

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

K081213

B. Purpose for Submission:

New Device

C. Measurand:

CD4 Lymphocyte

D. Type of Test:

Quantitative and Semi-quantitative, Flow Cytometry

E. Applicant:

BD BIOSCIENCES

F. Proprietary and Established Names:

BD FACSCCount CD4 reagents

G. Regulatory Information:

| Product Code | Classification | Regulation Section | Panel |
|--------------|----------------|--------------------|------------|
| GKZ | Class II | 864.5220 | HEMATOLOGY |

H. Intended Use:

1. Intended use(s):

BD FACSCCount 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 count and CD4 percentage). The reagents are for in vitro diagnostic use on a BD FACSCCount instrument.

2. Indication(s) for use:

Same as Intended Use.

3. Special conditions for use statement(s):

Not applicable.

4. Special instrument requirements:

BD FACSCCount CD4 Reagents are for use with BD FACSCCount Instrument.

I. Device Description:

BD FACSCCount CD4 reagents are intended for use in enumerating the absolute counts of CD4 T lymphocytes and to determine the percentage of lymphocytes that are CD4 T lymphocytes in unlysed whole blood using the BD FACSCCount instrument system. The product offers a single test that requires one convenient, ready-to-use reagent tube labeled CD4. It is intended for use on a BD FACSCCount instrument.

The reagent kit consists of the following components: 50 reagent tubes of CD4 PE/CD14 PE-Cy5/CD15 PE-Cy5 and fluorescent nucleic dye, 65 reagent tube caps, one 5-mL vial of 5% formaldehyde in phosphate-buffered saline (PBS), used as fixative solution and Package Insert.

In addition to the reagent Kit, a customer will receive four diskettes (one primary diskette and three back-up diskettes) that contain software that is used to start up and

operate the instrument. A copy of Users Guide that contains Safety Guide (translated) and Quick Reference Guide will also be provided with the software diskettes to further describe specific instruction for use of BD FACSCount system with BD FACSCount CD4 Reagents.

J. Substantial Equivalence Information:

| | | |
|---|--|--|
| Predicate | BD Tritest CD3FITC/CD4PE/CD45PerCP with and without Trucount tubes (K071143 and K071141) | |
| Describe the item being compared | | |
| <p>Predicate - BD TriTEST CD4/CD8/CD3 (Intended Use) is a three color direct immunofluorescence reagent for use with a suitably equipped flow cytometer to identify and determine the percentages and absolute counts of mature human T lymphocytes (CD3+), helper/inducer (CD3+CD4+) T lymphocytes in erythrocyte lysed whole blood. When used with BD Trucount tubes, absolute counts of these populations can be enumerated from a single tube. This BD Tritest reagent and BD Trucount tubes can be used with the BD FACSLoader. The reagent can be used with or without an isotype control.</p> <p>New Device - BD FACSCount CD4 Reagents on BD FACSCount (Intended Use) are two color direct immunofluorescence reagent for identifying and determining absolute counts in cells/μL and percentage of CD4+ T lymphocytes in unlysed whole blood.</p> | | |
| Similarities | | |
| | BD TriTEST CD3/CD4/CD45 with and without BD Trucount absolute count tubes on BD FACSCalibur | BD FACSCount CD4 Reagents on BD FACSCount (New) |
| Sample type | Whole blood preserved with EDTA, heparin, or ACD-Solution A | Whole blood preserved with EDTA only |
| System electronics | Analog | Same. |
| Sample Analysis | Automatic analysis. User is able to adjust the gating to optimize. | Automatic analysis with no user intervention |
| Differences | | |
| | BD TriTEST CD3/CD4/CD45 with and without BD Trucount absolute count tubes on BD FACSCalibur | BD FACSCount CD4 Reagents on BD FACSCount (New) |
| Reagent | BD TriTEST CD3FITC/CD4PE/CD45PerCP | BD FACSCount CD4 Reagents CD4PE/CD14PE-Cy5/CD15PECy5/fluorescent nuclear dye |
| Absolute count beads | Trucount Absolute Count beads | Known number of reference beads included in reagent |
| Control Beads | None | BD FACSCount Controls (low/mid/high) |
| Dynamic Range | 68 - 7.2×10^3 cells/ μ L CD4/CD3 positive cells | CD4 absolute count of: 50-5000cells/ μ L CD4 percentage of : 5-65% |

