

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

A. 510(k) Number:

k130373

B. Purpose for Submission:

New Submission

C. Manufacturer and Instrument Name:

Beckman Coulter, Inc.
Navios Flow Cytometer

D. Type of Test or Tests Performed:

Flow Cytometric Immuno-assay: Cell identification with quantitative cell counts

E. System Descriptions:

1. Device Description:

The Navios Flow Cytometer uses flow cytometric principles to determine qualitative and quantitative measurements of biological and physical properties of cells and other particles. These properties are measured when the cells pass through the laser beam(s) in single file.

The Navios Flow Cytometry System also offers an optional standalone offline software package, Navios software, which enables the user to reanalyze, display, print and export acquired listmode data.

The Navios tetra Software is an optional locked algorithm application plug-in that is designed for the Navios Flow Cytometer. It provides automated analysis and results for tetraCHROME reagents; this application cannot be modified by the user.

Flow-Set Pro Fluorospheres is a suspension of fluorospheres with uniform and stable size and fluorescence intensity. The stability of these product parameters allows for the standardization of light scatter and fluorescence intensity instrument settings. For automated, algorithm-based Beckman Coulter flow cytometry IVD systems such as Navios tetra Software, target value ranges for X-Mode (fluorescent intensity) are provided by Beckman Coulter with each Flow-Set Pro Fluorospheres lot on the Table of Application Target Settings.

The Navios Flow Cytometer system consists of:

- Navios Flow Cytometer
- Navios tetra Software
- Navios Software (off-line analysis tool)
- Flow-Set Pro Fluorospheres
- CYTO-STAT tetraCHROME reagents
- COULTER IMMUNOPREP Reagent System

- TQ-Prep Workstation (Accessory for Sample Preparation)
- PrepPlus™ 2 Workstation (Accessory for Sample Preparation)

2. Principles of Operation:

The Navios flow cytometer aligns cells in suspension by hydrodynamic focusing and passes them through a laser beam one cell at a time. The scattered and fluoresced light that emanates from these cells as they intersect the laser beam is collected by photomultiplier tubes and transduced to an electronic pulse and counted by the cytometer's computer. 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 CYTO-STAT tetraCHROME CD45-FITC/CD4-RD1/CD8- ECD/CD3-PC5 and CYTO-STAT tetraCHROME CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5. Monoclonal Antibody Reagents are each a combination of four murine monoclonal antibodies, each conjugated to a specific fluorochrome and specific for a different cell surface antigen. Red blood cells are lysed with the COULTER ImmunoPrep Reagent System. The remaining white blood cells are analyzed on a Navios flow cytometry system with the Navios tetra Software package. Navios Flow Cytometer Software and Navios tetra Software, in conjunction with quality control reagents, provide automated standardization of light scatter and fluorescence intensities and automated adjustment of color compensation settings. The Navios tetra Software, in conjunction with the Navios Flow Cytometer Software, and CYTO-STAT tetraCHROME CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and CYTO-STAT tetraCHROME CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5 Monoclonal Antibody Reagents, provides automated analysis of lymphocyte subpopulations.

3. Modes of Operation:

Batch or single tube

4. Specimen Identification:

Barcode reader or manual entry

5. Specimen Sampling and Handling:

The automated sample loader for the instrument uses a carousel that holds thirty-two 12 x 75-mm test tubes and includes a barcode reader.

6. Calibration:

Fluorescence intensity is calibrated by adjusting photomultiplier voltage using Flow-Set Pro Fluorospheres.

The absolute count of a population is based on the calibration factor (CAL Factor) and the number of Flow-Count fluorospheres particles within a user defined CAL region.

7. Quality Control:

IMMUNO-TROL Cells and IMMUNO-TROL Low Cells are assayed, lysable, whole-blood quality control products used to verify the activity of the CYTO-STAT tetraCHROME reagents and to verify the methods used for staining targeted cells, lysing

erythrocytes, and analyzing samples with flow cytometry.

