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

The **BD FACSCount Instrument System**[1] 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.

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

	Date	Outcome
Prequalification listing	12 November 2012	listed
Dossier assessment	20 April 2012	MR
Site inspection(s) of the quality management system	13 February 2017	MR
Product performance evaluation	1 October 2012	MR

MR: Meets Requirements

Report amendments and product changes

This public report has since been amended. Amendments may have arisen because of changes to the prequalified product for which the WHO has been notified and has undertaken a review. Amendments to the report are summarised in the following table, and details of each amendment are provided below.

Public report amendment	Summary of amendment	Date of report amendment
2.0	Addition of manufacturing in Cayey, Puerto Rico, to manufacture the FACSCount Reagent Kit.	22 March 2018
4.0	Labelling updates made to the BD FACSCount CD4 Reagent Kit and BD FACSCount Control Kit.	3 August 2023

[1] Please note the product will be discontinued from the market on 31 July 2024.

Intended use:

According to the claim of intended use from Becton, Dickinson and Company, BD Biosciences, *" BD FACSCount CD4 reagents are used to enumerate the absolute counts of CD4 Tlymphocytes and determine the percentage of lymphocytes that are CD4 Tlymphocytes in unlysed whole blood (CD4 counts and CD4 percentages). The reagents are intended for in vitro diagnostic use on a BD FACS Count instrument.*
The BDFACSCount control kit is intended for in vitro diagnostic use in setting up the BD FACSCount instrument and for checking linearity."

Assay Description:

According to the claim of assay description from Becton, Dickinson and Company, BD Biosciences, "*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 summarised 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 summarising 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 BD 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:

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.

Warnings/limitations:

Refer to the current version of the manufacturer's instructions for use.

Prioritisation for prequalification:
BD Biosciences submitted an application for prequalification of BD FACSCount System PQDx 0133-045-00. Based on the established WHO prioritisation criteria, BD FACSCount Instrument System with FACSCount Control Kit and BD FACSCount CD4 Reagent Kit was given priority for prequalification assessment.

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 the WHO in accordance with the internal procedure for 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 BD FACSCount Control Kit and BD FACSCount CD4 Reagent Kit.

Commitments for prequalification:

The manufacturer committed to amend and submit additional documentation on the 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 (at 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 11 May 2017.

Product performance evaluation

BD FACSCount System using the BD FACSCount CD4 Reagent Kit was evaluated in two WHO collaborating laboratories, namely the Institute of Tropical Medicine, Belgium and the 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 utilises BD BD FACSCount CD4 reagents in ready-to-use reagent tube format to enumerate absolute $CD4^+$ counts and determine CD4 percentage after 30 minutes of incubation. 100 µl of well-mixed unlysed whole blood and BD 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 of use was assessed.

The acceptance criteria for reproducibility studies was that the assay should have 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 than 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 BD FACSCount CD4 reagents and BD 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.

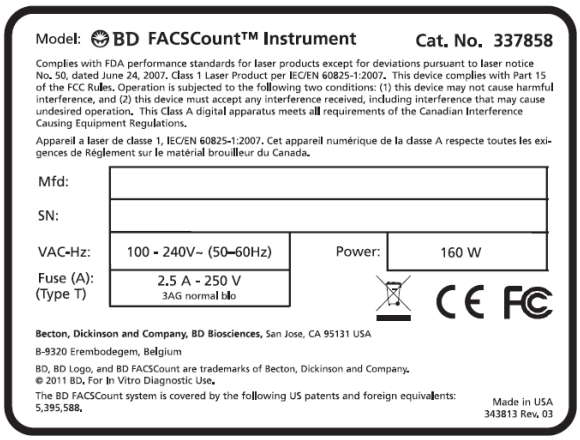
The mean inter-assay variability for CD4 less than 200/µL was 5.0% and 9.5% for FACSCount CD4 reagent absolute counts and FACSCount CD4 reagent percentage, respectively, in laboratory 1. In contrast, the mean was 7.1% and 7.9% for FACSCount CD4 reagent absolute counts and FACSCount CD4 reagent percentage,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

