

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

A. 510(k) Number:

k081526

B. Purpose for Submission:

New Device

C. Measurand:

Lymphocytes CD3+, CD3+CD4+, CD3+CD8+

D. Type of Test:

Quantitative and Qualitative, Flow Cytometry

E. Applicant:

Beckman Coulter, Inc.

F. Proprietary and Established Names:

Flow CARE™ TLG Reagent

G. Regulatory Information:

1. Regulation section:
21 CFR 864.5220, Automated Differential Cell Counter
2. Classification:
Class II
3. Product code:
GKZ, Counter, Differential Cell
4. Panel:
81 Hematology

H. Intended Use:

1. Intended use(s):
The FlowCARE TLG Reagent kit combines four fluorescent labeled monoclonal antibodies in a single reagent formulation. It is intended “For In Vitro Diagnostic Use” for the enumeration of CD3+, CD3+CD4+ and CD3+CD8+ absolute cell count and CD3+, CD3+CD4+ and CD3+CD8+ lymphocyte percentage in combination with a White Blood Cell (WBC) Count from a hematology instrument as a dual platform measurement, or independently when used in combination with Flow-Count™ Fluorospheres as a single platform measurement.

The FlowCARE TLG Reagent is designed for use on the COULTER® EPICS™ XL™/XL-MCL™ or a suitably equipped flow cytometer with a 488 nm laser capable of detecting light scatter (forward and side) and a minimum of four-color fluorescence emission detectable in the following ranges: 504 - 541 nm, 568 – 590 nm, 610 – 635 nm, and 660 – 680 nm. Users should refer to the manufacturer’s instrument manuals for specific instructions for setting PMT voltages and fluorescence compensation prior to analysis.

2. Indication(s) for use:
Same as Intended Use.
3. Special conditions for use statement(s):
Not applicable.
4. Special instrument requirements:

For use on the COULTER® EPICS™ XL™/XL-MCL™ or a suitably equipped flow cytometer with a 488 nm laser capable of detecting light scatter (forward and side) and a minimum of four-color fluorescence emission detectable in the following ranges: 504 - 541 nm, 568 – 590 nm, 610 – 635 nm, and 660 – 680 nm. Users should refer to the manufacturer’s instrument manuals for specific instructions for setting PMT voltages and fluorescence compensation prior to analysis.

I. Device Description:

The FlowCARE™ TLG Reagent consists of a four-color antibody reagent composed of CD45-FITC, CD4-PE, CD8-ECD, and CD3-PC5. The assay is performed on the EPICS XL or suitably equipped flow cytometer (see intended use for specifications) using appropriate quality control reagents in combination with an optional absolute count reagent, Flow-Count™ Fluorospheres, for determination of CD3+, D3+CD4+, and CD3+CD8+ absolute counts as a single platform measurement, or in combination with a White Blood Cell Count from a hematology analyzer as a dual platform measurement. The reagent is supplied in 0.5 mL vials.

J. Substantial Equivalence Information:

1. Predicate device name(s):
tetraONE™ System for EPICS XL Flow Cytometry System
2. Predicate K number(s):
k990172
3. Comparison with predicate:

Similarities		
Item	tetraONE System for EPICS XL Flow Cytometry System	FlowCARE TLG Reagent
Intended Use	Enumeration of three major T-lymphocyte subset populations (CD3+, CD3+CD4+, CD3+CD8+)	Same
Analytical Instrumentation	Deployed on EPICS® XL-MCL™ flow	Deployed on EPICS® XL™/XL-MCL™ or a suitably equipped flow cytometer with a 488 nm laser capable of detecting light scatter (forward and side) and a minimum of four-color fluorescence emission detectable in the following ranges: 504 - 541 nm, 568 – 590 nm, 610 – 635 nm, and 660 – 680 nm.
Analysis Reagents	Uses CYTO-STAT® tetraCHROME™ CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5	CD45-FITC, CD4-PE (RD1), CD8-ECD, and CD3-PC5 monoclonal dye conjugates are identical to CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 tetraCHROME™ reagent components.
Analysis Reagents	Uses Flow-Count™ Fluorospheres absolute count reagent	Same
Setup Reagents	<ul style="list-style-type: none"> • Flow-Set™ Fluorospheres • CYTO-COMPT™ Cell Kit • CYTO-COMPT™ Reagent Kit 	Same or equivalent reagents