8. Software:

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

Yes X or No _____

F. Regulatory Information:

1. Regulation section:

21 CFR 864.5220 – Automated Differential Cell Counter

2. Classification:

Class II

3. Product code:

OYE- flow cytometric reagents and accessories

PDX- flow cytometry calibrator

4. Panel:

81 Hematology

G. Intended Use:

1. Indication(s) for Use:

Navios Flow Cytometer:

The Navios Flow Cytometer is intended for use as an in vitro diagnostic device for immunophenotyping. It can be used in conjunction with the following monoclonal antibody reagents and software package:

- CYTO-STAT tetraCHROME CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and CYTO-STAT tetraCHROME CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5 monoclonal antibody reagents. These reagents provide identification and enumeration of CD3+CD4+, CD3+CD8+, CD3+, CD19+ and CD3-CD56+ lymphocyte percentages and absolute counts in peripheral whole blood. Absolute counts may be determined by the Navios flow cytometer using Flow-Count Fluorospheres (single platform technology method) or separate hematology results (dual platform method). These reagents are indicated for use in the immunologic assessment of patients having or suspected of having immune deficiency.
- Navios tetra Software for automated analysis and results with CYTO-STAT tetraCHROME CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and CYTO-STAT tetraCHROME CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5 monoclonal antibody reagents.

Navios tetra Software may be installed on an independent computer workstation for off-line analysis of listmode files generated by the Navios Flow Cytometer with the monoclonal antibody reagents and software package listed above. The

off-line analysis must be performed in accordance with the product labeling.

Navios tetra Software:

The Navios tetra Software is intended for use as an in vitro diagnostic device for immunophenotyping with CYTO-STAT tetraCHROME CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and CYTO-STAT tetraCHROME CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5 monoclonal antibody reagents on the Navios Flow Cytometer. It provides automated analysis and results for the identification and enumeration of CD3+CD4+, CD3+CD8+, CD3+, CD19+ and CD3-CD56+ lymphocyte percentages and absolute counts in peripheral whole blood. Absolute counts may be determined by the Navios flow cytometer using Flow-Count Fluorospheres (single platform technology method) or separate hematology results (dual platform method). It is indicated for use in the immunologic assessment of patients having or suspected of having immune deficiency.

Flow-Set Pro Fluorospheres

Flow-Set Pro Fluorospheres is a suspension of fluorescent microspheres used as an aid in standardizing forward scatter, side scatter, and fluorescence detectors (FL1-4) on the Cytomics FC 500 and Navios Flow Cytometers.

2. Special Conditions for Use Statement(s):

For prescription use only

Special instrument requirements:

Use with TQ-Prep Workstation (Accessory for Sample Preparation) and PrepPlus™ 2 Workstation (Accessory for Sample Preparation)

H. Substantial Equivalence Information:

1. Predicate Device Name(s) and 510(k) numbers:

Cytomics FC 500 with tetraCXP software, k030828
Flow-Set Fluorospheres, k944751

2. Comparison with Predicate Device:

Navios Flow Cytometer with Navios tetra Software and Navios Analysis Software

Similarities and Differences		
Item	Device	Predicate
Intended Use	Navios Flow Cytometer: The Navios Flow Cytometer is intended for use as an in vitro diagnostic device for immunophenotyping. It can be used in conjunction with the following monoclonal	Cytomics FC 500 Flow Cytometer with tetraCXP software: The tetraCXP Software for Cytomics FC 500 flow cytometry systems and CYTO-STAT tetraCHROMETM CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and CYTO-STAT tetraCHROMETM CD45-FITC/CD56-RD1/CD19-

Similarities and Differences		
Item	Device	Predicate
	<p>antibody reagents and software package:</p> <ul style="list-style-type: none"> • CYTO-STAT tetraCHROME CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and CYTO-STAT tetraCHROME CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5 monoclonal antibody reagents. These reagents provide identification and enumeration of CD3+CD4+, CD3+CD8+, CD3+, CD19+ and CD3-CD56+ lymphocyte percentages and absolute counts in peripheral whole blood. Absolute counts may be determined by the Navios Flow Cytometer using Flow-Count Fluorospheres (single platform technology method) or separate hematology results (dual platform method). These reagents are indicated for use in the immunologic assessment of patients having or suspected of having immune deficiency. •Navios tetra Software for automated analysis and results with CYTO-STAT tetraCHROME CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and CYTO-STAT tetraCHROME CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5 monoclonal antibody reagents. •Navios Software may be installed on an independent computer workstation for off-line analysis of listmode files 	<p>ECD/CD3-PC5 monoclonal antibody reagents combine four-color fluorescent monoclonal antibody reagents, quality control reagents, an optional absolute count reagent, and software for automated analysis of lymphocyte populations in whole blood using Cytomics FC 500 Flow Cytometry systems with CXP Software.</p> <p>The system with CYTO-STAT tetraCHROME CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 is intended "For In Vitro Diagnostic Use" and allows simultaneous identification and enumeration of total CD3+, total CD4+, total CD8+, dual CD3+/CD4+ and dual CD3+/CD8+ T lymphocyte population percentages and absolute counts.</p> <p>The system with CYTO-STAT tetraCHROME CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5 is intended "For In Vitro Diagnostic Use" and allows simultaneous identification and enumeration of total CD3+ (T), CD19+ (B), and CD3-/CD56+ (NK) lymphocyte population percentages and absolute counts. This reagent reflects the distribution of the three major subsets comprising the lymphocyte population upon which other lymphocyte enumeration studies are based and provides the total lymphocyte percentage.</p>

