510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

k141932

B. Purpose for Submission:

New assay and instrument

C. Measurand:

Lymphocyte percentage and absolute counts for CD3+, CD3+CD4+,CD3+CD8+, CD3-CD19+, CD3-CD56+ and/or CD16+, CD45+ Low SS, and CD45+ (absolute count only) cells.

D. Type of Test:

Qualitative and quantitative flow cytometric immunoassays

E. Applicant:

Beckman Coulter, Inc.

F. Proprietary and Established Names:

AQUIOS CL Flow Cytometer
AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5
AQUIOS Tetra-2+ Panel CD45-FITC/(CD56+CD16)-RD1/CD19-ECD/CD3-PC5
AQUIOS Lysing Reagent Kit
AQUIOS Immuno-Trol and Immuno-Trol Low Cells

G. Regulatory Information:

1. Regulation section:

   21 CFR §864.5220 - Automated differential cell counter
   (Flow cytometer, Lysing Reagent Kit, Tetra-1 Panel, Tetra-2+ Panel)

   21 CFR §864.8625 - Hematology quality control mixture
   (Immuno-Trol and Immuno-Trol Low Cells)

2. Classification:

   Class II

3. Product code:

   OYE, flow cytometric reagents and accessories
   JPK, mixture, hematology quality control

4. Panel:

   81 Hematology

H. Intended Use:

1. Intended use(s):

   AQUIOS CL Flow Cytometry System Indications for Use:

   The AQUIOS CL Flow Cytometer is intended for use with in vitro diagnostic flow cytometric applications using up to four fluorescent detection channels using a blue (488 nm) laser, two light scatter detection channels and electronic volume (EV). It is used in conjunction with the following reagents and software package.

   AQUIOS Tetra-1 Panel and AQUIOS Tetra-2+ Panel monoclonal antibody reagents are for use on the AQUIOS CL Flow Cytometer with peripheral whole blood for immunophenotyping. These reagents are indicated for use in the immunologic assessment of patients having, or suspected of having, immune deficiency. These reagents provide identification and enumeration of:

   - AQUIOS Tetra-1 Panel Monoclonal Antibody Reagent
     - Total CD3+, CD3+CD4+,CD3+CD8+, CD3+CD4+/CD3+CD8+ (ratio only) lymphocyte percentages and absolute counts.
     - CD45+ absolute count
     - CD45+ Low SS (lymphocytes) percentage and absolute count

   - AQUIOS Tetra-2+ Panel Monoclonal Antibody Reagent
     - Total CD3+, CD3-CD19+, CD3-CD56+ and/or CD16+ lymphocyte percentages and absolute counts.
     - CD45+ absolute count
     - CD45+ Low SS (lymphocytes) percentage and absolute count

   AQUIOS Flow Cytometry Software may be run on an independent computer workstation for off-line analysis of results generated by the AQUIOS CL Flow Cytometer with the monoclonal antibody reagents listed above. The off-line analysis must be performed in accordance with the product labeling.

   AQUIOS Tetra-1 and Tetra-2+ Monoclonal Antibody Reagents Indications for Use:
   AQUIOS Tetra-1 Panel and AQUIOS Tetra-2+ Panel monoclonal antibody reagents are for use on the AQUIOS CL Flow Cytometer with peripheral whole blood for immunophenotyping. These reagents are indicated for use in the immunologic assessment

of patients having, or suspected of having, immune deficiency. These reagents provide identification and enumeration of:

- AQUIOS Tetra-1 Panel Monoclonal Antibody Reagent
    - Total CD3+, CD3+CD4+,CD3+CD8+, CD3+CD4+/CD3+CD8+ (ratio only) lymphocyte percentages and absolute counts.
    - CD45+ absolute count
    - CD45+ Low SS (lymphocytes) percentage and absolute count

- AQUIOS Tetra-2+ Panel Monoclonal Antibody Reagent
    - Total CD3+, CD3-CD19+, CD3-CD56+ and/or CD16+ lymphocyte percentages and absolute counts.
    - CD45+ absolute count
    - CD45+ Low SS (lymphocytes) percentage and absolute count

AQUIOS Immuno-Trol Cells Indications for Use:
AQUIOS IMMUNO-TROL Cells are assayed, lysable whole blood quality control product for immunophenotyping analysis using monoclonal antibody reagents and flow cytometry. It provides a positive cell control that is processed in the same manner as a whole blood sample. This allows verification of instrument and reagent performance. It also verifies the methods used for staining targeted cells, lysing erythrocytes, and analyzing samples by the AQUIOS CL Flow Cytometer.