Similarities		
<i>Item</i>	<i>tetraONE System for EPICS XL Flow Cytometry System</i>	<i>FlowCARE TLG Reagent</i>
QC Reagents	<ul style="list-style-type: none"> • IMMUNO-TROL™ Control Cells • IMMUNO-TROL™ Low Control Cells 	Same or equivalent reagents

Differences		
<i>Item</i>	<i>tetraONE System for EPICS XL Flow Cytometry System</i>	<i>FlowCARE TLG Reagent</i>
Analysis Software	System II™ Automated analysis using cellSTAT3D™ algorithm	Manual analysis using customer created protocols according to package insert
Specimen Age	<ul style="list-style-type: none"> • ≤ 6 hours (with automated software) • ≤ 72 hours (without automated software, tetraCHROME CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5) 	≤ 120 hours (5 days)* * The specimen age limit for dual platform measurement is dependent upon the claims for the hematology analyzer but not to exceed five days.

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

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, December 4, 2001.

EP9-A2 Method Comparison and Bias Estimation Using Patient Samples, Approved Standard-Second Edition, NCCLS

EP5A Evaluation of Precision Performance of Clinical Chemistry Device Approved Guideline, NCCLS

International Council for Standardization in Haematology; Prepared by the ICSH Expert Panel on Cytometry. *Clinical Lab Haematology*, 16(2):157-174, 1994.

L. Test Principle:

This test depends on the ability of a monoclonal antibody to bind to the surface of cells expressing discrete antigenic determinants. Specific cell staining is accomplished by incubating whole blood with the monoclonal antibody reagent. The FlowCARE TLG Reagent is a combination of four murine monoclonal antibodies, each conjugated to a specific fluorochrome and specific for different cell surface antigens.

Red blood cells are lysed with the COULTER IMMUNOPREP™ Reagent System and COULTER TQ-Prep™ Workstation or equivalent. The remaining white blood cells are analyzed by flow cytometry using a sequential gating strategy. The total WBC gate is defined in the first histogram (log SS vs. CD45-FITC) by the inclusion of all CD45+ events with low, medium and high Side Scatter. A second histogram (CD3-PC5 vs. CD4-PE), gated on the lymphocytes defined in the first histogram, identifies CD3+CD4+ lymphocytes by setting a region to include dual positive events. A third histogram (CD3-PC5 vs. CD8-ECD), gated on the lymphocytes defined in the first histogram, identifies CD3+CD8+ lymphocytes by setting a region to include dual positive events. The CD3+ percent is obtained from either histogram 2

or 3. The CD3+, CD3+CD4+ and D3+CD8+ absolute count are calculated using Flow-Count Fluorospheres. Alternatively, the absolute counts may be calculated using the %lymphocytes obtained from the fourth histogram (log SS vs. CD45-FITC) gated on the total WBC. The total WBC count from the hematology analyzer is multiplied by the %lymphocytes and the % CD3+, D3+CD4+ or %CD3+CD8+.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
 - a. *Precision/Reproducibility:*

Within-Run Variability

The percent positive and absolute count values were determined using IMMUNO-TROL and IMMUNO-TROL Low Cells, run in duplicate, twice each day for up to 20 days at 3 geographically diverse sites using FlowCARE TLG Reagent. Measurements (% positive and absolute counts) for CD3+, CD3+CD4+, and CD3+CD8+ populations were within assay values as determined in the control package insert. See result below:

	IMMUNO-TROL		IMMUNO-TROL low	
	n	Mean \pm 1 SD	n	Mean \pm 1 SD
CD3+ Lymphocytes				
% positive	246	74.1 \pm 0.7	245	55.8 \pm 1.1
Absolute counts	246	781 \pm 22	245	463 \pm 15
CD3+CD4 Lymphocytes				
% positive	246	51.8 \pm 0.7	245	17.6 \pm 0.6
Absolute counts	246	546 \pm 17	245	146 \pm 7
CD3+CD8 Lymphocytes				
% positive	246	19.6 \pm 0.6	245	34.9 \pm 1.3
Absolute counts	246	207 \pm 8	245	290 \pm 10

- b. *Linearity/assay reportable range:*

Linearity testing was performed in accordance with Class II Special Controls Guidance Document: Premarket Notifications for Automated Differential Cell Counters for Immature or Abnormal Blood Cell Types; Final Guidance for Industry and FDA. Three replicates measurements were made at each of 13 serial dilutions of a concentration preparation of COULTER CYTO-TROL Control Cells to achieve a range of CD3+, CD3+CD4+, and CD3+CD8+ lymphocytes concentrations. Cells were stained with the FlowCARE tetraPLG Reagent and analyzed by flow cytometry.

All acceptance criteria were met as defined in Table below.

Linearity Limits (absolute counts)

Analyte/Units	Linearity	Range
CD3+CD4+ count, cells/ μ L	$r^2 \geq 0.95$	50 – 3000
CD3+CD8+ count, cells/ μ L	$r^2 \geq 0.95$	50 – 1100
CD3+ count, cells/ μ L	$r^2 \geq 0.95$	300 – 5000

- 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:*
Accuracy testing was performed in accordance with CLSI/NCCLS EP9-A, Method Comparison and Bias Estimation Using Patient Sample; Approved Guideline. The comparison of FlowCARE TLG Reagent to the predicate device was studied in 201 lysed normal and abnormal whole blood samples (gated on lymphocytes) collected from three sites. All acceptance criteria were met as defined in the following table:

Accuracy Limits (absolute counts)

Analyte/Units	Accuracy	Range
CD3+CD4+ count, cells/ μ L	0-300 \pm 30 bias >300 \pm 10% bias	50 – 3000
CD3+CD8+ count, cells/ μ L	0-300 \pm 30 bias >300 \pm 10% bias	50 – 1100
CD3+ count, cells/ μ L	0-300 \pm 30 bias >300 \pm 10% bias	300 – 5000

Regression Analysis (Absolute counts)

Marker	Slope	R ²
CD3+CD4+,	1.03	0.9922
CD3+CD8+	1.04	0.9917
CD3+	1.03	0.9882

(Single Platform)

- 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:
Whole blood specimens were collected from apparently healthy males and females. The population was geographically diverse and included individuals

from the Eastern United States, Western and Central Canada who were unselected as to race or age (n=37). Samples were stained with FlowCARE TLG Reagent. Values determined by flow cytometry (COULTER EPICS XL-MCL flow cytometer gated on lymphocytes) represent total CD3+, CD3+CD4+, and CD3+CD8+ cells and are provided in the Normal Whole Blood Table below.

A white blood cell count and five-part differential were obtained for each sample. Absolute counts were determined using the Flow-Count (Direct) Method. Values are expressed as percentage (%) of the total lymphocyte count and as absolute counts (cells/ μ L).

The values below are intended as representative only. Each laboratory should establish its own expected values from the local population of normal donors.

Normal Whole Blood

<i>Measurement</i>	<i>n</i>	<i>Min.</i>	<i>Max</i>	<i>Mean \pm1SD</i>
% Lymphocytes				
CD+3	37	53	91	74.6 \pm 8.6
CD3+CD4+,	37	30	63	49.5 \pm 7.5
CD3+CD8+	37	12	40	22.3 \pm 5.5
Absolute Count (cells/μL)				
CD+3	37	799	2494	1414.0 \pm 471.4
CD3+CD4+,	37	395	1588	916.0 \pm 302.4
CD3+CD8+	37	189	821	415.6 \pm 120.0

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.