

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

A. 510(k) Number:

k082091

B. Purpose for Submission:

New Device

C. Measurand:

Lymphocyte Subsets CD3+CD4+, CD3+CD8+

D. Type of Test:

Quantitative

E. Applicant:

ReaMetrix, Inc.

F. Proprietary and Established Names:

Dry Tri T-STAT (CD3/CD4/CD8) 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:
Hematology (81)

H. Intended Use:

1. Intended use(s):
The Dry Tri T-STAT (CD3/CD4/CD8) Reagent is a three-color immunofluorescence stain for the labeling and identification of helper/inducer (CD3+CD4+) and cytotoxic/suppressor (CD3+CD8+) T lymphocytes combined with a precise number of fluorescent counting beads to provide absolute CD4+ and CD8+ T-Cell counts. This reagent is intended to be used for flow cytometric analysis of erythrocyte-lysed human whole blood.
2. Indication(s) for use:
The Dry Tri T-STAT (CD3/CD4/CD8) Reagent is intended for in vitro diagnostic use, is a three-color immunofluorescence stain for the labeling and identification of helper/inducer (CD3+CD4+) and cytotoxic/suppressor (CD3+CD8+) T lymphocytes combined with a precise number of fluorescent counting beads to provide absolute CD4+ and CD8+ T-Cell counts. This reagent is intended to be used for flow cytometric analysis of erythrocyte-lysed human whole blood. The DryTri T-STAT Reagent can be used to monitor forms of immunodeficiency.
3. Special conditions for use statement(s):
For prescription use only.
4. Special instrument requirements:
Becton Dickinson FACScan, Becton Dickinson FACSCalibur and Beckman Coulter EPICS XL-MCL

I. Device Description:

The Dry Tri T-STAT (CD3/CD4/CD8) Reagent is formulated in buffered saline with sodium azide and stabilizers. It contains ATTO 488 – labeled CD4 monoclonal antibody, clone RPAT4; phycoerythrin (PE) – labeled CD8 monoclonal antibody, clone LT8; and PE-DY- 649 – labeled CD3 monoclonal antibody, clone UCHT1. The specificities of the monoclonal antibodies used in the Dry Tri T-STAT (CD3/CD4/CD8) were confirmed by the Human Leukocyte Differentiation Antigens (HLDA) Workshops (9). A precise number of fluorescent counting beads are included in the Dry Tri T-STAT reagent to allow single-platform determination of absolute CD4+ and CD8+ T-cell counts. The Dry-Tri T-STAT reagent is provided in dried form and dispensed in flow cytometer compatible sample tubes with each tube containing one ready-to-use test. Fifty (50) tests are supplied in each Dry Tri T-STAT (CD3/CD4/CD8) package.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Becton Dickinson TriTEST™ reagent CD4 FITC/CD8 PE/CD3 PerCP; &
TruCount Absolute counting tubes
Beckman Coulter CYTO-STAT® CD45-FITC/CD4-PE/CD8-ECD/CD3-PC5
TetraCHROME reagent
2. Predicate K number(s):
k971205
k030408
3. Comparison with predicate:

Similarities			
<i>Item</i>	<i>Dry Tri T-STAT reagent</i>	<i>Tri TEST reagent</i>	<i>CYTO-STAT TetraCHROME reagent</i>
Indication for use	Enumeration of CD4+and CD8+ T-cells	Same	Same
Flow cytometer requirements	488nm excitation laser with side scatter, forward scatter, 3 fluorescence channels	Same	Same
Analysis Reagent	3-color fluorochrome reagent	3-color fluorochrome reagent	4-color fluorochrome reagent
Data analysis	Manual analysis using customer created protocols according to Package Insert	Same	Same

Differences			
<i>Item</i>	<i>Tri T-STAT reagent</i>	<i>Tri TEST reagent</i>	<i>CYTO-STAT TetraCHROME reagent</i>
CD4 detection reagent	Monoclonal antibody RPA-T4 conjugated to Atto 488	Monoclonal antibody SK3 conjugated to PE	Monoclonal antibody SFC121T4D11 conjugated to phycoerythrin (PE)
CD8 detection reagent	Monoclonal antibody LT8 conjugated to PE	Monoclonal antibody SK1 conjugated to PE	Monoclonal antibody SFC121Thy2D3 conjugated to PE-Texas Red® -X
CD3 detection reagent	Monoclonal antibody UCHT1 conjugated to PE-Dyomics 649	Monoclonal antibody SK7 conjugated to PerCP	Monoclonal antibody UCHT1 conjugated to PE-Cy5
Analysis reagent	ReaCount fluorescent beads	TruCount tubes	Flow-Count fluorospheres
Sample age, pre-processing	Potassium EDTA, blood processed within 24 hr draw	Potassium EDTA, blood processed within 48 hr draw	Potassium EDTA, blood processed within 72 hr draw

