

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

**A. 510(k) Number:**

k090967

**B. Purpose for Submission:**

Modified 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

**E. Applicant:**

BD BIOSCIENCES

**F. Proprietary and Established Names:**

BD Multitest 6-Color TBNK Reagent with **optional** BD Trucount Tubes

**G. Regulatory Information:**

1. Regulation section:  
21 CFR 864.5220, Automated cell counter
2. Classification:  
Class II
3. Product code:  
GKZ, Automated differential cell counter
4. Panel:  
Hematology (81)

**H. Intended Use:**

1. Intended use(s):  
BD Multitest 6-color TBNK reagent with optional BD Trucount™ tubes is a six-color direct immunofluorescence reagent for use with the BD FACSCanto™ and BD FACSCanto™ II flow cytometers 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.  
BD Multitest 6-color TBNK reagent and BD Trucount tubes can be used with the BD FACS™ Loader.
2. Indication(s) for use:  
Same as Intended Use
3. Special conditions for use statement(s):  
For Prescription Use Only
4. Special instrument requirements:  
BD Multitest 6-Color TBNK reagent is for use with the BD FACSCanto™ and BD FACSCanto™ II systems.

**I. Device Description:**

BD Multitest 6-Color TBNK Reagent is a monoclonal antibody cocktail provided in 1 mL of buffered saline with 0.1% sodium azide and sufficient for 50 tests. It contains FITC-labeled CD3, clone SK7; PE-labeled CD16, clone B73.1 and CD56, clone NCAM 16.2; PerCP-Cy™5.5-labeled CD45, clone 2D1 (HLe-1); PE-Cy™7-labeled

CD4, clone SK3; APC-labeled CD19, clone SJ25C1; and APC-Cy7-labeled CD8, clone SK1.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
BD Multitest 6-Color TBNK Reagent with Trucount Tubes
2. Predicate K number(s):  
K060375
3. Comparison with predicate:

<b>Similarities</b>		
<i>Item</i>	<i>BD Multitest 6-Color TBNK Reagent with Optional Trucount Tubes</i>	<i>BD Multitest 6-Color TBNK Reagent with Trucount Tubes</i>
Intended Use	BD Multitest 6-color TBNK reagent with <b>optional</b> BD Trucount™ tubes is a six-color direct immunofluorescence reagent for use with the BD FACSCanto™ family of flow cytometers 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. BD Multitest 6-color TBNK reagent and BD Trucount tubes can be used with the BD FACST™ Loader.	Same
Clinical Application	Determining percentages or counts of CD3+CD4+ lymphocytes can be useful in monitoring human immunodeficiency virus (HIV)-infected individuals. Individuals with HIV typically exhibit a steady decrease of CD3+CD4+ lymphocyte counts as the infection progresses. CD3+CD4+ percentages or counts and total T and B lymphocytes are used to characterize and monitor some forms of immunodeficiency and autoimmune disease. NK lymphocytes identified as CD3- and CD16+ and/or CD56+ have been shown to mediate cytotoxicity against certain tumors and virus-infected cells. NK-mediated cytotoxicity does not require class I or class II major histocompatibility complex (MHC) molecules to be present on the target cells.	Same
Sample Type	Whole Blood	Same
Principles of Procedure	Monoclonal antibody cocktail staining of whole blood. Acquisition on a flow cytometer. Determination of lymphocyte population percentages.	Same
Reagent	BD Multitest 6-Color TBNK Reagent consisting of CD3-FITC/ CD16-PE + CD56-PE/ CD45-PE-Cy5.5/ CD4-PE-Cy7/ CD19-APC/ CD8-APC-Cy7 in 1 ml of buffered saline with 0.1% sodium azide	Same
Storage and Handling	Monoclonal antibody reagent: <ul style="list-style-type: none"> <li>• Store at 2-8°C</li> <li>• Stable opened or unopened until expiration date</li> <li>• Do not use after expiration date</li> <li>• Do not freeze or expose to direct light during storage or incubation</li> </ul>	Same
Instrumentation	BD FACSCanto or BD FACSCanto II	Same
Specimen Collection	Collect blood in EDTA BD Vacutainer™ tubes	Same

<b>Similarities</b>		
<b>Item</b>	<b>BD Multitest 6-Color TBNK Reagent with Optional Trucount Tubes</b>	<b>BD Multitest 6-Color TBNK Reagent with Trucount Tubes</b>
	Minimum volume = 50 uL Must be stained within 24 hours of draw and must be analyzed within 6 hours of staining	
Quality Control	BD recommends running two levels of liquid control material (procedural control), processed like patient samples, run at least once per testing day	Same
Limitations	1) Labs must establish their own reference ranges or intervals 2) Reagent has not been validated by BD for use with heparin or acid citrate dextrose (ACD) anticoagulants 3) Not intended for screening for leukemic cells or phenotyping leukemic patients 4) Absolute counts not comparable between labs using different manufacturer's equipment.	Same