K. Standard/Guidance Document Referenced (if applicable):

| |
|--|
| STANDARDS |
| Title and Reference Number |
| Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (EP09-A2) |
| How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline - Second Edition (C28-A2) |
| Stability Testing of In Vitro Diagnostic Reagents (13640) |
| Other Standards |

| GUIDANCE | | | |
|--|--------|----------|---|
| Document Title | Office | Division | Web Page |
| Class II Special Controls Guidance Document: Premarket Notifications for Automated Differential Cell Counters for Immature or Abnormal Blood Cells; Final Guidance for Industry and FDA | OIVD | DIHD | http://www.fda.gov/cdrh/ode/guidance/1184.html |
| Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices - Guidance for Industry and FDA Staff | ODE | | http://www.fda.gov/cdrh/ode/guidance/337.html |

L. Test Principle:

The single tube assay is performed by staining the specimen with the ready-to-use BD FACSCount CD4 reagents. 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 are exposed to the laser light, which causes the fluorochrome-labeled and fluorescently dyed cells to fluoresce. This fluorescent light provides the information necessary for the instrument to identify and count the CD4 lymphocytes and other (CD4 negative) lymphocytes. With the fluorescent nuclear dye bound to all nucleated blood cells, CD14 and CD15 conjugates in the reagent move granulocytes and monocytes away from the CD4 positive and negative lymphocytes population. The reagent tube also contains a known number of fluorescent reference beads, which are used as a fluorescence and quantitation standard for calculating the absolute count of CD4 positive lymphocytes. A precise volume of whole blood is stained directly in the reagent tube, and the results are provided automatically.

The processed sample is introduced to the FACSCount instrument and transported by the sheath fluid to the flow cell as a narrow sample stream. Therein, the elements of the sample, including cells, beads, and debris, are exposed to a focus laser beam. The exposure causes the sample elements to emit fluorescent light, which is collected and processed by the system.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The design of this study was based on the CLSI Guideline, EP5-A2, *Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline*. The within-run standard deviation (SD) and coefficient of variation (CV), and the With-in device

SD and CV of the following measurements were computed for:

- CD4 absolute count
- CD4 percentage

Precision of the CD4 absolute count and CD4 percentage was characterized as follows:

- Three BD FACSCount instruments and three operators were used.
- BD Multi-Check Control and Multi-Check CD4 Low control were stain in duplicate with BD FACSCount CD4 reagent.
- Two separate runs per day for a period of 21 days.

Within-device and within-run precision of CD4 absolute counts (cells/ μ L)

| | Low control CV (cell/ μ L) | Normal control CV (cells/ μ L) |
|---------------|-----------------------------------|---------------------------------------|
| Within device | 4.82 | 4.28 |
| Within run | 4.04 | 3.46 |

Within-device and within-run precision of CD4 percentage (cells/ μ L)

| | Low control CV (cell/ μ L) | Normal control CV (cells/ μ L) |
|---------------|-----------------------------------|---------------------------------------|
| Within device | 0.38 | 1.28 |
| Within run | 0.35 | 1.15 |

BD FACSCount CD4 reagents met acceptance criteria of precision study and demonstrated acceptable precision performance.

b. Linearity/assay reportable range:

One set with eleven artificially-prepared normal blood specimens with equally spaced CD4+ T-lymphocyte concentrations was used in the linearity evaluation. The study was designed to cover the entire reportable range of 50 - 5000 cells/ μ L. The artificially prepared 11 blood samples with different CD4+ T-lymphocyte concentrations were stained in 3 replicates with FACSCount CD4 reagents and acquired for evaluating assay linearity on three BD FACSCount instruments with BD FACSCount CD4 Clinical software. The linearity range for the FACSCount CD4 reagent was 37-5812 cells/ μ L for CD4 absolute count.

