

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE AND INSTRUMENT TEMPLATE**

A. 510(k) Number:

K041882

B. Purpose for Submission:

To seek clearance for the FlowCare™ CD4 Reagent and the FlowCare™ System, are components of an automated immune hematology system intended for *in vitro* diagnostic use in the direct enumeration of White Blood Cell populations and certain T-lymphocyte subsets from human whole blood.

C. Measurand:

White Blood Cell Count, Lymphocyte Percentage (of WBCs), Lymphocyte Number, CD4 T-Lymphocyte Count, CD4 Percentage (of total Lymphocytes)

D. Type of Test:

Quantitative, automated flow-based

E. Applicant:

PointCare Technologies, Inc.

F. Proprietary and Established Names:

FlowCare™ System and FlowCare™ CD4 Reagent Kit

G. Regulatory Information:

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

H. Intended Use:

1. Intended use(s):
The FlowCare™ System is an automated immune hematology system intended for *in vitro* diagnostic use in the direct enumeration of White Blood Cell populations and certain T-lymphocyte subsets from human whole blood.

The FlowCare™ CD4 Reagent Kit using the FlowCare™ System is intended for *in vitro* diagnostic use in clinical laboratory settings.

2. Indication(s) for use:
Whole blood samples can be analyzed with the FlowCare™ System for the following parameters:
White Blood Cell Count
Lymphocyte Percentage (of White Blood Cells)
Lymphocyte Number
CD4 T-Lymphocyte Count
CD4 Percentage (of total Lymphocytes)
3. Special condition for use statement(s):
N/A
4. Special instrument Requirements:
N/A

I. Device Description:

The FlowCare™ System consists of an analysis instrument, a touch screen computer and a standard inkjet printer.

The touch screen computer is the central processing unit for system operation and data analysis. The Windows-based software analyzed the patient sample for immune hematology parameters with optimized cluster finding algorithms.

The FlowCare™ CD4 Reagent Kit has been formulated specifically for use with the FlowCare™ System to provide optimal assay performance for White Blood Cell differential parameter analysis and CD4 counting.

J. Substantial Equivalence Information:

1. Predicate device name(s):
 - a. Coulter® Gen-S Hematology Analyzer
 - b. Coulter® LH 750 Hematology Analyzer
 - c. Coulter® tetraONE™ System for EPICS XL Flow Cytometry Systems
2. Predicate K number(s):
 - a. K962988
 - b. K032342 & K011342
 - c. K990172

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Sample	Same as predicate	Coulter LH 750 & Gen-S: Anticoagulated whole blood
Intended Use	Determination of WBC count, Lymphocyte count, Lymphocyte percentage, CD4 T-Lymphocyte count and CD4 percentage	Coulter LH 750 & Gen-S: Determination of WBC count, Lymphocyte count, Lymphocyte percentage plus other hematologic parameter Coulter tetraONE: Determination of CD4 percentage plus other lymphocyte populations
Principle of Operation	Light scatter	Coulter tetraONE: Light Scatter
Differences		
Item	Device	Predicate
CD4 Reagent	Anti-CD4 monoclonal antibody conjugated to colloidal gold substrate	Coulter tetraONE: Anti-CD4 monoclonal antibody labeled with fluorochrome
Instrumentation	Compact flow-based immune hematology analyzer with computer and software	Coulter LH 750 & Gen-S: Standard hematology analyzer with computer and software Coulter tetraONE: Standard flow cytometer with computer and software
Principle of Operation	Light scatter	Coulter LH 750 & Gen-S: Impedance

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)

L. Test Principle:

The FlowCare™ System reports CD4 T-Lymphocyte counts in conjunction with White Blood Cell (WBC) count and total Lymphocyte count information, on the basis

of software analysis of established light scatter measurements with the use of proprietary non-fluorescent reagents.

All assay steps are performed on whole blood with capped bar-coded reagent tubes designed for use on the FlowCare™ System. All assay aspiration, dispensing and mixing steps are automated. The whole blood specimen is introduced into the CD4 Reagent Tube, where an anti-CD4 monoclonal antibody, conjugated to a colloidal gold substrate, recognizes and bind to the surface of leukocytes with CD4 receptors, such as CD4 positive T-Lymphocytes and monocytes.

Following a binding step incubation, the reaction mixture is subjected to selective lysing of erythrocytes. The lysing preserves the white blood cells in their near-native state, for subsequent flow-based leukocyte differential analysis. The instrument software analyzes, under flow, different leukocyte cell populations based on their light scatter properties. Automated cell population cluster analysis is performed by the software and results provided with no operator interpretation.

