WHO Prequalification of Diagnostics Programme
PUBLIC REPORT

Product: BD FACSCount™ Instrument System with FACSCount™ Control Kit and BD FACSCount™ CD4 Reagent Kit *(Absolute and Percentage CD4+ Counts)*
Number: PQDx 0133-045-00

Abstract

The **BD FACSCount™ Instrument System** with **FACSCount™ Control Kit** and **BD FACSCount™ CD4 Reagent Kit** with product codes **337858, 340166, 339010** 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. This public report was amended 30 June 2016 to include an additional manufacturing site.

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™ CD4 reagent kit is intended for in vitro diagnostic use in enumerating the absolute counts of $CD4^+$, T lymphocytes and determine the percentage of lymphocytes in unlysed whole blood, using the BD FACSCount™ instrument. The presence of known number of fluorescent reference beads enable the software to automatically identify the lymphocyte population of interest and calculate the CD4 counts (cells/uL).

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.

The whole blood is added to the reagent tube flourochrome –labelled antibodies in the reagent which binds specifically to white blood cell surface antigens while a fluorescent nuclear dye binds to the nucleated blood cells. After fixation, the sample is run on the instrument. During sample acquisition, the cells pass through the laser light, which causes the labelled cells to fluoresce. This fluorescent light is analysed by the instrument to generate lymphocytes and CD4 T lymphocytes.

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

I**nstrumentation**:

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

Software:

- BD FACSCount™ Users Guide and Software (339011)

Reagents:

- BD FACSCount™ CD4 Reagent Kit (339010) (50 pairs CD4 PE / CD14 PE-CyTM5 / CD15 PE-Cy5 reagents), fixative (one 5-ml vial 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 K_2 or K_3 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™ CD4 Reagent Kit and BD FACSCount™ Control Kit should be stored at 2 to 8 ^{o}C.

Shelf-life:
BD FACSCount CD4 Reagent Kit: 15 months.

BD FACSCount™ Control kit: 24 months.

Summary of prequalification status for BD FACSCount™ Instrument System with FACSCount™ Control Kit and BD FACSCount™ CD4 Reagent Kit *(Absolute and Percentage CD4+ Counts)*

	Initial acceptance	
	Date	**Outcome**
PQ status amended	30 June 2016	listed
Status on PQ list	12 November 2012	listed
Dossier assessment	20 April 2012	MR
Inspection status	08 October 2012	MR
Laboratory evaluation	01 October 2012	MR

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

BD FACSCount™ Instrument System with FACSCount™ Control Kit and BD FACSCount™ CD4 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 0133-045-00. Based on the established WHO prioritization criteria, BD FACSCount™ Instrument System with FACSCount™ Control Kit and BD FACSCount™ CD4 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™ CD4 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 and Company, BD Biosciences 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™ CD4 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 "Protocol for multicenter laboratory assessment of dedicated and point-of-care CD4+ T-lymphocytes enumeration technologies" (PQDx_114) 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™ CD4 reagents in ready-to-use reagent tube format to enumerate absolute $CD4^+$ counts and determine CD4 percentage after 30 minutes incubation. 100 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 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 the a percentage coefficient of variation (%CV) 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 that 2.0%. 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 Scott percentage similarity methods.

In laboratory 1, a total of 7/240 (2.9%) samples stained with FACSCount CD4 reagent failed to run in the FACSCount instrument. In laboratory 2 a total of 9/200 (4.5%) samples stained with FACSCount CD4 reagent failed to run in the FACSCount instrument. Intra-laboratory variation studies showed mean %CV of 5.0% and 3.2% for FACSCount CD4 reagents absolute counts and FACSCount CD4 reagent percentage respectively in laboratory 1. In laboratory 2 the intra-laboratory variation was 6.8%, and 5.0% for FACSCount CD4 reagent absolute counts and FACSCount CD4 reagent percentage respectively.