AQUIOS IMMUNO-TROL Low Cells are assayed, lysable whole blood quality control product for immunophenotyping analysis using monoclonal antibody reagents and flow cytometry. It provides a positive cell control that is processed in the same manner as a whole blood sample. This allows verification of instrument and reagent performance. It also verifies the methods used for staining targeted cells, lysing erythrocytes, and analyzing samples by the AQUIOS CL Flow Cytometer.

AQUIOS Lysing Reagent Kit Indications for Use:
AQUIOS Lysing Reagent Kit is used as part of the AQUIOS flow cytometer system. The kit consists of two reagents used by AQUIOS flow cytometers to prepare whole blood samples for analysis of white blood cells.

2. Indication(s) for use:

    Same as Intended Use

3. Special conditions for use statement(s):

    For prescription use only

4. Special instrument requirements:

    All system components above must be used together.

I. Device Description:

The AQUIOS CL Flow Cytometry System is composed of the following components:

- AQUIOS CL Flow Cytometer
- AQUIOS System Software
- AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5
- AQUIOS Tetra-2+ Panel CD45-FITC/(CD56&CD16)-RD1/CD19-ECD/CD3-PC5
- AQUIOS Immuno-Trol Cells
- AQUIOS Immuno-Trol Low Cells
- AQUIOS Lysing Reagent Kit

J. Substantial Equivalence Information:

1. Predicate device name(s):
   CaliBRITE 3 Color and FACSComp
   CaliBRITE APC beads CaliBRITE 4 kit (unlabeled, FITC-, PE-,PerCP- and APC-labeled CaliBRITE beads), and FACSComp software (FACSCalibur)
   MultiTEST CD3/CD8/CD45/CD4
   Multitest CD3/CD16+56/CD45/CD19 Reagent and Multitest IMK Kit Lysing Solution with BD TruCount Tubes
   Immuno-Trol Control Cells
   Immuno-Trol Low Cells

2. Predicate 510(k) number(s):
   k961623
   k973483
   k974360
   k980858
   k984216
   k013842

3. Comparison with predicate:

   AQUIOS Flow Cytometric System

Similarities		
Item	AQUIOS Flow Cytometric System	FACSCalibur with CaliBRITE APC, CaliBRITE 4 kit and MultiTEST
Assay Methodology	Flow Cytometry	Same
Detection/Assay Principle	Immunofluorescence	Same
Specimen Type	EDTA anticoagulated whole blood	Same
Assay Reagents	AQUIOS Tetra: Two tube monoclonal antibody reagent assay: CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 CD45-FITC/ (CD56+CD16)-RD1/ CD19-	BD Trucount: Two tube antibody reagent assay: CD3-FITC/CD8-PE/CD45-PerCP/CD4-APC CD3-FITC/(CD16+CD56)-PE/CD45-

Similarities		
Item	AQUIOS Flow Cytometric System	FACSCalibur with CaliBRITE APC, CaliBRITE 4 kit and MultiTEST
	ECD/CD3-PC5	PerCP/CD19-APC
Sample Analysis	Automated gating of cellular populations	Same
Software	Patient and control data management, stores, permits review and reports to LIS	Same
Quality Control	Two levels, stabilized whole blood	Same
Result Reporting	Software assisted report generation	Same