Similarities and Differences		
Item	Device	Predicate
	generated by the Navios Flow Cytometer with the monoclonal antibody reagents and software package listed above. The off-line analysis must be performed in accordance with the product labeling.	
Regulation Number and Description, Product Code	864.5220 –Automated Differential Cell Counter, OYE	864.5220 –Automated Differential Cell Counter, GKZ
Safety Features	Interlocks and mitigation of hazards via software and hardware controls	Same
Controlling Software	Same software architecture.	The acquisition software enables the user to acquire data from the FC 500 Flow Cytometry instrument and to analyze, display, print and export acquired listmode data. The embedded software resides in the FC 500 instrument. It controls the instrument functionality including the multi-carousel loader (MCL) for sample introduction. The embedded software controls the instruments' lasers, acquisition system and fluidics. The instrument fluidics aspirates the sample, and performs instrument maintenance functions such as startup/shutdown. The embedded software also captures and provides the data to the workstation for processing.
System Configuration	Same except PC based workstation running Microsoft Windows Vista application specific software	Bench top <ul style="list-style-type: none"> • PC based workstation running Microsoft Windows 2000 application specific software • Printer
tetraCHROME Reagent Assay	Two tube monoclonal antibody reagent assay <ul style="list-style-type: none"> • CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 • CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5 Provides identification and	Same

Similarities and Differences		
Item	Device	Predicate
	enumeration of Total CD3+, CD3+CD4+, CD3+CD8+, CD3-CD56+, and CD19+ percentages and absolute count results	
tetraCHROME Reagents Specimen and Prepared Sample Stability Claims	Specimen stability – 24 hours room temperature <ul style="list-style-type: none"> • Prepared sample stability • 2 hours room temperature • 24 hours refrigerated temperature 	Same
Sample Preparation with Monoclonal Antibodies	Off-board sample preparation following instructions provided with cleared monoclonal antibody reagent	Same
Sample Presentation	Prepared sample added to a daughter tube	Same
Sample Mixing prior to Acquisition	Prepared sample is vortex mixed	Same
Sample Introduction	Tube sampler <ul style="list-style-type: none"> • Automated presentation with Multi-tube Carousel Loader (MCL) from 32 test tube capacity carousel • Manual presentation into a tube location on a MCL via tube access door 	Same
Aspiration Pathway	Same aspiration pathway used for automated and manual presentation	Same
Sample Identification	Bar-code reading of carousel position and labeled sample tube. User may also identify samples based on carousel location with a work list.	Same
Sample Analysis	Same except automated gating of tetraCHROME achieved with Navios tetra software	Principle of analysis – Flow Cytometric <ul style="list-style-type: none"> • Detection hardware – Lasers, fluidics, optics, electronics • Sample analysis pathway • Manual gating of cellular populations per reagent IFU or automated gating of cellular populations for tetraCHROME reagents with tetraCXP software
Lasers	IVD configuration – 488 nm	One Laser Standard Configuration