The mean inter-assay variability for CD4 less than 200/µL was 5.0%, and 9.5% for FACSCount CD4 reagent absolute counts, and FACSCount CD4 reagent percentage respectively in laboratory 1; while the mean was 7.1%, and 7.9% for FACSCount CD4 reagent absolute counts and FACSCount CD4 reagent percentage respectively in laboratory 2.

The mean instrument precision was 4.2% and 3.3% for FACSCount CD4 reagents absolute counts, and FACSCount CD4 reagents percentage respectively in laboratory 1; while the mean was 5.1% and 3.4% for FACSCount reagent absolute counts, FACSCount CD4 reagent absolute counts, and FACSCount CD4 reagent percentage respectively in laboratory 2. 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.

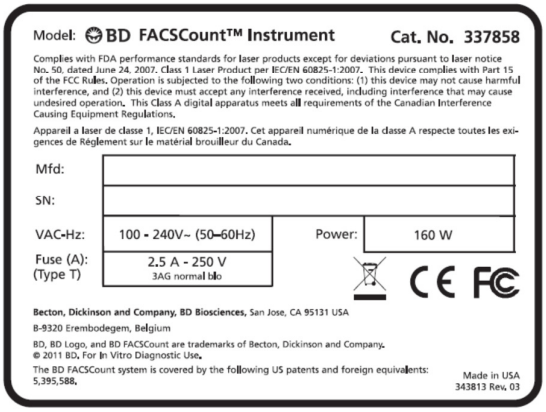
Change notification

In 2016, BD Biosciences., submitted a change notification related additional of a site of manufacture at Vicks Drive, Lot #1 Corner Road 735, Cayey, Puerto Rico, 0076. This change notification was assessed and product was found to meet WHO prequalification requirements.

