

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k060375

B. Purpose for Submission:

New Device

C. Measurand:

T, B, and natural killer (NK) cells as well as the CD4 and CD8 subpopulations of T cells

D. Type of Test:

Quantitative and Semi-quantitative flow cytometric assay

A. Applicant:

BD BIOSCIENCES

F. Proprietary and Established Names:

BD Multitest 6-Color TBNK Reagent with BD Trucount Tubes

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
GKZ	Class II	864.5220 Automated Differential Cell Counter	Hematology (81)

H. Intended Use:

1. Intended use(s):

BD Multitest 6-Color TBNK reagent with BD Trucount tubes is intended for in vitro diagnostic use with the BD FACSCanto system to identify and determine the percentages and absolute counts of T, B, and natural killer (NK) cells as well as the CD4 and CD8 subpopulations of T cells in peripheral blood.

2. Indication(s) for use:

For use with the BD FACSCanto flow cytometer.

For use with whole blood collected in K3 EDTA tubes.

For use in the identification and determination of percentages and absolute counts of T, B, and natural killer (NK) cells as well as the CD4 and CD8 subpopulations of T cells in peripheral blood.

For in vitro diagnostic use.

3. Special conditions for use statement(s):

Not Applicable

4. Special instrument requirements:

BD Multitest 6-Color TBNK reagent is for use with the BD FACSCanto system.

I. Device Description:

BD Multitest 6-color TBNK reagent with BD Trucount tubes is packaged in a kit consisting of one vial containing sufficient reagent to yield 50 tests, and two pouches, each containing 25 BD Trucount tubes. One BD Trucount tube is required for each test, and each tube is for single-use only.

The BD Multitest 6-color TBNK reagent is provided in 1 mL of buffered saline with 0.1% sodium azide. The following antibodies comprise the reagent:

Antibody	Clone	Fluorochrome
CD4	SK3	Phycoerythrin-Cyanine 7 (PE-Cy7)
CD8	SK1	Allophycocyanin-Cyanine 7 (APC-Cy7)
CD3	SK7	Fluorescein Isothiocyanate (FITC)
CD19	SJ25C1	Allophycocyanin (APC)
CD16+CD56	B73.1 / NCAM16.2	Phycoerythrin (PE)
CD45	2D1 (HLe-1)	Peridinin chlorophyll protein – Cyanine 5.5 (PerCP-Cy5.5)

BD Trucount tubes each contain a freeze-dried pellet of fluorescent beads held by a metal retainer at the bottom of the tube.

J. Substantial Equivalence Information:

Predicate	Item	Similarities	Differences
K980858 - BD Multitest IMK Kit with BD Trucount Tubes	Intended Use	Identification and determination of percentages and absolute counts of the following mature human lymphocyte subsets in erythrocyte-lysed whole blood: T lymphocytes (CD3+, CD3+CD4+, and CD3+CD8+), B lymphocytes (CD3-CD19+), and NK lymphocytes (CD3-CD16+CD56+)	Same
K980858 - BD Multitest IMK Kit with BD Trucount Tubes	Components (including Cluster Designation, Conjugation, and Clone)	<p>BD Multitest CD3/CD16+CD56/CD45/CD19 (1 vial, 50 tests) including CD3 FITC (SK7), CD16 PE (B73.1) + CD56 PE (NCAM16.2), CD45 PerCP (2D1), CD19 APC (SJ25C1), and buffer with 0.1% sodium azide</p> <p>BD Multitest CD3/CD8/CD45/CD4 (1 vial, 50 tests) including CD3 FITC (SK7), CD8 PE (SK1), CD45 PerCP (2D1), CD4 APC (SK3), and buffer with 0.1% sodium azide</p> <p>BD FACS Lysing Solution</p> <p>BD Trucount Tubes (100 tubes)</p>	<p>BD Multitest 6-Color TBNK Reagent (1 vial, 50 tests) including CD4 PE-Cy7 (SK3), CD8 APC-Cy7 (SK1), CD3 FITC (SK7), CD19 APC (SJ25C1), CD16 PE (B73.1) + CD56 PE (NCAM16.2), CD45 PerCP-Cy5.5 (2D1), and buffer with 0.1% sodium azide</p> <p>BD FACS Lysing Solution (not included with the reagent)</p> <p>BD Trucount Tubes (50 tubes)</p>
K980858 - BD Multitest IMK Kit with BD Trucount Tubes	Specificity	Specificities of antibodies have been verified by the International Workshop on Human Leukocyte Differentiation Antigens.	Same.
K980858 - BD Multitest IMK Kit with BD Trucount Tubes	Method to Identify Populations of Interest	Lyse/no-wash method using a two-tube panel with four-color antibody reagents (one tube stained with CD3/CD16+CD56/CD45/CD19, the other with CD3/CD8/CD45/CD4) to identify lymphocytes with specific cell-surface antigens, fluorescence triggering, and CD45 vs. SSC for gating. Uses fluorescent beads to quantify absolute counts	Same, except uses a one-tube panel with six-color antibody reagent