Similarities and Differences		
Item	Device	Predicate
	IVD configuration – Standard doubled diode <ul style="list-style-type: none"> • 22mW • 488nm • Vertical polarization • Mode 00 	Standard Argon Ion <ul style="list-style-type: none"> • 22mW • 488 nm • Vertical polarization • Mode 00
Optics	Same except collection also uses fiber optics	Free space delivery and collection of laser light
Electronics	40 MHz sampling Digital integrator circuitry w/ early stage ADC Yields ~25,500 events/sec	200 KHz sampling Analog integrator circuitry w/ late stage ADC Yields ~1,500 events/sec
Photomultiplier Tubes (PMTs) / Colors	IVD configuration – Standard 4 PMTs	Same
Maximum Parameter Detectors	IVD configuration – Six (FS, SS, FL1 – FL4)	Same
Off-line Analysis Software	Optional software kits for analysis on off-line workstations	Same
Data Handling	Electronics and software required to support data handling	Same
Data Reporting	FlowPAGE, Panel Report, Plots, and Statistics printouts	Same
Cleaning Cycle Between Samples	Executed with IsoFlow Sheath Fluid ensuring carryover specification is met	Same
Cleanse Cycle	Same except uses FlowClean cleaning reagent	Cleaning cycle performed with COULTER Clenz cleaning reagent as part of the daily shutdown process, before and after running samples with vital dyes that stain the tubing, and as part of troubleshooting.
Quality Control Techniques	Daily Instrument Checks Commercial Controls <ul style="list-style-type: none"> • Inter-laboratory Quality Assurance Program (IQAP) 	Same
Workstation	Software functionality to allow: <ul style="list-style-type: none"> • Patient data management – storage, review, reporting • Control data management – storage, review, reporting • System configuration 	Same

Similarities and Differences		
Item	Device	Predicate
	management <ul style="list-style-type: none"> • System service test and adjustment procedures. 	
Laser Optics	Achromatic Crossed Cylinder Lens - 488 spot size = 10 x 80mm	Crossed Cylinder Lens - 488 spot size = 10 x 80mm
Fluorescence Collection	Collection lens and light path 1st lens set: <ul style="list-style-type: none"> • Gel coupled to flow cell operates at NA1.2 • Forms images at 21x magnification 2nd lens set: <ul style="list-style-type: none"> • Located at fiber entry reduces the magnification per channel to 3x • Light passes through optical fiber to PMT area • Light leaves the fiber and is collimated • Collimated light is separated by filters into bands • PMTs detect light and convert to electrical signals 	Collection lens and light path 1st lens series: <ul style="list-style-type: none"> • Glued to flow cell operates at NA1.2 • Forms Images at 3x magnification • Image formed at .020 diameter pinhole 2nd lens series: <ul style="list-style-type: none"> • Image at pinhole is transformed into collimated beam • Collimated light is separated by filters into desired bands • PMTs detect light and convert to electrical signals
Color Separation	Collimated beam is separated into desired components with dichroic filters used in a linear topology	Collimated beam is separated into desired components with dichroic filters used in a tree topology
Sample Introduction	<ul style="list-style-type: none"> • Automated presentation with Multi-tube Carousel Loader (MCL) from 32 test tube capacity carousel • Manual presentation into a tube location on MCL via tube access door 	Same
Sample Analysis	Principle of analysis – Flow cytometric <ul style="list-style-type: none"> • Detection hardware – Lasers, fluidics, optics, electronics • Sample analysis pathway • Manual gating of cellular populations by user or automated gating of 	

Similarities and Differences		
Item	Device	Predicate
	cellular populations with algorithmic software	
Automated Algorithm Analysis for tetraCHROME reagents	Navios tetra Software	tetraCXP Software
Accessories	Optional software kits for analysis on off-line workstations	Same

Flow-Set Pro Fluorospheres

Similarities and Differences		
Item	Device	Predicate
Intended Use	Flow-Set Pro Fluorospheres is a suspension of fluorescent microspheres used as an aid in standardizing forward scatter, side scatter, and fluorescence detectors (FL1-4) on the Cytomics FC 500 and Navios Flow Cytometers.	Flow-Set Fluorospheres are a suspension of Fluorospheres (fluorescent microspheres) used as an aid in optimizing a flow cytometer for quantitative analysis of human leukocytes.
Regulation Number and Description, Product code	864.5220 – Automated Differential Cell Counter, PDX	864.8625– Hematology Quality Control Mixture, JPK
Reagent Components	Same except 3 µm (nominal diameter) polystyrene fluorospheres	3.6 µm (nominal diameter) polystyrene fluorospheres suspended in an aqueous medium containing surfactants and preservatives at 1 x 10 ⁶ fluorospheres/mL (nominal concentration)
Fluorescence Emission	Ranges from 515-800 nm when excited at 488 nm	Ranges from 525 nm to 700 nm when excited at 488 nm
Manufacturing Process	Manufactured by loading a solution of three dyes into a polystyrene particle.	Polymerized in the presence of a single, broad spectrum dye.
Sample Preparation	Must be thoroughly mixed prior to use. Samples in test tubes which stand for an extended period of time should be vortex mixed before use. No other sample preparation is necessary.	Same