Labelling

1. Labels

1.1 Instrument



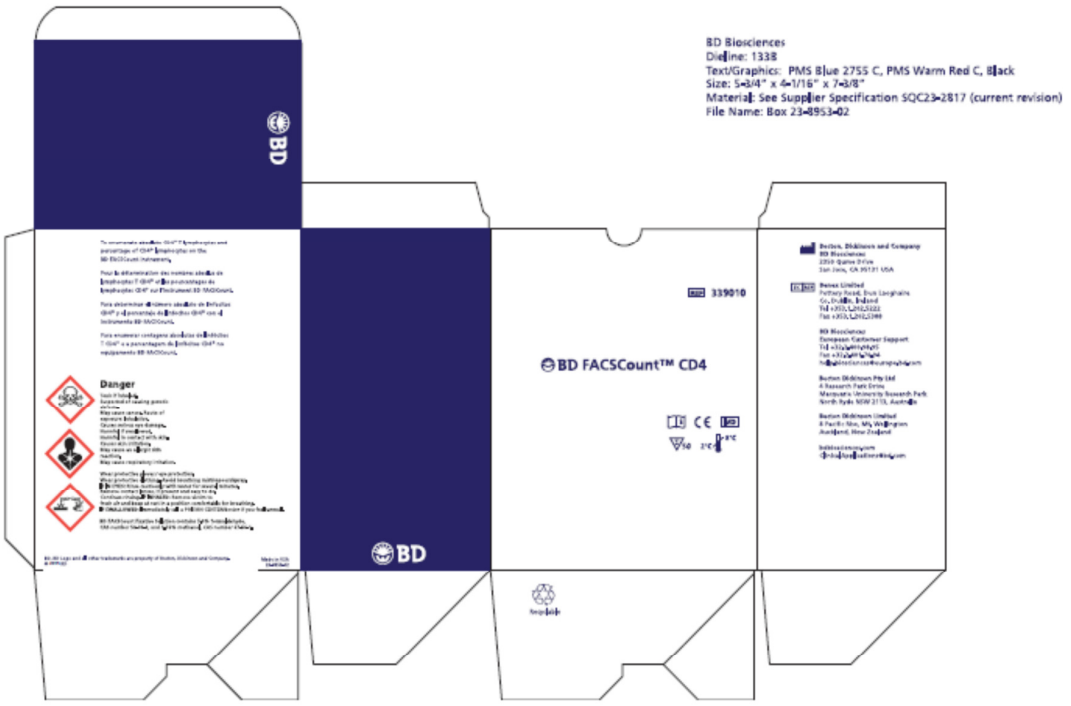
1.2 BD FACSCount™ Controls

1.3 BD FACSCount™ System – Fixative Solution

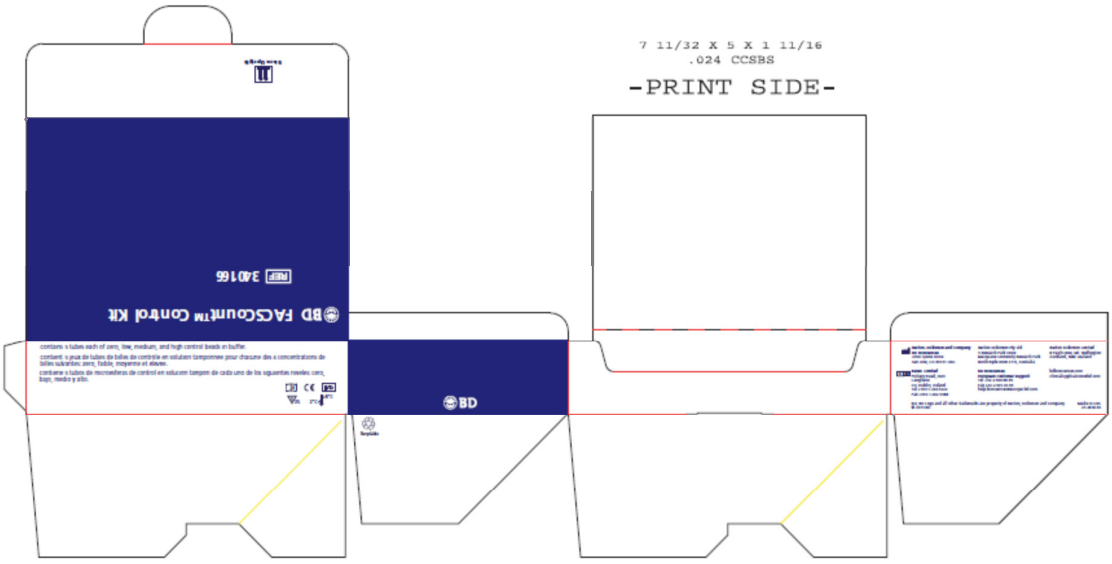
1.4 BD FACSCount™ Caps

1.5 BD FACSCount™ Reagents

1.6 Outer box for BD FACSCount™ Reagents



1.7 Outer box for BD FACSCount™ Controls



2. Instructions for use

BD FACSCount™ CD4

For enumerating absolute counts and determining percentages of CD4 T lymphocytes in unlysed whole blood

50 Tests—Catalog No. 339010

2/2015 23-8777-03

IVD

CE

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1. INTENDED USE

BD FACSCount™ CD4 reagents are used to enumerate the absolute counts of CD4 T lymphocytes and determine the percentage of lymphocytes that are CD4 T lymphocytes in unlysed whole blood (CD4 counts and CD4 percentages). The reagents are intended for in vitro diagnostic use on a BD FACSCount™ instrument.

Clinical Applications

CD4 counts and CD4 percentages 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 and percentage of CD4 T lymphocytes are the cellular parameters most closely associated with HIV disease progression and patient prognosis.[4] The number of CD4 T lymphocytes declines in HIV infection.[5-7]

2. PRINCIPLES OF THE PROCEDURE

A single test requires one ready-to-use reagent tube.

When whole blood is added to the reagent tube, fluorochrome-labeled antibodies in the reagents bind specifically to white blood cell surface antigens, and a fluorescent nuclear dye binds to the nucleated blood cells. After a fixative solution is added, the sample is run on the instrument. During sample acquisition, the cells pass through the laser light, which causes the labeled cells to fluoresce. This fluorescent light provides the information necessary for the instrument

to identify and count the lymphocytes and CD4 T lymphocytes.