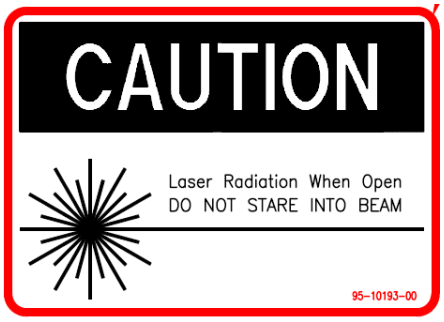
The mean instrument precision was 4.2% and 3.3% for FACSCount CD4 reagents absolute counts and FACSCount CD4 reagents percentage, respectively, in laboratory 1. In comparison, the mean was 5.1% and 3.4% for FACSCount reagent absolute counts, FACSCount CD4 reagent absolute counts, and FACSCount CD4 reagent percentages, respectively, in laboratory 2. The carry-over was less than 2% in both laboratories. Regarding the agreement with the reference method, the correlation coefficients were high, with minimal bias in both laboratories.

Labelling

1. Labels

1.1 Instrument





1.2 BD FACSCount CD4 Reagent Kit labels (339010)

To enumerate absolute $CD4^+$ T lymphocytes and percentage of $CD4^+$ lymphocytes on the BD FACSCount instrument.

Pour la détermination des nombres absolus de lymphocytes T $CD4^+$ et les pourcentages de lymphocytes $CD4^+$ sur l'instrument BD FACSCount.

Para determinar el número absoluto de linfocitos $CD4^+$ y el porcentaje de linfocitos $CD4^+$ con el instrumento BD FACSCount.

Para enumerar contagens absolutas de linfócitos T $CD4^+$ e a percentagem de linfócitos $CD4^+$ no equipamento BD FACSCount.

Danger

Toxic if inhaled.
Suspected of causing genetic defects.
May cause cancer. Route of exposure: Inhalative.
Causes serious eye damage.
Harmful if swallowed.
Harmful in contact with skin.
Causes skin irritation.
May cause an allergic skin reaction.
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: Immediately call a POISON CENTER/doctor if you feel unwell.

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

Made in USA
23-8953-03

BD

REF 339010

BD FACSCount™ CD4

IVD

50

2°C 8°C

Becton, Dickinson and Company
BD Biosciences
2350 Qume Drive
San Jose, CA 95131 USA

EC REP **Benex Limited**
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Tel +353.1.202.5222
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bdbiosciences.com
ClinicalApplications@bd.com

Recyclable

BD FACSCount™ CD4

REF 339010

IVD

eifu.bd.com

℞ Only

Σ 50

2 °C – 8 °C

LOT

Counts - Numération - Recuentos - Contagens
CD4:

Made in USA
23-23806-00

BD FACSCount™ CD4

REF 339010

IVD

eifu.bd.com

℞ Only

Σ 50

2 °C – 8 °C

Made in USA
23-23809-00

1.3 BD FACSCount Control Kit labels (340166)

Store Upright

BD

BD

Recyclable

Made in USA
23-23813-00

BD FACSCount™ Control Kit

REF 340166

IVD

eifu.bd.com

2 °C – 8 °C

Σ 25

℞ Only

23-23812-00

BD FACSCount™ System - Fixative Solution REF 340167

Σ 50

50 µL

IVD

2 °C – 8 °C

eifu.bd.com

℞ Only

Becton, Dickinson and Company, BD Biosciences
2350 Qume Drive, San Jose, CA 95131 USA

Made in USA
91-0140/23-23807-00

Placement of 2D Bar Code

(01)00382903401673
(17)xxxxxx
(10)xxxxxx
(30)xxx(240)xxxx

LOT

2. Instructions for use[2]

[2] English version of the IFU was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

BD FACSCount™ Reagent Kit

For enumerating absolute counts of CD4, CD8, and CD3 T lymphocytes in unlysed whole blood

50 Tests—Catalog No. 340167

2021-08
English

23-23276(01)

IVD

℞ Only

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 decreases and the relative percentage of CD8 T lymphocytes increases in HIV infection, resulting in a decrease 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[6,7] is present on T-helper/inducer lymphocytes and monocytes.[8,9]