Similarities and Differences		
Item	Device	Predicate
Target Value Ranges	Same as Flow-Set with target value ranges provided for Navios tetra Software	Procedure provided for user to establish target value ranges for each manually gated application. Additionally, target value ranges provided for algorithm-based IVD software applications such as tetraCXP for FC 500 Flow Cytometer
Final Product Form	Liquid, ready to use	Same
Open Vial Stability	65 days	Same
Closed Vial Stability	10 months	18 months

I. Special Control/Guidance Document Referenced (if applicable):

CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

CLSI EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods, Approved Guideline, Second Edition 2004.

CLSI C28-A3, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline.

CLSI EP15-A2 User Verification of Performance for Precision and Trueness; Approved Guideline – Second Edition

NCCLS EP09-A2-IR, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Second Edition (Interim Revision)

CLSI H26-A2, Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Guideline-Second Edition; Section 5.7 – Carryover

CLSI EP17-A2 Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline, Second Edition

CLSI H42-A2, Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline-Second Edition

J. Performance Characteristics:

1. Analytical Performance:

a. *Accuracy:*

Method comparison with predicate device:

Several method comparison analyses were performed in order to support the comparability of the automated, algorithm gating methods and manual gating method with tetraCHROME reagents (tetraCHROME CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5) and Flow-Count fluorospheres (single platform absolute counting method).

- i. Method Comparison Study 1: Navios Flow Cytometer with Navios tetra Software (algorithm) vs. FC 500 Flow Cytometer with tetraCXP Software (algorithm). Three sites (one in the US and two in Canada) collected a total of 291 specimens (75% abnormal) for the assessment of agreement to the predicate method. Results met all acceptance criteria. Navios Flow Cytometer has demonstrated acceptable clinical performance accuracy as compared to the predicate, FC 500 Flow Cytometer.

		Total CD3+	Total CD3+	CD3+/ CD4+	CD3+/ CD4+	CD3+/ CD8+	CD3+/ CD8+
units		cells/ μ L	%	cells/ μ L	%	cells/ μ L	%
n		291	291	291	291	291	291
Correlation		0.990	0.990	0.994	0.997	0.993	0.998
Mean	tetraCXP	1366	77.96	570	31.70	763	44.24
	Navios tetra	1402	77.12	601	32.27	780	43.58
Slope (95% Confidence Limits)		1.006 (0.994 - 1.018)	0.992 (0.962 - 1.022)	1.045 (1.016 - 1.075)	0.978 (0.960 - 0.995)	1.019 (1.003 - 1.035)	0.995 (0.985 - 1.004)
Intercept (95% Confidence Limits)		29.182 (17.490 - 40.873)	-0.220 (-2.611 - 2.171)	10.820 (0.8592 - 0.782)	1.297 (0.837 - 1.758)	3.995 (-4.812 - 12.802)	-0.377 (-0.749 - -0.005)

		Total CD3+	Total CD3+	CD3- /CD56+	CD3- /CD56+	CD19+	CD19+
units		cells/ μ L	%	cells/ μ L	%	cells/ μ L	%
n		291	291	291	291	291	291
Correlation		0.989	0.994	0.993	0.992	0.995	0.995
Mean	tetraCXP	1375	78.00	137	7.87	217	12.03
	Navios tetra	1420	77.48	145	8.03	228	12.15
Slope (95% Confidence Limits)		1.027 1.0091.046	1.000 0.9861.015	1.047 1.0271.067	1.003 0.9731.033	1.036 1.0221.050	1.022 0.9951.050
Intercept (95% Confidence Limits)		8.779 (-10.940 - 28.498)	-0.553 (-1.726 - 0.620)	2.350 (0.875 - 3.824)	0.160 (0.003 - 0.317)	3.228 (0.474 - 5.983)	-0.010 (-0.157 - 0.137)

- ii. Method Comparison Study 2: Navios Flow Cytometer with Navios tetra Software (algorithm) vs. FC 500 Flow Cytometer with Manual Gating. Results met all acceptance criteria. Navios Flow Cytometer with Navios tetra Software has demonstrated acceptable clinical performance accuracy as compared to the predicate, FC 500 Flow Cytometer with Manual Gating.
- iii. Method Comparison Study 3: Navios Flow Cytometer with Manual Gating vs. FC 500 Flow Cytometer with Manual Gating. Results met all acceptance criteria. Navios Flow Cytometer with Manual Gating has demonstrated acceptable clinical performance accuracy as compared to the predicate, FC 500 Flow Cytometer with Manual Gating.