In addition, the reagent tubes contain a known number of fluorescent reference beads to which a precise volume of whole blood is added. The software automatically identifies the lymphocyte populations of interest and calculates the CD4 counts (cells/μL) by comparing cellular events to bead events. Results include CD4 counts and CD4 percentages.

3. REAGENTS

Reagents Provided, Sufficient for 50 Tests

The following are provided:

- 50 reagent tubes containing CD4 PE/CD14 PE-Cy™5*/CD15 PE-Cy5, fluorescent nuclear dye, and reference beads
- 65 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.
- One 5-mL vial of 5% formaldehyde in phosphate-buffered saline (PBS), used as fixative solution

The CD4 antigen,[8,9] 55 kilodaltons (kDa),[10] is present on a T-lymphocyte subset[11,12] that comprises 28% to 58%[13] of normal peripheral blood lymphocytes.[9,10] The CD4 antigen is present in low density on the cell surface of monocytes and in the cytoplasm of monocytes.

CD4, clone SK3,[8] is derived from the hybridization of mouse NS-1 myeloma cells with spleen cells from BALB/c mice immununized with human peripheral blood T lymphocytes. CD4 is composed of mouse IgG_1 heavy chains and kappa light chains.

CD14 recognizes a human monocyte/macrophage antigen, with a molecular weight of 55 kDa.[14] The CD14 antigen is present on the majority of normal peripheral blood monocytes.[15]

CD14, clone MϕP9, is derived from the hybridization of mouse Sp2/0 myeloma cells with spleen cells from BALB/c mice immunized with peripheral blood monocytes from a patient with rheumatoid arthritis. CD14 is composed of mouse IgG_{2b} heavy chains and kappa light chains.

CD15 recognizes a human myelomonocytic antigen.[16] The structure recognized by CD15 antibodies is lacto-N-fucopentose III.[16] The CD15 antigen is present on the majority of mature peripheral blood eosinophils and neutrophils and is present at low density on circulating monocytes.

CD15, clone MMA, is derived from the hybridization of mouse P3-X63-Ag8.653 myeloma cells with spleen cells from BALB/c mice immunized with the U-937 histiocytic cell line. CD15 is composed of mouse IgM heavy chains and kappa light chains.

The nuclear dye binds to nucleic acid and fluoresces.

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Concentration values are listed in the following table:

Reagent	Concentration
Beads	1.29 x 10^5 beads/mL
CD4	0.1 µg/mL
CD15	0.625 µg/mL
CD14	0.625 µg/mL
Oxazine	4.3 µg/mL

Precautions

- For In Vitro Diagnostic Use.
- The antibody reagents contain sodium azide as a preservative; however, care should be taken to avoid microbial contamination, which could cause erroneous results.

Fixative contains 5.0% formaldehyde, CAS number 50-00-0 and 1.76% methanol, CAS number 67-56-1.

	Danger
	H331 Toxic if inhaled.
	H341 Suspected of causing genetic defects. H350 May cause cancer. Route of exposure: Inhalative.
	H318 Causes serious eye damage.
	H302 Harmful if swallowed. H312 Harmful in contact with skin. H315 Causes skin irritation. H317 May cause an allergic skin reaction. H335 May cause respiratory irritation.
	Wear protective gloves / eye protection.Wear protective clothing. Avoid breathing mist/vapours/spray. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.

WARNING The reagent solution contains a nuclear dye. The toxicological properties of this dye have not been investigated. If inhaled or ingested, contact a physician immediately. If skin or eye contact occurs, wash with copious amounts of water.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[17,18] 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.

Storage and Handling

The reagent is stable until the expiration date shown on the label when stored at 2°C–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.

Reagents or Materials Required but Not Provided

- BD Vacutainer® EDTA blood collection tubes or equivalent

- Disposable pipet tips (Catalog No. 340292) or equivalent
- Vortex mixer (See Recommended Brands of Materials in the *BD FACSCount System User's Guide For Use with BD FACSCount CD4 Reagents*.)
- BD FACSFlow™ sheath fluid (Catalog No. 342003) or equivalent
- BD FACSCount™ controls (Catalog No. 340166)
- BD FACSCount system

4. INSTRUMENT

BD FACSCount CD4 reagents are designed for use on a BD FACSCount instrument. We recommend running BD FACSCount controls daily. Be sure to use the BD FACSCount CD4 protocol disk with the most recent control data when running samples stained with CD4 reagents on the BD FACSCount instrument. See the *BD FACSCount System User's Guide For Use with BD FACSCount CD4 Reagents* for detailed instructions.