Differences		
Item	AQUIOS Flow Cytometric System	FACSCalibur with CaliBRITE APC, CaliBRITE 4 kit and MultiTEST
Intended Use	The AQUIOS CL Flow Cytometer is intended for use with in vitro diagnostic flow cytometric applications using up to four fluorescent detection channels using a blue (488 nm) laser, two light scatter detection channels and electronic volume (EV). It is used in conjunction with the following reagents and software package. AQUIOS Tetra-1 Panel and AQUIOS Tetra-2+ Panel monoclonal antibody reagents are for use on the AQUIOS CL Flow Cytometer with peripheral whole blood for immunophenotyping. These reagents are indicated for use in the immunologic assessment of patients having, or suspected of having, immune deficiency. These reagents provide identification and enumeration of: - AQUIOS Tetra-1 Panel Monoclonal Antibody Reagent: Total CD3+, CD3+CD4+,CD3+CD8+, CD3+CD4+/ CD3+CD8+ (ratio only) lymphocyte percentages and absolute counts. CD45+ absolute count, CD45+ Low SS (lymphocytes) percentage and absolute count AQUIOS Tetra-2+ Panel Monoclonal Antibody Reagent: Total CD3+, CD3-CD19+, CD3-CD56+ and/or CD16+ lymphocyte percentages and absolute counts. CD45+ absolute count, CD45+ Low SS	The BD Multitest IMK kit is a four-color direct immunofluorescence reagent kit for use with a suitably equipped flow cytometer to identify and determine the percentages and absolute counts of the following mature human lymphocyte subsets in erythrocyte-lysed whole blood: T lymphocytes (CD3+), B lymphocytes (CD19+), helper/inducer T lymphocytes (CD3+CD4+), suppressor/ cytotoxic T lymphocytes (CD3+CD8+), and natural killer (NK) lymphocytes (CD3-CD16+ and /or CD56+). BD Trucount tubes are used for determining absolute counts. BD Multitest reagents and BD Trucount tubes can be used with the BD FACS Loader.

Differences		
Item	AQUIOS Flow Cytometric System	FACSCalibur with CaliBRITE APC, CaliBRITE 4 kit and MultiTEST
	(lymphocytes) percentage and absolute count AQUIOS Flow Cytometry Software may be run on an independent computer workstation for off-line analysis of results generated by the AQUIOS CL Flow Cytometer with the monoclonal antibody reagents listed above. The off-line analysis must be performed in accordance with the product labeling.	
Product Code	OYE	GKZ
Signal Detection	FS Diode SS Solid State Detector (488nm LP) FL1 (FITC) 525 nm BP FL2 (RD1) 575/30nm BP FL3 (ECD) 620/30nm BP FL4 (PC5) 695/30nm BP	FSC Diode SSC 488/10 nm FL1 (FITC) 525/30nm FL2 (PE) 575/30nm FL3 (PerCP) 620/30nm LP FL4 (APC) 675/30nm
Detectors	Seven (EV. FS. SS. FL1-FL4)	Six (FS. SS. FL1-FL4)
Lasers	488nm solid state laser	488nm argon ion and 635 diode laser
System Configuration	AQUIOS System with Reagent Cart and All-in-One Touch Screen Computer loaded with AQUIOS System Software, Database, and Tetra Tests (Tetra 1, Tetra 2, and Tetra Combo)	BD FACSCalibur with FACS Loader Option and BD FACStation™ Mac Pro computer and Cell Quest SW
Sample Preparation	Automated – onboard	Manual
Specimen Identification	Barcode or manual entry	Manual entry
Standardization	Fixed settings with daily verification	Automated daily voltage/gain and compensation adjustments
Quantification	Syringe-based (volumetric)	Bead-based
Off-line Analysis	Offline AQUIOS system software	BD MULTISet and BD Cell Quest Pro
Software/ Operating System	AQUIOS System Software/ Microsoft Windows 7	FACSComp/ Mac OS9
Sample Vessel	96 well plate	daughter tube
Antibody Incubation	15 minutes- 45 minutes	15 minutes
Lyse Reagent	AQUIOS Lysing Reagent Kit	BD FACS Lysing solution
Lyse Incubation	$\leq$3 minutes	15 minutes
Specimen Stability	24 hours, room temperature	48 hours, room temperature
Prepared sample stability	3 minutes	24 hours
Antibody closed	150 days	As labelled

Differences		
Item	AQUIOS Flow Cytometric System	FACSCalibur with CaliBRITE APC, CaliBRITE 4 kit and MultiTEST
vial stability		
Antibody open vial stability	30 days	Not claimed
Antibody on-board stability	72 hours cumulative, 8 hours continuous	n/a

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

1. FDA Class II Special Controls Guidance Document: Premarket Notifications for Automated Differential Cell Counters.
2. CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline;
3. CLSI H26-A2, Validation, Verification, and Quality Assurance of Automated Hematology Analyzers, Approved Guideline
4. CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline
5. CLSI EP09-A3, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline.
6. CLSI EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline
7. CLSI EP25-A; Evaluation of Stability of In Vitro Diagnostic Reagents. Approved Guideline.
8. CLSI EP28-A3c Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline

L. Test Principle:

Immunofluorescence (and electronic volume sensing) assay for identification and enumeration of monoclonal antibody labeled leukocytes. Labeled cells in suspension are passed single file through a laser beam and the resultant scattered and fluoresced light is separated with dichroic filters and quantified with photomultiplier tubes to determine the proportion and number of cells bearing the labeled antibodies. Volumetric metering of the sample permits single platform determination of absolute cell count.