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 as a disulfide-linked bimolecular complex with a 32-kilodalton (kDa) α subunit.[10,11] The majority of peripheral blood $CD8^+$ T lymphocytes expresses an α/β heterodimer (32, 30 kDa), while $CD8^+CD16^+$ natural killer (NK) lymphocytes and $CD8^+$ T-cell receptor (TCR)-γ/δ^+ T lymphocytes express an α/α homodimer (30 kDa). $CD8^+$TCR-α/β^+ T lymphocytes can express either an α/α homodimer or an α/β heterodimer.[10,11]

CD3, clone SK7,[12-15] 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/TCR complex.[16] The antigen recognized by CD3 antibodies is noncovalently associated with either α/β or γ/δ TCR (70 to 90 kDa).[17]

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

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

4. STORAGE AND HANDLING

Each antibody 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.

5. REAGENTS

Reagent Provided, Sufficient for 50 Tests

The BD FACSCount™ reagent kit contains the following:

- CD4 PE/CD3 PE-Cy5* 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

Reagents or Materials Required but Not Provided

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

Concentration values are listed in the following table.

Reagent	Concentration (µg/mL)
CD4 PE	0.075
CD3 PE-Cy5	0.625
Beads	1.29 x 10^5 beads/mL
CD8 PE	0.312
CD3 PE-Cy5	0.625
Beads	2.58 x 10^5 beads/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.

Contains 0.494% Ethylenediamine, ethoxylated and propoxylated, CAS number 26316-40-5, 5.0% formaldehyde, CAS number 50-00-0 and 1.76% methanol, CAS number 67-56-1.

Go to regdocs.bd.com to download the Safety Data Sheet.

	Danger
	H317: May cause an allergic skin reaction. H302: Harmful if swallowed. H315: Causes skin irritation. H319: Causes serious eye irritation. H317: May cause an allergic skin reaction. H341: Suspected of causing genetic defects. H350: May cause cancer. H335: May cause respiratory irritation. H402: Harmful to aquatic life.

* Cy™ is a trademark of GE Healthcare. This product is subject to proprietary rights of GE Healthcare and Carnegie Mellon University, and is made and sold under license from GE Healthcare. This product is licensed for sale only for in vitro diagnostics. It is not licensed for any other use. If you require any additional license to use this product and do not have one, return this material, unopened, to BD Biosciences, 2350 Qume Drive, San Jose, CA 95131, and any money paid for the material will be refunded.

	Danger
Prevention	P261: Avoid breathing dust/fume/gas/mist/vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves/protective clothing/eye protection/face protection. P264: Wash thoroughly after handling. P270: Do not eat, drink or smoke when using this product. P201: Obtain special instructions before use. P202: Do not handle until all safety precautions have been read and understood. P281: Use personal protective equipment as required. P271: Use only outdoors or in a well-ventilated area. P273: Avoid release to the environment.
Response	P302+P352: IF ON SKIN: Wash with plenty of water/… P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P321: Specific treatment (see on this label). P363: Wash contaminated clothing before reuse. P304+P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P337+P313: If eye irritation persists: Get medical advice/attention. P301+P312: IF SWALLOWED: Call a POISON CENTER/doctor/… if you feel unwell. P330: Rinse mouth. P312: Call a POISON CENTER/doctor if you feel unwell.
Storage	P233: Keep container tightly closed. P403: Store in a well-ventilated place. P405: Store locked up.
Disposal	P501: Dispose of contents/container to an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal.

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[18,19] 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 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™ workstation. 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. Change 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–25°C).