Predicate	Item	Similarities	Differences
K980858 - BD Multitest IMK Kit with BD Trucount Tubes	Control	Recommend use of commercially available whole blood control with established values for subset percentages and absolute counts	Recommend use of two levels of commercially available whole blood controls with established values for subset percentages and absolute counts. BD specifically recommends the use of BD Multi-Check and BD Multi-Check CD4 Low controls
K041074 - BD FACSCanto System with BD FACSCanto Clinical Software	Instrument and Software	BD FACSCanto flow cytometer with BD FACSCanto clinical software version 1.0	Same, except uses version 2.0 of BD FACSCanto clinical software
K041074 - BD FACSCanto System with BD FACSCanto Clinical Software	System Setup	BD FACSCanto flow cytometer with BD FACSCanto clinical software version 1.0 and BD FACS 7-color setup beads	Same, except uses version 2.0 of BD FACSCanto clinical software
K980858 - BD Multitest IMK Kit with BD Trucount Tubes	Sample and Stain Stability	Anticoagulated blood stored at room temperature (20–25°C) must be stained within 48 hours of draw and then analyzed within 24 hours of staining	Anticoagulated blood stored at room temperature (20–25°C) must be stained within 24 hours of draw and then analyzed within 6 hours of staining
K980858 - BD Multitest IMK Kit with BD Trucount Tubes	Results	CD3+, CD3+CD4+, and CD3+CD8+ T lymphocytes; CD3-CD19+ B lymphocytes, and CD3-CD16+CD56+ NK lymphocytes expressed as percentages of total lymphocytes or as absolute counts (cells/ μ L) in whole blood	Same
K980858 - BD Multitest IMK Kit with BD Trucount Tubes	Sample Type	Erythrocyte-lysed whole blood, collected in K3 EDTA blood collection tubes	Same

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

STANDARDS
Title and Reference Number
Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (EP09-A2)
Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline (EP5-A)
How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline - Second Edition (C28-A2)

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
Guidance for Industry and FDA Staff; Replacement Reagent and Instrument Family Policy	OIVD		http://www.fda.gov/cdrh/oivd/guidance/950.html

L. Test Principle:

The BD FACSCanto system for six-color immunophenotyping consists of a BD FACSCanto flow cytometer, BD Multitest 6-color TBNK reagent, BD Trucount tubes, a Windows-based PC workstation, BD FACSCanto clinical software version 2.0 for automated acquisition and analysis.

Determining lymphocyte subset percentages and absolute counts requires: (1) obtaining a whole blood sample; (2) cell-surface antigen staining with the six-color monoclonal antibody reagent in a BD Trucount tube; (3) erythrocyte lysis; and (4) flow cytometric acquisition and analysis of list mode data.

When whole blood is added to the reagent, the fluorochrome-labeled antibodies in the reagent bind specifically to leukocyte surface antigens. During acquisition, the cells travel past the laser beam and scatter the laser light. The stained cells fluoresce. These scatter and fluorescence signals, detected by the instrument, provide information about the cell's size, internal complexity, and relative fluorescence intensity. BD Multitest reagents employ fluorescence triggering, allowing direct fluorescence gating of the lymphocyte population to reduce contamination of unlysed or nucleated red blood cells in the gate. A precise volume of sample is stained directly in a BD Trucount tube. The lyophilized pellet in the tube dissolves, releasing a known number of fluorescent beads. During analysis, the absolute number (cells/ μ L) of positive cells in the sample can be determined by comparing cellular events to bead events. BD FACSCanto clinical software (version 2.0 or higher) determines absolute counts.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision was determined by running duplicate measurements of commercially available control at different levels of analyte concentration twice a day for a period of 21 operating days on three different instruments.

Standard deviations with 95% confidence limits were calculated for the within-run precision and the total precision of the lymphocyte subset percentages.

Percent CVs with 95% confidence limits were calculated for the within-run precision and the total precision of the lymphocyte subset percentage.