5. PROCEDURE

Collecting Blood

Collect blood aseptically by venipuncture, using EDTA blood collection tubes.[19] A minimum of 100 µL of whole blood is required for this procedure. Follow the collection tube manufacturer's guidelines for the minimum volume of blood to be collected to ensure proper specimen dilution, especially when determining absolute counts with reference beads.

Anticoagulated blood stored at room temperature (20°C–25°C) must be stained within 24 hours of draw and must be analyzed within 48 hours of staining.

NOTE Do not use previously fixed and stored patient samples. Whole blood samples refrigerated before staining can give aberrant results. Specimens obtained from patients taking immunosuppressive drugs can yield poor resolution.[20] Blast cells can interfere with test results. Hemolyzed specimens should not be used.

Performing Quality Control

We recommend performing a control run using BD FACSCount controls to check system accuracy and linearity. Run controls each day before you run patient samples or whenever you open a new reagent lot. See the *BD FACSCount System User's Guide For Use with BD FACSCount CD4 Reagents* for detailed information on performing a BD FACSCount control run.

Preparing Tubes

NOTE We recommend that you prepare no more than 15 reagent tubes at one time.

1. Label the tab of each reagent tube with the patient accession number or number that identifies the tube of blood.
2. Vortex each tube upside down for 6 seconds and upright for 6 seconds.

   NOTE Set the vortex speed to a setting that causes the liquid to rise to the top of the tube.
3. Open each reagent tube with the coring station.

Adding Blood

1. Invert the EDTA tube 5 to 10 times to make sure that the whole blood is adequately mixed.

2. Pipette 50 µL of whole blood into the reagent tube labeled with the corresponding patient accession number.

   Reverse pipetting is critical to accuracy. We recommend using the BD FACSCount pipet that is provided with the BD FACSCount system.

   Pipette whole blood onto the side of the tube just above the liquid reagent.

   If an electronic pipet is not available, follow these instructions for manual reverse pipetting.

   - Depress the button to the second stop. When you release the button, excess sample is drawn up into the tip.
   - Depress the button to the first stop to expel a precise volume of blood. This leaves excess blood in the tip.

   Always change to a new tip between tubes. Discard tips in an appropriate biohazard container.

3. Cap the tube and vortex upright for 6 seconds.
4. Repeat steps 1 through 3 to prepare a sample tube for each patient specimen.
5. Incubate the tubes for 30 minutes at room temperature (20°C–25°C) in the workstation. Close the cover to protect the reagents from light.

   NOTE Correct incubation time is critical and must be at least 30 minutes but no longer than 40 minutes for each sample tube.

Adding Fixative

1. Uncap each sample tube and pipette 50 µL of fixative solution into each tube.

   Always change to a new tip between tubes. Discard tips in an appropriate biohazard container.

2. Recap each tube and vortex upright for 6 seconds.

Run the sample tubes on the BD FACSCount instrument within 48 hours of adding fixative. Store samples at room temperature, protected from light, until they are run on the instrument.

Running Patient Samples

See the *BD FACSCount System User's Guide For Use with BD FACSCount CD4 Reagents* for detailed information on running patient samples.

Make sure you enter the patient accession number in the software before you begin.

1. Vortex the CD4 tube upright for 6 seconds.

   WARNING Inadequate suspension of white blood cells can result in inaccurate results.

2. Uncap the tube and set the cap aside.
3. Place the sample tube in the sample holder and press Run.

   A software message will indicate when the analysis is complete.

4. Remove the sample tube and recap it.

   Discard the sample tube in an appropriate biohazard container.