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

10. Change 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 instructions for use (IFU) 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.[20]

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)

Parameter	Sex	Age	n	Mean (cells/µL)	95% Range[a]
$CD4^+CD3^+$[b]	Female	18–65	57	798	470–1,298
	Male	18–65	92	702	355–1,213
$CD8^+CD3^+$[c]	Both	18–40	92	433	208–796
	Both	41–65	58	346	144–699
$CD3^+$	Both	18–65	151	1,206	688–1,955
CD4/CD8 Ratio[c]	Both	18–40	92	1.87	0.92–3.41
	Both	41–65	58	2.49	0.83–6.10

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 2 Within-sample reproducibility for BD FACSCount™ reagent parameters (five normal subjects) as absolute counts

Parameter	Mean[a](cells/µL)	n	SD as an estimate of within-sample reproducibility[b]	df[c]	CV[d]
$CD4^+CD3^+$	720	150	26	135	3.56
$CD8^+CD3^+$	473	150	17	135	3.61
$CD3^+$	1,261	150	28	135	2.21
CD4/CD8 ratio	1.76	150	0.09	135	5.06

a. Mean is the pooled mean. For example, $\overline{Y}$ = 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)

$$SD = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + \ldots (n_k - 1)s_k^2}{n_1 + n_2 + \ldots + n_k - k}}$$

s_i^2 = variance of the ith sample for $1 \leq i \leq k$

k = number of samples

n_i = 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

$$= \frac{SD}{Y} \times 100$$

Table 3 Within-sample reproducibility for BD FACSCount™ reagent parameters (nine abnormal subjects) as absolute counts

Parameter	Mean[a] (cells/µL)	Subjects	n	SD as an estimate of within-sample reproducibility[b]	df[c]	CV[d]
$CD4^+CD3^+$	484	9	81	19	54	3.91
$CD8^+CD3^+$	1,067	9	81	27	54	2.57
$CD3^+$	1,657	9	81	48	54	2.91
CD4/CD8 ratio	0.53	9	81	0.03	54	6.03

a. Mean is the pooled mean. For example, $\overline{Y}$ = 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

$$= \frac{SD}{Y} \times 100$$

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

Parameter	Instrument	Mean[a] (cells/µL)	SD[b]	CV[c]
$CD4^+CD3^+$	1	729	28	3.82
	2	715	22	3.05
	3	715	27	3.75
	instrument variation	720	0[d]	0.00

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

Parameter	Instrument	Mean[a] (cells/µL)	SD[b]	CV[c]
$CD8^+CD3^+$	1	480	18	3.80
	2	480	17	3.55
	3	460	16	3.44
	instrument variation	473	0[d]	0.00
$CD3^+$	1	1,268	27	2.16
	2	1,272	20	1.57
	3	1,242	34	2.77
	instrument variation	1261	0[d]	0.00
CD4/CD8 ratio	1	1.78	0.09	5.00
	2	1.74	0.09	5.18
	3	1.76	0.09	4.98

a. Mean is the pooled mean, for example, $\overline{Y}$ = 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)

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

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. 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)

Parameter	Mean[a] (cells/µL)	Variance Component	SD[b]	%CV[c]
$CD4^+CD3^+$	328	Within site	11.0	3.38
	325	Between site	0.0[d]	0.00
$CD8^+CD3^+$	752	Within site	28.4	3.77
	752	Between site	4.6[d]	0.62
$CD3^+$	1,080	Within site	40.2	3.73
	1,080	Between site	6.0[d]	0.56
CD4/CD8 ratio	0.337	Within site	0.0137	4.08
	0.337	Between site	0.0045[d]	1.34

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. Site component of variation estimated from an analysis of the variance model

BD FACSCount™ Reagents vs Comparative Method

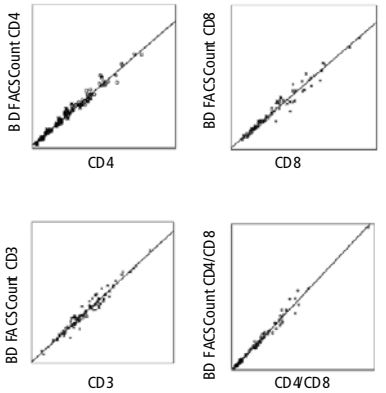
The same blood samples, from both normal and abnormal subjects, were analyzed with BD Simultest™ IMK-lymphocyte reagents (using BD Simulset™ software) on the BD FACScan™ flow cytometer, the Sysmex® NE-8000 hematology counter, and with BD FACSCount™ reagents on the BD FACSCount™ system. The BD Simultest™ 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