The AQUIOS CL Flow Cytometer is a flow cytometer that has detectors for 4-color analysis. In addition to detection of fluorescence parameters, the system also has the ability to detect relative cell diameter using Forward Scatter (FS), relative cell granularity using Side Scatter (SS), and relative electronic volume (EV) using the Coulter Principle. The AQUIOS CL 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 AQUIOS System Software is designed for the AQUIOS CL flow cytometer. It includes the algorithms and test definitions that provide automated analysis and results for AQUIOS Tetra-1 and 2+ reagents; this application cannot be modified by the user.

The AQUIOS CL Flow Cytometer uses on-board sample preparation as part of the overall system

workflow. The AQUIOS Lysing Reagent Kit is comprised of two ready to-use reagents: Reagent A lyses the red blood cells, Reagent B quenches the solution, slowing the lyse reaction down in preparation for analysis.

The AQUIOS Flow Cytometry System also offers an optional standalone offline workstation. This workstation is identical to the workstation that is physically connected to the instrument and can be used for off-line analysis of results generated by the AQUIOS CL Flow Cytometer with AQUIOS Tetra-1 and Tetra-2+ reagents and AQUIOS System software according to the product labeling. Samples are aliquotted, stained, incubated and lysed onboard using a single-use reagent cassette. AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 reagent provides identification and enumeration of CD3+/CD4+, CD3+/CD8+, and CD3+ lymphocyte percentages and absolute counts in peripheral whole blood. AQUIOS Tetra-2+ Panel CD45-FITC/ (CD56+CD16)-RD1/CD19-ECD/CD3-PC5 provides identification and enumeration of CD3+, CD3-/CD19+ and CD3-/CD56+ and/or CD16+ lymphocyte percentages and absolute counts in peripheral whole blood.

AQUIOS Immuno-Trol and Immuno-Trol Low Cells are assayed, lysable whole blood quality control product for immunophenotyping analysis using monoclonal antibody reagents and flow cytometry. They provide a positive cell control that is processed in the same manner as a whole blood sample. This allows verification of instrument and reagent performance. It also verifies the methods used for staining targeted cells, lysing erythrocytes, and analyzing samples by the AQUIOS CL Flow Cytometer.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Assay Repeatability and Reproducibility Using Control Material
   Assay reproducibility was assessed using two levels of control material [AQUIOS Immuno-Trol Cells (IT), and AQUIOS Immuno-Trol Low Cells (ITL)]. Lot variability of AQUIOS Tetra and AQUIOS Lyse was incorporated and assessed as part of the overall variability of the study design.
   Three lots each of Tetra-1 reagent, Tetra-2+ reagent, and Lyse were tested. Each control level was run in duplicate twice each day (morning and afternoon) for a minimum of 20 days. A new vial of control material was opened each day of testing. The test was executed on three AQUIOS CL flow cytometers, with one at each of the three test sites. Less than 1% of results failed the quality parameters. As instructed in the label, they were excluded from the analysis; in laboratory practice these samples would not be resulted and, as instructed in the labeling, would be re-run on the AQUIOS CL Flow Cytometer.