- iv. Method Comparison Study 4: Navios Flow Cytometer with Manual Gating vs. Navios Flow Cytometer with Navios tetra Software (algorithm). Results met all acceptance criteria. Navios Flow Cytometer with Manual Gating has demonstrated acceptable clinical performance accuracy as compared to the Navios Flow Cytometer with Navios tetra Software (algorithm).

b. Precision/Reproducibility:

To assess variability, three precision studies were conducted:

- i. Precision Study 1: CLSI EP05-A2 precision study with control material using Navios tetra software

Precision studies with control materials were conducted in accordance with CLSI EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline. Two levels of control material (IMMUNO-TROL Cells and IMMUNO-TROL Low Cells) were evaluated in the study. The controls were prepared with tetraCHROME reagents (CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5) and Flow-Count Fluorospheres. They were run in duplicate twice each day (morning and afternoon) for a minimum of 20 days. The test was executed on three Navios Flow Cytometers with Navios tetra Software at the three independent test sites. All parameters met the precision and reproducibility criteria.

Acceptance Criteria:

Analyte / Units of Measure	% Positive cells (Total CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/CD56+, CD19+)		Absolute cell counts (CD3+/CD4+, CD3+/CD8+, Total CD3+, CD3-/CD56+, CD19+)	
Mean	≤ 20%	> 20%	< 300 cells/μL	≥ 300 cells/μL
Repeatability Specification (%CV)	≤ 10%	≤ 5%	≤ 10%	≤ 5%
Reproducibility Specification (%CV)	≤ 15%	≤ 10%	≤ 15%	≤ 10%

- ii. Precision Study 2: CLSI EP05-A2 precision study with control materials using the tetraCHROME reagents manual gating method.

To assess the precision of manually gated data on the Navios Flow Cytometer, the listmode data collected at Ohio State University, Columbus, Ohio in Precision Study 1, CLSI EP05-A2 was replayed and manually gated by three qualified operators according to the tetraCHROME reagents and Flow-Count Fluorospheres Instructions for Use (IFU). The acceptability criteria are as stated above for precision study 1. All parameters met the precision and reproducibility criteria.

- iii. Precision Study 3: Whole Blood Repeatability

Whole blood specimen prepared with tetraCHROME CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and tetraCHROME CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5

reagents and Flow Count fluorospheres from three test sites (Ohio State University, Columbus, Ohio, London Health Sciences Centre, London, Ontario, Canada, and Foothills Hospital, Calgary, Canada) were run in duplicate on the Navios Flow Cytometer with Navios tetra Software. The dataset consisted of 336 whole blood specimens including samples representing the CD4 measuring range and analyte concentrations at the medical decision points. Whole blood repeatability followed the approach per CLSI H26-A2 ‘Validation, Verification and Quality Assurance of Automated Hematology Analyzers’ standard, of providing estimates of repeatability using biological samples representing normal and medical decision points. All parameters met the precision and reproducibility criteria.

Acceptance Criteria:

Analyte / Units of Measure	% Positive cells (Total CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/CD56+, CD19+)		Absolute cell counts (CD3+CD4+, CD3+/CD8+, Total CD3+, CD3-/CD56+, CD19+)	
	≤ 20%	> 20%	< 300 cells/μL	≥ 300 cells/μL
Mean	≤ 20%	> 20%	< 300 cells/μL	≥ 300 cells/μL
Repeatability Specification (%CV)	≤ 10%	≤ 5%	≤ 10%	≤ 5%

Precision and reproducibility of Flow-Set Pro fluorospheres

Three lots of Flow-Set Pro fluorospheres were tested on a Navios Flow Cytometer over twenty days according to methods defined in CLSI EP5 to assess repeatability and reproducibility. All parameters demonstrated CV’s < 15% and as such met the manufacturer’s acceptance criteria. Flow-Set Pro fluorospheres were also tested on an Epics FC500 Flow Cytometer and found to meet the manufacturer’s acceptance criteria.

