<th>r</th>
<th>n</th>
<th>Range of Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Comparative Method(^a)</td>
</tr>
<tr>
<td>CD4^+CD8^+</td>
<td>0.88</td>
<td>21.15</td>
<td>0.99</td>
<td>98</td>
<td>75–1,526</td>
</tr>
<tr>
<td>CD8^+CD3^+</td>
<td>0.87</td>
<td>14.74</td>
<td>0.98</td>
<td>99</td>
<td>187–2,210</td>
</tr>
<tr>
<td>CD3^+</td>
<td>0.90</td>
<td>50.85</td>
<td>0.98</td>
<td>101</td>
<td>274–2,824</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td>1.03</td>
<td>0.01</td>
<td>0.99</td>
<td>94</td>
<td>0.08–6.70</td>
</tr>
</tbody>
</table>

\(^a\) The data was collected using the BD SimulSens NK-lymphocyte reagents on a BD FACsCan™ flow cytometer and a

**NOTE:** Six percent of samples evaluated failed quality control criteria of the comparative method. No samples analyzed at this clinic site failed the BD FACSCount system quality control.

Figure 1: Fitted distribution plots for the BD FACSCount system versus comparative method.

**Stability of Stained Whole Blood**

Whole blood from five normal and seven abnormal subjects was aliquoted, prepared, and analyzed using the BD FACSCount system. The preparations were stored at room temperature (20°C to 25°C, 68°F to 77°F) in the dark for up to 48 hours and the analysis repeated. At 24 hours, all of the values obtained fell within 10% of the mean at time zero. At 48 hours, all of the values obtained fell within 15% of the mean at time zero. The CD4 range fell within 60 and 883, the CD8 range fell within 294 and 1,095, and the CD3 range fell within 512 and 1,815.

**Stability of Whole Blood from Draw**

Whole blood from five normal and seven abnormal subjects was aliquoted. Some aliquots were then prepared and analyzed using the BD FACSCount system. The remaining aliquots were stored at room temperature (20°C to 25°C, 68°F to 77°F) for up to 48 hours and the preparation and analysis repeated. At 24 hours, all of the values obtained fell within 10% of the mean at time zero. At 48 hours, all of the
9. LIMITATIONS

CAUTION The pipet used in the sample preparation procedure must be properly calibrated to ensure that it is dispensing exactly 50 µL of blood.

- Perform blood and control bead delivery by reverse pipetting. (The BD FACSCount pipet is preprogrammed to operate in the reverse pipetting mode.) Pipetting precision and accuracy must be verified. See the BD FACSCount System User's Guide for information on pipetting.
  - The CD4 tube must be run before the CD8 tube.
  - Collect samples in BD Vacutainer K2 or K3 EDTA blood collection tubes, or equivalent. A minimum of 200 µL of whole blood is required for the test.
  - Do not store whole blood longer than 48 hours before preparing.
  - Do not store whole blood on a blood rocker or other mixing device.
  - Do not refrigerate whole blood before preparing.
  - Do not dilute whole blood or use any volume other than 50 µL.
  - Store prepared samples at room temperature (20°C to 25°C, 68°F to 77°F) in the dark and run on the BD FACSCount instrument within 48 hours of preparation.
  - The reagents used in this test system are light sensitive. Minimize exposing the reagent tubes to light.
  - Do not re-run patient samples more than twice on the BD FACSCount instrument after they have already been run.
  - We recommend that each laboratory establish its own reference ranges for
people outside the age range of 18 to 65 years and for pregnant women.

- Performance has not been established on persons undergoing monoclonal antibody therapy.
- Use only BD FACSCOUNT reagents and controls with the BD FACSCOUNT instrument.
- CD4 counts below 50 cells/µL and CD3 and CD8 counts below 100 cells/µL are for information only.

**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.

**TROUBLESHOOTING**


**REFERENCES**


1. INTENDED USE

The BD FACSCount™ control kit is intended for in vitro diagnostic use in setting up the BD FACSCount™ instrument and for checking linearity.

2. PRINCIPLES OF THE PROCEDURE

Four bead concentrations (Zero, Low, Medium, and High) are added to normal blood stained with BD FACSCount reagents and used daily when the instrument is first turned on, and whenever a new lot of reagent is opened. Data for the last control run is stored on the BD FACSCount instrument protocol diskette.

3. REAGENTS

Reagent Provided, Sufficient for 25 Runs

The BD FACSCount control kit includes four bead concentrations (Zero, Low, Medium, and High), contained in two tube pairs with color-coded tops.