   Sponsor's Acceptance Criteria:

Parameter:	CD45+		Total CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/CD56+CD16+, CD3-/CD19+		Total CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/CD56&CD16+, CD3-/CD19+	
	≤ 5000 cells/µL	>5000 cells/µL	≤ 20%	> 20%	< 300 cells/µL	≥ 300 cells/µL
Repeatability (%CV)	≤ 5%	≤ 3%	≤ 10%	≤ 5%	≤ 10%	≤ 5%
Reproducibility (%CV)	≤ 9%	≤ 4%	≤ 15%	≤ 10%	≤ 15%	≤ 10%

Marker	CD45+Low SS %				CD45+Low SS cells/µL			
Level	≤5%	>5-<25%	25-50%	>50%	≤300	>300 - 300	1300-2600	>2600
Repeatability (%CV)	≤20%	≤10%	≤5%	≤3.5%	≤20%	≤10%	≤50%	<3.5%
Reproducibility (%CV)	≤15%		≤7%		≤15%		≤7%	

Assay Precision Results:

					Repeatability		Between Runs	Between Days	Between Sites	Total
Level	Tube	Analyte	Unit	N	mean	CV%	CV%	CV%	CV%	CV%
IT	AQUIOS Tetra-1	CD3+	%	311	72.4	1.2	0.6	0.3	0.2	1.4
			Cells/µL	311	831	2.7	1.3	<1%	1.4	3.3
		CD3+CD4+	%	311	48.4	1.8	0.6	<1%	0.1	1.9
			Cells/µL	311	555	2.9	1.6	<1%	1.3	3.6
		CD3+CD8+	%	311	22.2	3.1	0.5	0.2	0.3	3.2
			Cells/µL	311	254	3.9	0.5	0.5	1.8	4.4
		CD45+	Cells/µL	311	4845	2.2	1.5	<1%	1.5	3.0
		CD45+ Low SS	%	311	23.7	1.6	0.5	<1%	0.2	1.7
			Cells/µL	311	1147	2.9	1.3	<1%	1.3	3.4
	AQUIOS Tetra-2+	CD3+	%	311	72	1.4	0.3	0.5	0.4	1.6
			Cells/µL	311	833	2.8	1.3	<1%	2.2	3.8
		CD3-CD19+	%	311	13.7	3.4	0.7	<1%	1.7	3.9
			Cells/µL	311	158	4.4	1.3	<1%	3.1	5.5
		CD3-CD56+/CD16+	%	311	12.3	5.3	<1%	1.5	0.7	5.5
			Cells/µL	311	142	6.7	0.5	1.5	2.6	7.3
IT Low	AQUIOS Tetra-1	CD3+	%	307	58.0	2.1	0.6	0.5	0.1	2.2
			Cells/µL	307	388	3.1	1.4	<1%	1.5	3.7
		CD3+CD4+	%	307	18.3	3.8	1.0	1.5	0.1	4.2
			Cells/µL	307	123	4.5	1.9	<1%	0.6	5.0
		CD3+CD8+	%	307	35.6	3.1	0.7	<1%	0.8	3.2

					Repeatability		Between Runs	Between Days	Between Sites	Total
Level	Tube	Analyte	Unit	N	mean	CV%	CV%	CV%	CV%	CV%
			Cells/µL	307	238	9.2	1.6	<1%	2.0	4.7
		CD45+	Cells/µL	307	5064	2.3	1.3	<1%	1.6	3.1
		CD45+ Low SS	%	307	13.2	2.2	1.1	<1%	0.3	2.5
			Cells/µL	307	669	3.2	1.4	0.2	1.2	3.7
	AQUIOS Tetra-2+	CD3+	%	307	57.5	2.1	0.6	0.2	0.5	2.2
			Cells/µL	307	387	2.9	1.6	0.5	1.6	3.7
		CD3-CD19+	%	307	17.3	4.5	<1%	<1%	0.0	4.5
			Cells/µL	307	117	5.1	<1%	<1%	1.2	5.2
		CD3-CD56+/CD16+	%	307	23.7	4.3	<1%	0.6	0.0	4.3
			Cells/µL	307	159	5.8	1.2	<1%	1.2	6.0

At all levels of control material tested, all markers met the sponsor's acceptance criteria for repeatability and reproducibility (between runs, between days, between sites, and total).