WBC populations are differentiated by the software on the basis of their forward scatter and time-of-flight signatures. The software positively gates on the lymphocyte cell cluster, excluding in the process, monocytes. CD4 positive T-lymphocytes are distinguished from CD4 negative T-lymphocytes, based on their greater degree of scatter on extinction versus right angle scatter, due to the bound CD4-colloidal gold particle conjugate bound onto their cell surfaces.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A precision study was performed to assess the within-run precision and total precision of the FlowCare™ System by replicate measurements of control materials. Commercially available hematology (STRECK STaK-Chex®, tri-level – low, normal high) controls were used for WBC count, Lymphocyte count and Lymphocyte percentage of WBC count. CD4 quality controls (STRECK CD- Chex® Plus, bi-level – low, normal) quality controls were analyzed on two instruments, in triplicate runs, three times per day over three days. A high level is not available with the STRECK CD-CHEX controls. The testing was performed at PointCare Technologies, Inc., in Ashland, MA

The statistical measures of precision include the grand means along with standard deviations and coefficients of variation for both within-run precision and total precision.

The % CV for within-run precision and total precision demonstrate acceptable performance.

All % CVs for within-run and within-day precision demonstrated acceptable performance. Results of the precision studies met acceptance criteria.

In addition, a precision study was performed to assess the within-day precision of the FlowCare™ System by replicate measurements of whole blood samples at four concentration levels. The samples were analyzed on two instruments with three separate runs. In each run, three replicate measures were performed for each level of blood sample. Testing was performed at PointCare Technologies, Inc., in Ashland, MA.

All whole blood samples were analyzed for WBC count, Lymphocyte count, Lymphocyte percentage of WBC count, CD4 count and CD4 percentage of Lymphocyte count.

The results demonstrated acceptable performance for within-day precision.

b. Linearity/assay reportable range:

A linearity study was performed to assess the performance of the FlowCare™ System over a wide range of cell concentrations for the measured parameters of WBC count, Lymphocyte count and CD4 count. Additionally, CD4 count was further evaluated in the low range. For both the full range and low range study, a minimum of six concentration levels were analyzed in quadruplicate on two instruments.

Samples for the full range study were prepared using a concentrated whole blood sample diluted with autologous platelet poor plasma to achieve the desired concentration levels. The 50% (normal range) sample was used to determine the expected values at the other concentration levels.

For the CD4 count low range study, a whole blood sample was obtained from a donor known to have a CD4 count of approximately 400 cells/ μ L. The low range concentration levels were prepared by diluting the whole blood sample with autologous platelet poor plasma. The undiluted whole blood sample was used to determine the expected values at the other concentration levels.

The sample for each concentration was analyzed four times in random sequence on each of the two FlowCare instruments. Testing was performed at PointCare Technologies, Inc., in Ashland, MA.

The results for each parameter were evaluated for linearity by the method of Snedecor and Cochran (Statistical Methods, Iowa State University Press, Sixth Edition, 1967, Chapter 15, section 15.4). The method involves fitting a linear regression, a quadratic regression and a simple one-way analysis of variance to the replicate results of the assayed parameter, at each of the expected concentration levels. The test for departure from linearity is an F-test which compares the mean square error from the quadratic regression against the pooled mean square error. Large values of the F-test lead to rejection of a linear fit. Acceptable linearity was demonstrated in all cases.

Least squares regressions analysis was also performed for each parameter. In all cases, linearity was demonstrated, and the expected and actual FlowCare values were shown to be highly correlated.

Full Range Linearity:

Regression Statistics:	S/N 1816			S/N 2185		
	WBC#	Lymph#	CD4#	WBC#	Lymph#	CD4#
N	21	21	21	22	19	20
Correlation	0.998	0.967	0.981	0.984	0.994	0.992
Slope	1.018	0.960	1.002	0.957	0.969	0.950
Intercept	-131.732	-29.898	-85.463	204.348	-9.586	-7.461

Low Range Linearity:

Regressions Statistics:	CD4 Count	
	S/N 1816	S/N 1858
N	27	27
Correlation	0.994	0.994
Slope	1.042	0.975
Intercept	-12.932	13.295

FlowCare System Reportable Ranges:

Parameter	Reportable Range	Units
WBC Count	1.0 - 23.0	$10^3/\mu\text{L}$
Lymphocyte%	10 - 75	%
Lymphocyte Count	0.3 - 6.0	$10^3/\mu\text{L}$
CD4 Count	50 - 3000	μL
CD4%	0 – 80	%

c. Traceability (controls, calibrators, or method):

The CD4 monoclonal antibody used in the FlowCare CD4 Reagent Kit is derived from clone number RPA-T4. The monoclonal antibody recognized the human CD4 cell surface antigen, a 55 kD glycoprotein expressed by the helper-inducer subset of T-Lymphocytes and more weakly by monocytes.

The antigen specificity of the CD4 monoclonal antibody has been previously established by the Fourth and Fifth International Workshops for Leukocyte Typing.