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

H20-A *Reference Leukocyte Differential Count (Proportional) and Evaluation of Instrument Methods*, Approved Standard, CLSI

H42-A *Clinical Applications of Flow Cytometry: Quality Assurance and Immunophenotyping of Lymphocytes*, Approved Guideline, CLSI

L. Test Principle:

The Dry Tri T-STAT (CD3/CD4/CD8) reagent consists of murine monoclonal antibodies that specifically recognize the human leukocyte surface antigens, CD3, CD4, and CD8. Each of the monoclonal antibodies is labeled with a unique fluorochrome. Specific cell subsets are stained when blood is combined with the reagent and each monoclonal antibody binds to the binds to the cell determinant molecules on the cell surface. Specific cell subsets are identified when passed through a flow cytometer laser beam.

The Dry Tri T-STAT (CD3/CD4/CD8) reagent also contains a precise number of fluorescent beads. When the reagent is combined with a known volume of blood the reagent provides for the single platform determination of the absolute cell concentrations of the stained subsets. The volume of sample analyzed can be determined by multiplication of the total sample volume by the fraction of total beads that were detected during the analysis.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The reproducibility of measurement was determined for three concentrations of CD3+CD4+ and CD3+CD8+ T-cells counts analyzed in replicates of ten. The results are tabulated below.

	Level	Mean (cells/ μ L)	SD	%CV
CD3+CD4+ T-cells	Low	29	1.7	6
	Medium	607	23.6	3.9
	High	1173	66.3	5.7
CD3+CD8+ T-cells	Low	655	45.1	6.9
	Medium	770	37.1	4.8
	High	1557	30	1.9

b. *Linearity/assay reportable range:*

Assay linearity was determined by testing six point dilution series, 64-1680 CD3+CD4+ T-cells/ μ L and 32-1640 CD3+CD8+ T-cells/ μ L. The measurement at each level was performed in triplicate. The assay was determined to be linear for CD3+CD4+ and CD3+CD8+ T-cells with correlation coefficients (r^2) of 0.995 and 0.999, respectively.

The two dilution series spanned the specified reportable ranges of 65-1500 CD3+CD4+ T-cells/ μ L and 40-1500 CD3+CD8+ T-cells/ μ L.

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

Reagent stability was tested by staining control material cells over 52 weeks. The mean fluorescence intensity (MFI) of the cells was measured and tracked, in triplicate. The data presented demonstrated the relative MFI of the CD3-PE-Dyomics 649, CD4-Atto488 and CD8-PE conjugates were not diminished of the 52 week period.

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:*

The absolute CD3+CD4+ and CD3+CD8+ T-cells concentrations for 291 blood samples (both HIV positive and negative) at 4 sites were determined using the Dry Tri T-STAT (CD3/CD4/CD8) reagent and compared to those

values determined using the predicate reagents. Regression analysis reported in the table below indicates substantial equivalence.

Parameter	Slope	Y-Intercept	r ²
Site 1 (n=45)			
CD3+CD4+ T-cells	0.87	-3.28	0.989
CD3+CD8+ T-cells	0.89	35.0	0.962
Site 2 (n=96)			
CD3+CD4+ T-cells	0.97	-8.47	0.933
CD3+CD8+ T-cells	1.04	-58.3	0.912
Site 3 (n=100)			
CD3+CD4+ T-cell	0.98	5.85	0.915
Site 4 (n=50)			
CD3+CD4+ T-cells	0.90	12.99	0.987
CD3+CD8+ T-cells	0.88	2.49	0.960

- 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 absolute CD4+ and CD8+ T lymphocyte concentrations were measured for a normal population of adults in southern India, unselected for age or gender ranging from 21 to 80 years old, using the Dry Tri T-STAT (CD3/CD4/CD8) reagent. Values obtained from this diverse population are displayed below and consistent with the reported normal range.

T-Cell Subset	n	Minimum (cells/μL)	Maximum (cells/μL)	Mean±SD (cells/μL)
CD3+CD4+ T-cells	112	457	2076	995± 298
CD3+CD8+ T-cells	112	196	1402	634±3300

It is recommended that each laboratory establish its own expected values from the local population of donors.

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.