Assay Repeatability using Whole Blood:

The study included 82 whole blood specimens covering the CD3+/CD4+ measuring range with emphasis on the medical decision points. The whole blood specimens were prepared in replicates of five with AQUIOS Tetra reagents (AQUIOS Tetra-1 and AQUIOS Tetra-2+) run as a Tetra Combo panel. The test was executed on four AQUIOS CL flow cytometers at four test sites. Data were analyzed and presented to demonstrate the degree of imprecision across the measuring range (data not depicted) and were further summarized to illustrate the imprecision by percentile of results. The sponsor's acceptance criteria for repeatability are the same as listed for reproducibility, above. The results of the repeatability study using whole blood samples are listed in the following table:

				Repeatability		95% CI	
Tube	Analyte	Unit	Percentile	Mean	CV%	Lower	Upper
AQUIOS Tetra-1	CD3+	%	25^{th}	62.2	1.6	1.3	1.9
			50^{th}	72.0	1.4	1.1	1.7
			75^{th}	78.8	1.3	1.0	1.6
		Cells/µL	25^{th}	364.4	3.8	3.3	4.2
			50^{th}	612.3	3.0	2.5	3.4
			75^{th}	1061	2.3	1.9	2.7
	CD3+CD4+	%	25^{th}	13.1	4.6	4.1	5.1
			50^{th}	25.1	3.1	2.6	3.5
			75^{th}	41.9	2.3	1.8	2.7
		Cells/µL	25^{th}	76.8	7.0	6.3	7.6
			50^{th}	207.9	4.4	3.8	5.0
			75^{th}	415	3.2	2.6	3.8
	CD3+CD8+	%	25^{th}	19.8	5.3	3.9	6.7
			50^{th}	38.8	3.3	2.0	4.6
			75^{th}	52.7	2.6	1.4	3.9

				Repeatability		95% CI	
Tube	Analyte	Unit	Percentile	Mean	CV%	Lower	Upper
		Cells/µL	25th	158.6	4.1	3.0	5.1
			50th	377.6	2.0	1.4	2.7
			75th	654	1.3	0.8	1.8
	CD45+	Cells/µL	25th	3652.8	2.4	2.1	2.7
			50th	4875.9	2.3	2.1	2.6
			75th	7163.6	2.2	1.9	2.5
	CD45+ Low SS	%	25th	12.0	2.6	2.3	3.0
			50th	20.0	1.9	1.6	2.2
			75th	570.0	1.5	1.2	1.7
		Cells/µL	25th	570.0	3.7	3.3	4.1
			50th	950.1	2.9	2.5	3.3
			75th	1465.8	2.3	2.0	2.7
AQUIOS Tetra-2+	CD3+	%	25th	63.0	1.6	1.3	1.8
			50th	71.6	1.4	1.2	1.6
			75th	79.0	1.3	1.1	1.5
		Cells/µL	25th	362.0	3.3	3.0	3.6
			50th	601.6	2.8	2.6	3.1
			75th	1055.4	2.4	2.1	2.7
	CD3- CD19+	%	25th	7.9	7.5	6.2	8.8
			50th	11.6	6.4	5.1	7.7
			75th	18.8	5.2	3.4	6.7
		Cells/µL	25th	61	80.1	7.0	9.1
			50th	114	6.0	4.9	7.0
			75th	180	4.8	3.8	5.7
	CD3- CD56+/ CD16+	%	25th	8.1	5.9	4.8	7.0
			50th	12.4	4.5	3.6	5.5
			75th	20.1	3.4	2.5	4.3
		Cells/µL	25th	71.4	5.8	5.0	6.6
			50th	128.7	4.4	3.7	5.1
			75th	213.6	3.5	2.8	4.1

At all levels of whole blood tested, all markers met the sponsor's acceptance criteria for repeatability.

The repeatability data were also analyzed to assess performance of CD3+CD4+ cells/µL at medical decision points. The sponsor's acceptance criteria were that CV's are <10% for values <500 cells/µL and <5% for values >500 cells/µL. The results which are presented in the following table demonstrate that the manufacturer's acceptance criteria were met at all levels.

Tube	Analyte	Unit	Level	CV%	Lower CI	Upper CI
AQUIOS Tetra-1	CD3+CD4+	Cells/µL	50	8.5	7.8	9.2
			100	6.1	5.6	6.7

			200	4.5	3.9	5.0
			500	2.9	2.3	3.5

Lot-to-lot Reproducibility:

Absolute count of CD45+, as well as percent positive and absolute counts of CD45+ Low SS and the lymphocyte subsets (CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/CD56+CD16+, CD3- /CD19+) were analyzed from three lots of each monoclonal antibody (Tetra-1, Tetra-2+) tested in a minimum of three replicates for up to 40 days on a single AQUIOS CL flow cytometer with a single lot of AQUIOS Immuno-Trol (IT) and AQUIOS Immuno-Trol Low (ITL). The sponsor's acceptance criteria for reproducibility are as stated for precision and reproducibility.