5. Repeat steps 1 through 4 for the remaining samples.

6. EXPECTED RESULTS

Reference Ranges

The reference ranges for BD FACSCount CD4 reagents shown in Table 1 were determined at BD Biosciences in San Jose,

CA. Subjects were healthy adults between the ages of 18 and 65 years.

Table 1 Representative reference ranges for BD FACSCount CD4 reagents

Parameters	n[a]	Mean	95% Reference Range
Absolute CD4 (cells/µL)	141	906.65	380–1,704
Percent CD4	141	44.90	30.13–60.23

a. n=sample size

7. PERFORMANCE CHARACTERISTICS

Performance of the reagents was established by testing at BD Biosciences in San Jose, CA and at three clinical laboratories in the US.

Accuracy (Agreement)

CD4 absolute counts were enumerated and percentages were determined with BD FACSCount CD4 reagents on the BD FACSCount instrument using BD FACSCount CD4 software v1.0. Results were compared with results from the BD Tritest™ CD3 FITC/CD4 PE/CD45 PerCP reagent in BD Trucount™ tubes on the BD FACSCalibur™ flow cytometer using BD Multiset™ software.

Whole blood samples were collected at random at three clinical laboratories. Regression statistics are reported in Table 2.

Table 2 Regression analysis of test versus predicate for CD4 absolute counts and percentages

Parameters	n	R^2	Slope	Intercept	Range
Absolute CD4 (cells/µL)	101	0.981	0.971	12.695	59–3,405
Percent CD4	99	0.99	0.999	–0.391	5.51–64.69

Figure 1 Regression plot of test versus predicate for CD4 absolute counts (x-axis = BD FACSCount CD4 Absolute Counts, y-axis = BD Tritest CD4 Absolute Counts)

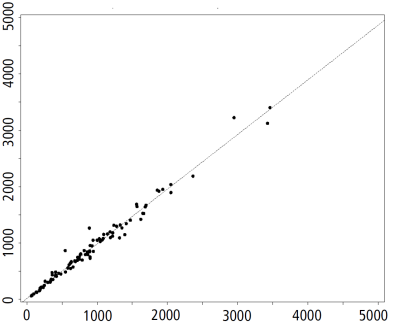
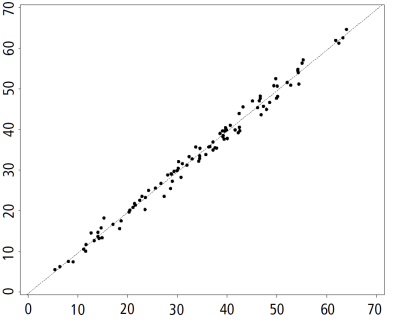


Figure 2 Regression plot of test versus predicate for CD4 percentages (x-axis = BD FACSCount CD4 Percentages, y-axis = BD Tritest CD4 Percentages)



Precision

Estimates of precision were determined at one site, BD Biosciences, using BD Multicheck™ low and normal controls. Two replicates of each control were analyzed in each run, and two runs were performed per day for a total of 21 days. Three different instruments with three different operators were used, each for seven of the

21 days. One reagent lot and one lot of BD FACSCount control beads were used for the duration of the study.

Coefficients of variation (CVs) and standard deviations (SDs) are provided for CD4 absolute counts and CD4 percentages for within-device* and within-run precision in Table 3 and Table 4.

Table 3 Within-device and within-run precision CD4 absolute counts

	Low control CV (cells/µL)	Normal control CV (cells/µL)
Within device	4.82	4.28
Within run	4.04	3.46

Table 4 Within-device and within-run precision CD4 percentages

	Low control SD (%)	Normal control SD (%)
Within device	0.38	1.28
Within run	0.35	1.15

Stability

A stability study was conducted at two clinical laboratories to assess the stability of the BD FACSCount CD4 reagents, and the following were 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 both

Whole blood samples were tested up to 24 hours post draw, and stained samples were tested up to 48 hours post stain. All samples were maintained at room temperature (20°C–25°C) before staining or acquisition.

Based on the results of this study, we recommend staining whole blood samples within 24 hours of draw and analyzing stained samples within 48 hours of staining.