<b>Differences</b>		
<b>Item</b>	<b>BD Multitest 6-Color TBNK Reagent with Optional Trucount Tubes</b>	<b>BD Multitest 6-Color TBNK Reagent with Trucount Tubes</b>
Principles of Procedure	Staining in a 12x75 mm tube. Manual entry of lymphocyte data from another method used to calculate lymphocyte population absolute counts (cells/uL).	Staining in a BD Trucount tube (a 12x75 mm tube containing a lyophilized bead pellet). Beads are used to calculate lymphocyte population absolute counts (cells/uL)
Storage and Handling	No Trucount tubes used	Trucount tubes: Store at 2-25°C, keep dry
Software	BD FACSCanto clinical software (minimum version 2.4)	BD FACSCanto clinical software (minimum version 2.0)
Results	1) Lymphocyte population <b>percentages</b> (T, B, NK, CD4+ T cells, CD8+ T cells)  2) Lymphocyte population <b>absolute counts</b> (cells/uL). (If lymphocyte data from another method is manually entered)	1) Same  2) Lymphocyte population <b>absolute counts</b> (cells/uL)

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

CLSI EP5-A2: *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition*

CLSI EP9-A2 *Method Comparison and Bias Estimation Using Patient Samples; Approved Standard-Second Edition*

ISO 14971: "Medical Devices - Application of Risk Management to Medical Devices"

Deciding When to Submit a 510(k) for a Change to an Existing Device (K97-1)

A New 510(k) Paradigm - Alternate Approaches to Demonstrating Substantial Equivalence in Premarket Notifications

Guidance for the Content of Premarket Submissions for Software Contained in

## Medical Devices - Guidance for Industry and FDA Staff

### 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

#### **L. Test Principle:**

When whole blood is added to the reagent, the fluorochrome-labeled antibodies in the reagent bind specifically to leucocyte surface antigens. The stained samples are treated with BD FACST<sup>™</sup> Lysing Solution to lyse erythrocytes prior to acquisition and analysis on the BD FACSCanto or BD FACSCanto II flow cytometer. During acquisition, the cells travel past two spatially-separated laser beam and scatter the laser light. The stained cells fluoresce. These scatter and fluorescence signals, detected by the flow cytometer, provide information about the cell's size, internal complexity, and relative fluorescence intensity. During analysis by BD FACSCanto clinical software, the lymphocyte population percentages are determined. Lymphocyte population absolute counts may be determined if lymphocyte data from another method is manually entered. BD Multitest reagents employ fluorescence triggering, allowing direct fluorescence gating of the lymphocyte population<sup>10-12</sup> to reduce contamination of unlysed or nucleated red blood cells in the gate.

When BD Trucount tubes are used, the lyophilized pellet in the tube dissolves, releasing a known number of fluorescent beads. During analysis, the absolute number (cells/ $\mu$ L) of positive cells in the sample can be determined by comparing cellular events to bead events. Then BD FACSCanto clinical software (minimum v2.0) determines absolute counts.

When BD Trucount tubes are not used, samples must be stained in 12 x75-mm BD Falcon<sup>™</sup> polystyrene tubes or equivalent. BD FACSCanto clinical software (minimum v2.4) can also determine absolute counts if lymphocyte data from another method is provided.

#### **M. Performance Characteristics (if/when applicable):**

##### 1. Analytical performance:

###### a. *Precision/Reproducibility:*

Precision was determined by running commercially available control samples stained with BD Multitest 6-Color TBNK Reagent on both the BD FACSCanto and BD FACSCanto II flow cytometers. Each control sample was measured in duplicate during two runs per day, over a span of 21 days, using three of each instrument type, and three rotating operators. Each control sample was analyzed separately with the following response variables:

- CD3+ T lymphocyte subset percentage
- CD4+ T lymphocyte subset percentage
- CD8+ T lymphocyte subset percentage
- CD16+CD56+ NK lymphocyte subset percentage
- CD19+ B lymphocyte subset percentage

For each lymphocyte subset percentage, the acceptance criterion for the study was total within-device standard deviation (SD)  $\leq 2.5$  with upper 95% confidence

limits  $\leq 2.5$  (they were  $< 2.0$ ). In addition, all analyzed components of total precision (including day, between-run and within-run precision) had upper 95 % confidence limits for SD  $< 2.0$ . All precision results met the study acceptance criteria.

- b. *Linearity/assay reportable range:*  
Not applicable.
  - c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*  
Not applicable.
  - 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 to determine the method bias between the systems. Sixty two (62) surplus or prospectively procured samples were collected from two sites. Three discrete bins were identified to provide even distribution of data across the range of CD4. A minimum of 10 and a maximum of 20 samples were collected per bin. Of the 62 samples, 52 met the bin classification criteria. See bin ranges below:

Bins	Range
1	$\geq 1\% - <15\%$
2	15% - 30%
3	$>30\%$

Accuracy was assessed by analyzing the bias between the device and the predicate system. The overall mean bias was determined for lymphocyte subset percentages on each instrument. A 95% confidence interval was conducted and subjected to the acceptance criteria.