Summary of linearity evaluation resulted in linear ranges

| | Actual Lower Limit of Linear Range | Actual Upper Limit of Linear Range | Average Coefficient of Determination (R^2) |
|-----------------------|---------------------------------------|---------------------------------------|---|
| CD4 absolute count | 37 cells/ μ l | 5812 cells/ μ l | 0.997 |

Linearity was assessed and observed to be linear within the reportable CD4+ absolute count range (50 – 5000 cells/ μ L).

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

A study was performed to generate product claim data for stability of whole blood specimens and stained samples. Specimens were collected at the 2 external sites, and specimen that met protocol requirements were included in the analysis. Each sample was collected in duplicates for each time point of T0/T0, T24/T0, T24/T24, T0/T48 and T24/T48. The protocol was established to cover the analytical range. The FACSCCount CD4 reagents met the acceptance criteria of Age of Blood and Age of Stain study and demonstrated acceptable performance up to T24/T48. The Age of Blood claim will be 24 hours, and Age of Stain claim will be 48 hours in the product labeling.

- d. *Detection limit:*
Not applicable.
 - e. *Analytical specificity:*
Not applicable.
 - f. *Assay cut-off:*
Not applicable.
2. Comparison studies:
- a. *Method comparison with predicate device:*
A comparison study was performed at 3 sites to demonstrate an agreement of the test method to the predicate by using a comparative evaluation of the same specimens stained in parallel with the BD FACSCCount CD4 Reagents and the predicate. (Using lyse/ no-wash preparation procedure for predicate and no-lyse/no-wash procedure for test method, according to each product package insert). Samples were stained within 24 hours of venipuncture and analyzed within 24 hours of staining. System configuration: **Test:** BD FACSCCount Flow Cytometer with BD FACSCCount CD4 clinical software v1.0 with BD FACSCCount CD4 Reagents, **Predicate:** BD FACSCalibur flow cytometer with Multiset software with BD TriTEST CD3/CD4/CD45 reagents with Trucount absolute count tubes.

Four discrete bins were identified to provide an even distribution of data across the range for CD4. See below:

| Bin | Range of CD4 absolute count |
|------------|------------------------------------|
| 1 | >=50 to =< 250 cells/μL |
| 2 | >250 to <=750 cells/μL |
| 3 | >750 to <=1200 cells/μL |
| 4 | >1200 to <=5000 cells/μL |
| Bin | Range of CD4 percentage |
| 1 | >=5% to =< 25% |
| 2 | >25 to <=65% |

Statistical analyses were performed based on the recommendations of CLSI document *EP9-A2: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition. Vol. 22, No. 19, 2002.*

Summary of Method Comparison study results:

| Parameter | n | R ² | Slope | Intercept |
|-------------------------------|-----|----------------|-------|-----------|
| CD4 Absolute Count (cells/μL) | 101 | 0.981 | 0.971 | 12.695 |
| CD4 Percentage (%) | 99 | 0.99 | 0.999 | -0.391 |

BD FACSCCount CD4 Reagents met the acceptance criteria of Method Comparison study and demonstrated acceptable agreement with Predicate device

- b. *Matrix comparison:*
Not applicable.
- 3. Clinical studies:
 - a. *Clinical Sensitivity:*
Not applicable.
 - b. *Clinical specificity:*
Not applicable.
 - c. Other clinical supportive data (when a. and b. are not applicable):
Not applicable.
- 4. Clinical cut-off:
Not applicable.
- 5. Expected values/Reference range:
The reference ranges for BD FACSCCount CD4 reagents shown in below were determined at BD Biosciences in San Jose, CA. The 141 Subjects were healthy adults between the ages of 18 and 65 years.

Representative reference ranges for BD FACSCCount CD4 reagents.

| Parameter | N | Mean | 95% Reference Range |
|-------------------------|-----|--------|---------------------|
| Absolute CD4 (cells/μL) | 141 | 906.65 | 380-1704 |
| Percent CD4 | 141 | 44.90 | 30.13-60.23 |

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.