					Within Lot		Between Lots	Total
Level	Tube	Analyte	Unit	N	mean	CV%	CV%	CV%
IT	AQUIOS Tetra-1	CD3+	%	219	73	1	1	1
			Cells/µL	219	804	4	<1%	4
		CD3+CD4+	%	219	49	2	1	2
			Cells/µL	219	535	5	<1%	5
		CD3+CD8+	%	219	22	3	1	3
			Cells/µL	219	225	5	<1%	5
		CD45+	Cells/µL	219	4923	4	<1%	4
		CD45+ Low SS	%	219	22	2	1	2
			Cells/µL	219	1098	4	<1%	4
	AQUIOS Tetra-2+	CD3+	%	248	73	1	<1%	1
			Cells/µL	248	861	3	<1%	3
		CD3-CD19+	%	248	15	3	1	3
			Cells/µL	248	171	4	1	4
		CD3-CD56+/CD16+	%	248	11	5	1	5
			Cells/µL	248	126	6	1	6
IT Low	AQUIOS Tetra-1	CD3+	%	156	58	2	<1%	2
			Cells/µL	156	367	5	<1%	5
		CD3+CD4+	%	156	18	4	<1%	4
			Cells/µL	156	112	6	<1%	6
		CD3+CD8+	%	156	37	3	<1%	3
			Cells/µL	156	233	5	<1%	5
		CD45+	Cells/µL	156	4930	4	<1%	4
		CD45+ Low SS	%	156	13	2	<1%	2
			Cells/µL	156	638	5	<1%	5
	AQUIOS Tetra-2+	CD3+	%	120	62	2	<1%	2
			Cells/µL	120	397	3	1	3
		CD3-CD19+	%	120	19	4	4	4
			Cells/µL	120	121	5	2	5
		CD3-CD56+/CD16+	%	120	18	6	1	6
			Cells/µL	120	113	7	1	8

Results of the lot-to-lot reproducibility study met the sponsor's acceptance criteria and demonstrated acceptable reproducibility.

Laser stability:
Both short-term laser performance (e.g. within-day stability; measured signals at 30 min, 3 hr, 6 hr, 8 hr, 24 hr) and long-term laser performance (e.g. between-day stability; measured signals at 15 minutes, 30 minutes, 3 hours, 6 hours, and 8 hours for a period of five (5) days) were conducted on two AQUIOS CL instruments. The sponsor's acceptance criteria of mean channel of the integral signal intensity does not vary more than ± 10% from the average signal channel number obtained over a period of 24 hours for short-term laser performance, and five days for long-term laser performance and the HPCV of the integral signals is ≤ 3.0 % was met for each of the instruments tested.

b. Linearity/assay reportable range:

Linearity of fluorescence signals:
Instrument photomultiplier tube linearity was assessed in triplicate with SPHERO™ Rainbow Calibration Particles (RCPs, Spherotech, Chicago, IL). The study was conducted on three (3) AQUIOS CL flow cytometers at Beckman Coulter, Miami, Florida. The sponsor's acceptance criteria of $R^2 > 0.900$ for FL1, FL2, FL3, and FL4 was met for all instruments tested.

Assay linearity:
White blood cell pools were mixed with red blood cells to create specimens having concentrations of lymphocytes that spanned the measuring range. Each of the concentrations was prepared and analyzed in triplicate on three AQUIOS CL flow cytometers as a Tetra Combo panel with AQUIOS Tetra-1 and AQUIOS Tetra-2+ reagents. Linearity of all reported analytes was determined on 3 separate instruments using the method described in CLSI EP06-A2, Evaluation of the Linearity of Quantitative Measurement Procedures. Linearity was evaluated by fitting linear and non-linear (quadratic and cubic) models and assessing that the deviations from linearity (difference between the non-linear and linear fits) were within the acceptance criteria. Deviations from linearity were calculated for the markers that were not statistically linear and were compared to the linearity acceptance limits. The sponsor's acceptance criteria were:

$R^2 \geq 0.95$;
For result values in the range 0-300, the acceptable bias is ± 30 cells/µL
For result values >300, the acceptable bias is ± 10%