c. *Linearity:*

- i. Instrument linearity was assessed with two Immunobrite beads of different fluorescent intensities analyzed at 5 different voltages covering the dynamic range of each PMT. The mean channel fluorescent intensity ratios of pairs was calculated at five different voltages levels across three decades on all PMTs. Lower and Upper Limits are calculated from the mean value +5%. The results of this study demonstrate that the intensity differences between the beads were constant across the range of voltages assessed.
- ii. Linearity of the tetraCHROME reagents on the Navios Flow Cytometer with Navios tetra software and the manual gating method was established in accordance with CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach. Using the following linearity acceptance limits:
 - $R^2 \geq 0.95$;
 - 0-300 cells/μL: ± 30 cells/μL bias
 - >300 cells/μL: ± 10% bias

The analytical system was found to be linear for each parameter as indicated in the following table:

tetra	Parameters	Navios tetra	Manual Gating
CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5	CD3+/μL	50-4653	49-4605
	CD3+/CD4+/μL	33-3147	33-3148
	CD3+/CD8+/μL	15-1408	15-1389
CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5	CD3+/μL	64-5218	65-5323
	CD3-/CD56+/μL	11-969	11-994
	CD19+/μL	11-1081	10-996

iii. Analytical Measuring Range

Based on the LOD/LOQ studies the following are reported as the analytical measuring range for this device.

tetra	Parameters	Analytical Measuring Range
CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5	CD3+/μL	65-4500
	CD3+/CD4+/μL	35-3000
	CD3+/CD8+/μL	15-1300
CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5	CD3+/μL	65-4500
	CD3-/CD56+/μL	35-900
	CD19+/μL	35-900

d. Carryover:

- i. A carryover study was performed using Flow Check Pro fluorospheres on each of 4 Navios flow cytometers according to the methodology presented in CLSI Document: H26-A2, Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard – Second Edition, Section 5.7 Carryover was demonstrated to be less than the acceptance limit of 0.1% from one sample to another.
- ii. A carryover study was performed to evaluate 1) specimen carryover and 2) reagent carryover using whole blood analyzed with the tetraCHROME system on each of 3 Navios Flow Cytometers according to the methodology presented in CLSI Document: H26-A2, Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard – Second Edition, Section 5.7 Scatter and fluorescence carryover was demonstrated to be less than the acceptance limit of 1% from one specimen to another when the numbers of gated events is 10,000 or greater.

e. Interfering Substances:

Not applicable

2. Other Supportive Instrument Performance Data Not Covered Above:

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

Flow-Set Pro Fluorospheres

- i. Open vial stability was assessed across three lots of Flow-Set Pro Fluorospheres. Each lot was tested and fluorescence intensity of the associated peaks was assessed on Days 0, 40, 60, 65 and 70. The acceptance criteria are as follows:

	Specification
Mean signal intensity and MFI	$\pm 20\%$ from baseline
Monodispersity	<ul style="list-style-type: none"> • $\geq 80\%$ for FS and FL1-FL 4 • $\geq 60\%$ for SS

- ii. Closed vial stability was assessed as above and the drift from time zero to 10 months for all channels on the three lots (TL1, TL2, and TL4) were shown to be within the $\pm 20\%$ allowable drift, indicating that the stability specification are met.
- iii. Flow Set Pro fluorospheres meet the open vial claim of 65 days and the closed vial claim of 10 months.

b. *Detection limit:*

- i. Limit of Blank (LoB): Two lots of ImmunoTrol erythrocytes were treated with CytoStat tetraCHROME reagents, Immunoprep reagents and Flow-Count to determine the LoB. The LoB was established for each marker as the upper 95% of the data as recommended in the CLSI EP17-A Guideline.
- ii. Limit of Detection (LoD) and Limit of Quantitation (LoQ): Eight dilutions of 2 lots of ImmunoTrol cells were prepared in ImmunoTrol erythrocytes and were treated with 3 lots of CytoStat tetraCHROME reagents, Immunoprep reagents and Flow-Count to measure the LoD and LoQ. LOD and LOQ were determined on two Navios Flow Cytometers by analyzing 2 freshly prepared replicates of each sample over 4 days. The equation provided in section 5.1 of EP17-A was used to calculate the low limit of quantitation (LLOQ) - $\text{Spec} + 2\text{SDs} = \text{Total Error}$.