**Mean Bias and the 95 % Confidence Interval for the BD FACSCanto.**

Lymphocyte Subset Percentage	Mean Bias	Standard Deviation of Bias	n	Lower Confidence Limit of Mean Bias	Upper Confidence Limit of Mean Bias	Criteria
CD3	-0.26	1.92	52	-0.71	0.18	$\pm 10.00$
CD4	0.01	1.15	52	-0.26	0.27	$\pm 3.00$
CD8	0.52	4.60	52	-0.55	1.59	$\pm 10.00$
CD16+CD56	0.13	0.93	52	-0.08	0.35	$\pm 3.00$
CD19	-0.02	0.84	52	-0.22	0.17	$\pm 3.00$

**Mean Bias and the 95 % Confidence Interval for the BD FACSCanto II**

Lymphocyte Subset Percentage	Mean Bias	Standard Deviation of Bias	n	Lower Confidence Limit of Mean Bias	Upper Confidence Limit of Mean Bias	Criteria
CD3	-0.21	2.48	52	-0.78	0.37	±10.00
CD4	-0.01	1.22	52	-0.38	0.19	±3.00
CD8	1.41	3.63	52	0.56	2.25	±10.00
CD16+CD56	0.03	1.06	52	-0.21	0.28	±3.00
CD19	-0.17	0.91	52	-0.39	0.04	±3.00

**Regression analysis on samples that met the bin criteria**

Instrumentation	Lymphocyte Subset	n	Unit	r <sup>2</sup>	Slope	Intercept
BD FACSCanto	Total CD3 <sup>+</sup>	52	%	0.988	1.000	-0.173
	CD3 <sup>+</sup> CD4 <sup>+</sup>	52	%	0.995	0.979	0.567
	CD3 <sup>+</sup> CD8 <sup>+</sup>	52	%	0.992	0.989	0.542
	CD3 <sup>-</sup> (CD16+CD56) <sup>+</sup>	52	%	0.988	0.997	0.178
	CD3 <sup>-</sup> CD19 <sup>+</sup>	52	%	0.989	1.037	-0.553
BD FACSCanto II	Total CD3 <sup>+</sup>	52	%	0.982	1.012	-0.919
	CD3 <sup>+</sup> CD4 <sup>+</sup>	52	%	0.994	0.996	-0.001
	CD3 <sup>+</sup> CD8 <sup>+</sup>	52	%	0.993	1.006	0.31
	CD3 <sup>-</sup> (CD16+CD56) <sup>+</sup>	52	%	0.986	1.034	-0.485
	CD3 <sup>-</sup> CD19 <sup>+</sup>	52	%	0.985	0.985	0.039

All accuracy results for lymphocyte subset percentages for both BD FACS Canto and BD FACSCanto II met the acceptance criteria. The regression statistics show that the slopes are close to 1, the intercepts are within assay imprecision as they are less than the within-device precision criteria of 2.5 and the r<sup>2</sup> values are greater than 0.95.

- 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 intervals for BD Multitest 6-color TBNK reagent with BD

Trucount tubes shown in the following table were determined at BD Biosciences in San Jose, CA. Subjects were healthy adults between the ages of 18 and 65 years.

Representative reference intervals for BD Multitest 6-color TBNK

Lymphocyte Subset	n	Mean (%)	95% Range
CD3 <sup>+</sup> CD4 <sup>+</sup>	123	46.4	28.2–62.8
CD3 <sup>+</sup> CD8 <sup>+</sup>	123	24.0	10.2–40.1
Total CD3 <sup>+</sup>	123	71.1	49.1–83.6
CD3 <sup>-</sup> CD19 <sup>+</sup>	123	14.9	6.5–27.0
CD3 <sup>-</sup> (CD16 <sup>+</sup> + CD56) <sup>+</sup>	123	11.7	4.2–25.2
Lymphocyte Subset	n	Mean (cells/ $\mu$ L)	95% Range
CD3 <sup>+</sup> CD4 <sup>+</sup>	123	1,106	441–2,156
CD3 <sup>+</sup> CD8 <sup>+</sup>	123	583	125–1,312
Total CD3 <sup>+</sup>	123	1,705	603–2,990
CD3 <sup>-</sup> CD19 <sup>+</sup>	123	354	107–698
CD3 <sup>-</sup> (CD16 <sup>+</sup> + CD56) <sup>+</sup>	123	266	95–640

It is recommended that laboratories must establish their own normal reference intervals for the BD Multitest 6-color TBNK reagent parameters that can be affected by gender of patient, age of patient, and preparative technique. Race of patient and individual variations of epitope expression can also have an effect, although sufficient data is not available to establish this. Age, gender, clinical characteristics, and race of patients should be known when a reference range is determined.

**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.