Parameter	Slope	Intercept	r	n	Range of Data	
					Comparative Method[a]	BD FACSCount™ Instrument
$CD4^+CD3^+$[b]	0.88	21.15	0.99	98	75–1,526	57–1,310
$CD8^+CD3^+$[c]	0.87	14.74	0.98	99	187–2,210	155–1,924
$CD3^+$[d]	0.90	50.95	0.98	101	241–3,824	232–3,457
CD4/CD8 ratio	1.03	0.01	0.99	94	0.08–6.70	0.08–6.82

a. The data was collected using the BD Simultest™ IMK-lymphocyte reagents on a BD FACScan™ flow cytometer and a Sysmex® NE-8000 hematology counter.
b. Comparative method calculated from percent $CD4^+CD3^+$ times absolute lymphocyte count (cells/μL).
c. Comparative method calculated from percent $CD8^+CD3^+$ times absolute lymphocyte count (cells/μL).
d. Comparative method calculated from the average percent $CD3^+$ times absolute lymphocyte count (cells/μL).
NOTE Six percent of samples evaluated failed quality control criteria of the comparative method. No samples analyzed at this clinical site failed the BD FACSCount™ system quality control.

Figure 1 Fitted distribution plots for the BD FACSCount™ system vs 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–25°C) 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–25°C) 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 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; the CD3 range fell within 512 and 1,815.

Repeated Use

Whole blood from 5 normal and 14 abnormal subjects was collected. Each sample was prepared and analyzed three times using the BD FACSCount™ system. Both the second and third analyses were within 10% of the first analysis.

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]

The CD4 antibody reacts with monocytes, as well as helper/inducer T lymphocytes.[22] One normal subject has been reported to have no reaction with CD4.[23] However, this lack of reactivity has not been observed in studies of over 300 subjects.[20] Because the CD3 antibody does not react with monocytes, monocytes do not interfere with the assay.

The CD8 antibody reacts with suppressor/cytotoxic T lymphocytes as well as a subset of NK lymphocytes.[24] Because the CD3 antibody does not react with NK lymphocytes, they do not interfere with the assay.

User-Reportable Ranges

The following ranges meet BD performance characteristics:

- CD4: 50 to 2,000 $CD4^+$ cells/µL
- CD8: 100 to 2,000 $CD8^+$ cells/µL
- CD3: 100 to 3,500 $CD3^+$ cells/µL

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® 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–25°C) 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.

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TROUBLESHOOTING

See Chapter 7 in the *BD FACSCount™ System User's Guide* for troubleshooting information.

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SYMBOLS GLOSSARY

SYMBOLS GLOSSARY [L006715(06) 2021-08]
Some symbols listed below may not apply to this product.
US Customers only: For symbol glossary, refer to **bd.com/symbols-glossary**

Symbol	Meaning
	Manufacturer
EC REP	Authorized representative in the European Community
CH REP	Authorised representative in Switzerland
	Date of manufacture
	Use-by date
LOT	Batch code
REF	Catalogue number
SN	Serial number
STERILE	Sterile
STERILE A	Sterilized using aseptic processing techniques
STERILE EO	Sterilized using ethylene oxide
STERILE R	Sterilized using irradiation
STERILE	Sterilized using steam or dry heat
	Do not resterilize
NON STERILE	Non-sterile
	Do not use if package is damaged and consult *instructions for use*
STERILE	Sterile fluid path
STERILE EO	Sterile fluid path (ethylene oxide)
STERILE R	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
LATEX	Contains or presence of natural rubber latex
IVD	In vitro diagnostic medical device
CONTROL -	Negative control
CONTROL +	Positive control
	Contains sufficient for *<n>* tests
	For IVD performance evaluation only
	Non-pyrogenic

Symbol	Meaning
#	Patient number
	This way up
	Do not stack
	Single sterile barrier system
PVC DEHP BBP	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	CE marking; Signifies European technical conformity
	Device for near-patient testing
	Device for self-testing
R_x Only	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
MD	Medical device
	Contains hazardous substances
	Ukrainian conformity mark
FC	Meets FCC requirements per 21 CFR Part 15
c UL us	UL product certification for US and Canada
UDI	Unique device identifier

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