Linearity

Linearity of the BD FACSCount CD4 reagent assay was assessed for the BD FACSCount instrument within a $CD4^+$ cell concentration of 50 to 5,000 cells/µL. Results were observed to be linear across the range.

Cross Reactivity

The specificity of these monoclonal antibodies has been established by blind testing at a number of laboratories by the International Leucocyte Workshop Group.[21]

User-Reportable Ranges

We conducted performance testing for the following ranges:

- Absolute counts: 50 to 5,000 $CD4^+$ cells/µL
- Percentages: 5% to 65%

Performance characteristics outside these ranges have not been established.

8. LIMITATIONS

CAUTION The pipet used in the sample preparation procedure must be properly calibrated to ensure 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

* For this study, *within-device precision* has the same meaning as *total precision*.

precision and accuracy must be verified. (See the *BD FACSCount System User's Guide For Use with BD FACSCount CD4 Reagents*).

- The vortex used must be set to a speed that causes the liquid to rise to the top of the reagent tube. Inadequate suspension of white blood cells can result in inaccurate results.
- Collect samples only in EDTA blood collection tubes. A minimum of 100 µL of whole blood is required for the test.
- Prepare samples within 24 hours of draw and analyze samples within 48 hours of preparation.
- Correct incubation time is critical and must be at least 30 minutes but no longer than 40 minutes for each sample. For this reason, we recommend preparing no more than 15 control and sample tubes at one time.
- Do not refrigerate whole blood before preparing.
- Do not dilute whole blood or use any volume other than 50 µL.
- The reagents used in this test system are light sensitive. Minimize exposing the reagent tubes to light.
- We recommend that each laboratory establish its own normal reference ranges.
- Product performance has not been established on persons undergoing monoclonal antibody chemotherapy.
- Use BD FACSCount CD4 reagents and controls only with the BD FACSCount instrument.
- Do not mix reagent lots when running controls or samples.
- BD conducted performance testing for the following ranges:
- Absolute counts: 50 to 5,000 $CD4^+$ cells/µL
- Percentage: 5% to 65%

  Performance characteristics outside these ranges have not been established. Any results outside these ranges will cause the following statement to appear on the Sample Run or Control Run printout: *Results are outside the product validated range*.

TROUBLESHOOTING

Refer to the troubleshooting section in the *BD FACSCount System User's Guide For Use with BD FACSCount CD4 Reagents* for troubleshooting 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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BD FACSCount™ Control Kit

For setting up the BD FACSCount instrument and for checking linearity

25 Runs—Catalog No. 340166

1/2014 23-10487-01

IVD

CE

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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

Concentration values are listed in the following table:

BD FACSCount Control	Concentration (beads/mL)
Low bead	2.0×10^7 to 3.0×10^7
Medium bead	2.0×10^7 to 3.0×10^7
High bead	2.0×10^7 to 3.0×10^7

Reagents or Materials Required But Not Provided

- BD Vacutainer® 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:

Kit (assay)	Catalog No.
BD FACSCount™ Reagent Kit (CD4/CD8/CD3)	340167
BD FACSCount™ CD4 Reagents (CD4)	339010

Preparing Controls

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[1,2] 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.

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

1. Stain a whole blood sample from a normal donor following the instructions in the reagent instructions for use (IFU).

   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.

Pair	Reagent	Control
1	CD4 tube	Zero
	CD8 tube	Low
2	CD4 tube	Medium
	CD8 tube	High

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

Tube	Control
1	Low
2	Medium
3	High

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 (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.
9. When analysis is complete, remove the *Low* tube and recap it.
10. Repeat steps 1 through 9 for the rest of the controls.

    At the end of the control run, the results are displayed and printed. Discard the reagent pairs in an appropriate biohazard container.

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TROUBLESHOOTING

See the appropriate BD FACSCount user's guide for troubleshooting information.

REFERENCES

1. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Third Edition.* Wayne, PA: Clinical and Laboratory Standards Institute; 2005. CLSI document M29-A3.
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