Repeatability of Lymphocyte Subset Percentages

Lymphocyte Subset	N	Low Sample SD	Normal Sample SD
CD4	42	0.64	0.95
CD8	42	1.07	0.65
CD3	42	1.17	0.86
CD19	42	0.89	0.62
CD16+CD56	42	0.90	0.61

Within-Device Precision for Lymphocyte Subset Percentages

Lymphocyte Subset	n	Low Sample SD	Normal Sample SD
CD4	42	0.69	1.23
CD8	42	1.29	0.81
CD3	42	1.23	0.90
CD19	42	0.89	0.62
CD16+CD56	42	0.96	0.62

Repeatability of Lymphocyte Subset Absolute Counts

Lymphocyte Subset	n	Low Sample %CV	Normal Sample %CV
CD4	42	7.6	4.7
CD8	42	4.1	4.7
CD3	42	4.0	4.2
CD19	42	5.7	5.3
CD16+CD56	42	7.0	7.9

Within-Device Precision of Lymphocyte Subset Absolute Counts

Lymphocyte Subset	n	Low Sample %CV	Normal Sample %CV
CD4	42	8.0	4.8
CD8	42	5.0	5.4
CD3	42	4.4	4.2
CD19	42	6.0	5.7
CD16+CD56	42	8.0	7.9

b. Linearity/assay reportable range:

Three whole blood samples were diluted with autologous plasma to known dilutions. High and low pools were created to span the expected CD4+ range of 200 to 3,000 cells/ μ L. Three replicates at each level were stained and acquired for each specimen. All samples from each specimen were acquired on the same day. Two BD FACSCanto instruments were used in this study. See linear ranges below:

Lymphocyte Subset	Linear Range (cells/ μ L)	R ²
CD4	4 – 2,234	0.998
CD8	158 – 1,125	0.991
CD3	498 – 3,356	0.996
CD19	71 – 447	0.989
CD16+CD56	0 – 1,559	0.999

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

Not applicable.

d. Detection limit:

Analytical limits at low levels were validated by accuracy, precision, and linearity studies.

e. Analytical specificity:

Not applicable.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

Whole blood samples (117) were collected and analyzed at two clinical evaluation sites. Lymphocyte subset percentage and absolute counts were enumerated with BD Multitest 6-color TBNK reagent in BD Trucount tubes and analyzed on the BD FACSCanto flow cytometer using BD FACSCanto clinical software. The results were compared with results from the BD Multitest™ IMK kit with BD Trucount tubes, and also were analyzed on the BD FACSCanto flow cytometer using BD FACSCanto clinical software. The results are as follows:

Lymphocyte Subset	n	Unit	R2	Slope	Intercept	Range
CD3+ CD4+	117	cells/μL	.995	0.965	6.0	4–1,593
		%	.998	1	0.0423	1–67
CD3+CD8+	117	cells/μL	.994	0.956	7.01	51–2,146
		%	.996	0.983	0.00592	11–83
Total CD3+	117	cells/μL	.995	0.968	13.5	107–3,403
		%	.996	0.985	0.895	34–88
CD3–CD19	117	cells/μL	.992	0.973	6.97	1–1,207
		%	.993	0.999	0.33	0–36
CD3–(CD16+ CD56)+	117	cells/μL	.99	0.98	-0.291	7–918
		%	.992	0.985	0.0603	2–51

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:

A total of 123 donor samples were collected and evaluated. Samples with an even gender distribution and representative of a healthy adult population – between the ages of 18 and 65 years – were tested and used to establish the lymphocyte subset reference intervals. Each sample was stained in duplicate, and the averaged values of the duplicates were used in determining the reference interval.

Reference Intervals for Lymphocyte Subset Percentages

Lymphocyte Subset	n	Mean (%)	95% Reference Interval (%)
CD4	123	46.4	28.2 – 62.8
CD8	123	24.0	10.2 – 40.1
CD3	123	71.1	49.1 – 83.6
CD19	123	14.9	6.5 – 27.0
CD16+CD56	123	11.7	4.2 – 25.2

Reference Intervals for Subset Absolute Counts

Lymphocyte Subset	n	Mean (cells/ μ L)	95% Reference Interval (cells/ μ L)
CD4	123	1106	441 – 2156
CD8	123	583	125 – 1312
CD3	123	1705	603 – 2990
CD19	123	354	107 – 698
CD16+CD56	123	266	95 – 640

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.