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 multi-site prospective study was conducted to evaluate the performance of the FlowCare System and reagents for the determination of White Blood Cell count, Lymphocyte count, Lymphocyte percentage of White Blood Cells, CD4 T-Lymphocyte count, and CD4 percentage of total Lymphocytes. Results from the FlowCare method were compared to those from currently available, FDA-cleared reference methods.

The study was conducted across four investigational sites in the U.S. and Sub-Saharan Africa using normal and abnormal whole blood samples tested in parallel.

Reference methods for WBC counts, Lymphocyte counts and Lymphocyte %: Coulter[®] Gen-S, Coulter[®] LH750, Sysmex[®] E2500

Reference methods for CD4%: Coulter[®] EPICS XL tetraONE™ SYSTEM, and tetraCHROME™ 4-color reagents, BDIS FacsCalibur™ and MultiTest™ 4-color reagents

Reference method for CD4 count: Dual Platform
(Reference Lymphocyte counts x Reference CD4%)

Study Procedure

For each investigational site, blood specimens were analyzed by the reference methods in accordance with the procedures recommended by the respective instrument manufacturers and those further defined by the standard operating procedures and quality control programs of the site. Analyses by the FlowCare method were performed following training provided by PointCare Technologies, Inc. and in accordance with procedures described in the preliminary instrument operator manual and reagent kit package insert.

Samples analyzed in the clinical study were obtained from the routine adult populations available in each investigational site. Blood samples were collected into EDTA whole blood specimen collection tubes. Prior to analysis, the samples were kept at ambient room temperature for analysis within 10 hours of the subject blood draw.

The whole blood samples tested at the two sites in the United States were obtained primarily from a healthy donor population. In contrast, the samples tested at the two sites in Africa were from a population predominantly composed of immunocompromised individuals including those known or suspected of being infected with HIV or with other clinical conditions that may be associated with altered CD4 T-Lymphocyte counts and other hematological parameters.

Analyses of the pooled data for all sites show comparable means and ranges for the FlowCare and reference method parameter. (See table below)

Parameter	N	Corr. Coeff.	Slope	Intercept	Mean		Range	
					FlowCare	Reference	FlowCare	Reference
All Sites								
WBC#	403	0.9623	1.116	-335.13	6194.5	5851.9	1900-19700	1820-18100
LYM#	403	0.9145	1.081	-44.62	1956.8	1850.1	400-6500	500-5320
LYM%	403	0.9487	0.955	1.405	33.1	33.2	4.5-80.2	6.2-78.8
CD4#	425	0.9121	1.034	9.289	678.5	647.1	0-2318	7-2090
CD4%	401	0.9507	0.950	1.406	35.9	36.3	0.4-73.9	1.0-68.0

b. Matrix comparison:
Not applicable

3. Clinical studies:

a. Clinical sensitivity:
Not applicable

b. Clinical specificity:
The antigen specificity of the CD4 monoclonal antibody has been previously established by the Fourth and Fifth International Workshops for Leukocyte Typing.

c. Other clinical supportive data (when a and b are not applicable):

4. Clinical cut-off:
Not applicable

5. Expected values/Reference range:
Whole blood specimens were collected from apparently healthy males and females in the Northeastern United States, without selection on the basis of age or race. Expected results for FlowCare parameter are presented based on a 95% normal distribution and compare closely with expected results observed with reference methods.

FlowCare Parameter	N	Range		Mean \pm SD
		Minimum	Maximum	
WBC Count	206	4400	12100	6882.5 \pm 1618.76
Lymph Count	206	1100	3300	1963.6 \pm 525.62
Lymph %	206	16.4	45.3	28.8 \pm 6.21
CD4 Count	207	468	1702	928.5 \pm 284.53
CD4%	205	31.3	65.8	47.7 \pm 7.46

N. Instrument Name:

FlowCare™ System

O. System Descriptions:

1. Modes of Operation:

Fully-automated cap piercing, closed tube system with position recognition

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes ☒X_____ or No _____

3. Sample Identification:

Barcode reading and tube position verification

4. Specimen Sampling and Handling:

Fully-automated closed tube piercing, automated mixing and control reaction temperature

5. Calibration:

The system is factory calibrated for Total WBC count, Lymphocyte count and CD4 count.

6. Quality Control:

Commercial Hematology quality control (QC) material should be analyzed for WBC count and Lymphocyte count parameter, and a commercial CD4 QC material for assessing CD4 Reagent Kit performance.

Whole blood specimens may be used instead of commercial controls, provided there are corresponding values available from other instruments for reference purposes.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "L. Performance Characteristics" Section Of The SE Determination Decision Summary:

A carryover study was performed to assess the effect of a whole blood sample on background counts in subsequent analyses for the measured parameters. Negligible carryover was observed.

Q. Proposed Labeling:

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

R. Conclusion:

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