Navios tetra	CD3+	CD3+/CD4+	CD3+/CD8+	CD3- /CD56+	CD19+	CD3+
LoB (cells/uL)	1	1	1	0	0	1
LoD (cells/uL)	2	2	2	1	1	2
LLOQ (cells/uL)	7	9	6	16	12	6
LLOQ Upper 95% Confidence (cells/uL)	14	15	10	33	15	10

Manual	CD3+	CD3+/CD4+	CD3+/CD8+	CD3- /CD56+	CD19+	CD3+
LoB (cells/uL)	1	1	0	0	0	1
LoD (cells/uL)	2	2	1	1	1	2
LLOQ (cells/uL)	4	6	5	13	17	3
LLOQ Upper 95% Confidence (cells/uL)	6	8	8	24	29	12

c. *Specimen Sampling and Handling:*

- i. Thirty specimens having CD4 counts across the AMR were prepared within 8 hours and held at room temperature for 24, 48 and 72 hours and prepared again in

order to verify the expected stability as reported in the literature. Specimens held 24 and 48 hours were refrigerated and tested again after an additional 24 and 48 hours. The acceptance criteria for the reported parameters are as follows:

Analyte / Units of Measure	Stability Limits
CD3+/CD4+ count, cells/ μ L	± 30 cells/ μ L or $\pm 10\%$, whichever is greater
CD3+/CD4+ %	± 2 percentage points
CD3+/CD8+ count, cells/ μ L	± 45 cells/ μ L or $\pm 15\%$, whichever is greater
CD3+/CD8+ %	± 3 percentage points
Total CD3+ count, cells/ μ L	± 45 cells/ μ L or $\pm 15\%$, whichever is greater
Total CD3+ %	± 3 percentage point drift
CD3-/CD56+ count, cells/ μ L	± 45 cells/ μ L or $\pm 15\%$, whichever is greater
CD3-/CD56+ %	± 2 percentage points
CD19+ count, cells/ μ L	± 45 cells/ μ L or $\pm 15\%$, whichever is greater
CD19+ %	± 2 percentage points

All parameters were verified to demonstrate the claimed stability of 24 hours for whole blood stored at room temperature and 24 hours for prepared specimens stored at refrigerated temperature.

- ii. Twenty five specimens having CD4 counts across the AMR were prepared within 8 hours and held at room temperature for 24 and 32 hours and prepared again in order to verify the expected stability as reported in the literature. Twenty three specimens prepared as above were also stored an additional 48 hours at refrigerated temperatures and analyzed using the automated and manual methods. The acceptance criteria were as stated above. All parameters were verified to demonstrate the claimed stability of 24 hours for whole blood stored at room temperature and 2 hours for prepared specimens stored at room temperature.

d. *Laser Performance:*

Laser performance was assessed on two (2) Navios Flow Cytometers using Flow-Check Pro Fluorospheres and it was demonstrated that integral signal intensity does not vary more than $\pm 5\%$ from the average integral signal channel number obtained over a period of 24 hours for Short-term Laser Performance and 8 days for Long-term Laser.

e. *PrepPlus 2 Specimen Processor:*

Studies were performed to assess the accuracy of the PrepPlus 2 specimen processor according to procedures detailed in CLSI EP15. Forty specimens were processed with the PrepPlus 2 and compared to those prepared with manual pipette. The acceptance criteria for the study are as follows:

Analyte / Units of Measure	Accuracy Specifications
Cell count (Total CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/CD56+, CD19+)	0-300 cells/ μ L: ± 40 bias >300 cells/ μ L: $\pm 13\%$ bias
Percent (Total CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/CD56+, CD19+)	0-40%: ± 1.5 percentage point bias >40%: ± 2.5 percentage point bias

The results demonstrated that PrepPlus 2 performs comparably to manual pipette for sample preparation when used with the Navios flow cytometer and tetraCHROME reagents and Flow Count for absolute count.

f. Single versus Dual Platform Method Comparison:

Forty one specimens (abnormal and normal) were selected for study. Absolute counts were determined using both Flow-Count beads (single platform) and compared to the absolute counts as calculated as the product of the subset percentage and the lymphocyte count from the LH 780 Hematology Analyzer (K061616) (dual platform). The methods were considered equivalent if the absolute value of the upper or lower 95 % confidence limit of the bias is statistically smaller than the allowable difference between the two methods ($\pm 10\%$). The two methods met the acceptance criteria for all reported parameters.

K. Proposed Labeling:

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

L. Conclusion:

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