- Pair one:
  Zero (yellow top): 0 beads/μL
  Low (red top): ~50 beads/μL

- Pair two:
  Medium (blue top): ~250 beads/μL
  High (purple top): ~1,000 beads/μL

Reagents or Materials Required but Not Provided

- BD Vacutainer® K2 or K3 EDTA blood collection tubes, or equivalent
- Disposable pipet tips
- Vortex mixer
- BD FACSCount system
4. PROCEDURE

Use this procedure to prepare and run controls for the following kits:

<table>
<thead>
<tr>
<th>Kit (assay)</th>
<th>BD Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD FACSCount Reagent Kit CD4/CD8/CD3</td>
<td>340167</td>
</tr>
<tr>
<td>BD FACSCount CD4 Reagents CD4</td>
<td>339010</td>
</tr>
</tbody>
</table>

Preparing Controls

**WARNING** All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing and gloves.

**WARNING** Patient blood samples must be collected in BD Vacutainer K2 or K3 EDTA blood collection tubes (or equivalent), and stored no longer than 48 hours at room temperature (20°C to 25°C, 68°F to 77°F). Results obtained from samples that do not meet these criteria can be inaccurate.

**WARNING** Sodium azide is harmful if swallowed (R22). Keep away from foodstuffs, beverages, and feed. Keep container tightly closed (S7). Do not breathe gas, fumes, vapor, or spray (S23). Wear protective clothing, gloves, and eye/face protection (S36/37/39). In case of contact with eyes, rinse immediately with plenty of water and seek medical advice (S26). Take off immediately all contaminated clothing (S27). Contact with acids liberates very toxic gas (R32). This material and its container must be disposed of as hazardous waste (S60). Contact with acidic solutions and metal compounds over time may form potentially explosive metal azides. Should any of this material be introduced into a sanitary sewer system, flush with copious amounts of water.

1. Stain a whole blood sample from a normal donor following the instructions in the reagent package insert.

**NOTE** Stained samples can be stored for up to 24 hours before adding control beads.

2. Remove one pair of Zero/Low control beads and one pair of Medium/High control beads from the control kit and place them in the control area of the workstation.

3. Uncap the stained sample tubes and discard the caps in an appropriate biohazard container.

4. Set the vortex mixer to a midrange speed and vortex the Zero/Low control bead pair upside down for 5 seconds, then upright for 5 seconds.

5. If you are running the CD4/CD8/CD3 assay, open the Zero control beads (yellow top) with the coring station and pipette 50 μL into the sample tube labeled Zero.

**NOTE** The Zero control is not necessary for the CD4 assay.

6. Open the Low control beads (red top) with the coring station and pipette 50 μL into the sample tube labeled Low.

7. Vortex the Medium/High control bead pair upside down for 5 seconds, then upright for 5 seconds.

8. Open the Medium control beads (blue top) with the coring station and
pipette 50 μL into the sample tube labeled Medium.

9. Open the High control beads (purple top) with the coring station and pipette 50 μL into the sample tube labeled High.

10. Cap the sample tubes with new caps.

11. Cap the two tube pairs of the BD FACSCount control beads and store upright.

For subsequent uses of the control beads, vortex upright for 5 seconds.

**Verifying Control Tubes**

- For the CD4/CD8/CD3 assay, you should have two sample tube pairs containing the control beads listed in the following table.

<table>
<thead>
<tr>
<th>Pair</th>
<th>Reagent</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CD4 tube</td>
<td>Zero</td>
</tr>
<tr>
<td></td>
<td>CD8 tube</td>
<td>Low</td>
</tr>
<tr>
<td>2</td>
<td>CD4 tube</td>
<td>Medium</td>
</tr>
<tr>
<td></td>
<td>CD8 tube</td>
<td>High</td>
</tr>
</tbody>
</table>

- For the CD4 assay, you should have three sample tubes containing the control beads listed in the following table.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Low</td>
</tr>
<tr>
<td>2</td>
<td>Medium</td>
</tr>
<tr>
<td>3</td>
<td>High</td>
</tr>
</tbody>
</table>

Run the tubes on the BD FACSCount instrument within 2 hours of adding control beads to the reagent tubes.

Store samples at room temperature in the workstation until they are run on the instrument. Vortex upright for 5 seconds immediately before running.

**Running Controls**

BD FACSCount reagents and control beads are each assigned specific lot codes and specific bead counts. Carefully enter the lot codes and bead counts before running controls or samples. This information is stored and does not need to be changed between runs unless a new lot of controls or a new lot of reagents is used. See the appropriate BD FACSCount user’s guide for instructions on entering lot codes and bead counts.

After you enter the normal control ID, the instrument prompts you for the first pair of controls.

**WARNING** Be sure you have added control beads to the reagent tubes (see Preparing Controls on page 2).

1. Vortex the Zero/Low tube pair upright for 5 seconds.

2. If you are running the CD4/CD8/CD3 assay, uncap the Zero tube and set the cap aside.

If you are running the CD4 assay, skip to step 6.

3. Place the Zero tube in the run position of the sample holder.

4. Press [RUN].

The software displays the event rate (events/second) and total events.

5. When analysis is complete, remove the Zero tube and recap it.

6. Uncap the Low tube and set the cap aside.

7. Place the Low tube in the run position.

8. Press [RUN].