Using these criteria, the linear range was determined for each phenotypic subset and the results are listed in the following table:

AQUIOS Tetra Reagent	Marker	Linearity Ranges cells/µL

AQUIOS Tetra-1	Total CD3+	53 – 4,798
	CD3+/CD4+	34 – 3,031
	CD3+/CD8+	20 – 1,658
	CD45	312 – 26,967
	CD45+ Low SS	76 – 6,988
AQUIOS Tetra-2+	Total CD3+	53 – 4,798
	CD3- /CD56 and/or CD16+	9 – 1,028
	CD3-/CD19+	13 – 1,099

Measuring Range

The claimed analytical measuring range was determined by taking into account the Linearity results for the high end (rounded down to the nearest hundred), and using the higher value between the low linearity number or LoQ number and then rounding to the nearest point above the aforementioned numbers in increments of five for the low value.

AQUIOS Tetra Reagent	Marker	Measuring Range cells/µL
AQUIOS Tetra-1	Total CD3+	55 – 4,700
	CD3+/CD4+	35 – 3,000
	CD3+/CD8+	45 – 1,600
	CD45	350 – 26,500
	CD45+ Low SS	80 – 6,500
AQUIOS Tetra-2+	Total CD3+	55 – 4,700
	CD3- /CD56 and/or CD16+	20 – 1,000
	CD3-/CD19+	25 – 1,000

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

Stability:

Reagent and control materials were subjected to conditions as stated below and tested for drift relative to baseline values. Details of each study are provided as follows:

AQUIOS Tetra Reagents:

AQUIOS Tetra Reagents Closed Vial Stability:

Four lots each of AQUIOS Tetra-1 and Tetra-2+ were stored at 2-8°C and tested for closed vial (CV) stability over ten (10) months. At each time point, one new vial of each Tetra reagent lot was tested on the AQUIOS CL flow cytometer with AQUIOS Immuno-Trol (IT) and Immuno-Trol Low (ITL) controls. Triplicate assessments were made at baseline followed by at least 10 replicates at subsequent time points.

AQUIOS Tetra Reagents Closed Vial Stability with temperature stress:

To study the effects of shipping, one lot of each of the AQUIOS Tetra-1 and Tetra-2+ reagents was exposed to a summer (22 to 39°C) and winter (-20 to +22°C) shipping profile after the T0 time point testing, then returned to 2-8°C storage and included in subsequent testing points. Triplicate assessments were made at baseline followed by at least 10 replicates at subsequent time points.

AQUIOS Tetra Reagents Open Vial Stability:
For open vial stability testing, new vials of each control lot were opened towards the end of the products closed vial stability claim (>150 days). Ten replicates were tested at each time point over the course of 30 days.

AQUIOS Tetra Reagents On-board Stability:
To test on-board stability, once the vials reached their designated closed and open vial dating, one vial of each of three lots was subjected to thermal cycling and testing on the AQUIOS CL flow cytometer. At each on-board stability test point including on board T0, each Tetra reagent lot was tested on the AQUIOS CL flow cytometer with IT and ITL testing 10 replicates for each control.

The acceptance criteria for all stability studies measuring drift are depicted in the following tables:

	Count/µL						
	CD3+ CD4+	**CD3+ CD8+**	**CD3- CD19+**	**CD3-CD56+ CD16+**	**CD3+**	**CD45+**	**CD45+ Low SS**
IT ± Ranges	21%	30%	27%	34%	25%	25%	34%
ITL ± Ranges	22%	30%	27%	34%	25%	25%	34%

	Percent					
	CD3+ CD4+	**CD3+ CD8+**	**CD3- CD19+**	**CD3-CD56+ CD16+**	**CD3+**	**CD45 +**
IT ± Ranges	3	6	3	5	7	5
ITL ± Ranges	4	6	3	5	7	5

Conclusions: All of the above AQUIOS Tetra-1 and Tetra-2+ stability studies passed the sponsor's acceptance criteria and so support the following closed, open, and onboard claims:

AQUIOS Tetra-1 and Tetra-2+ Stability Claims

	Closed Vial	Open Vial	On Board	
			Cumulative	Continuous
Claim	180 days	30 days	72 hours	8 hours
Storage Temperature	2-8°C	2-8°C	18-26°C (Controlled Room Temperature)	

AQUIOS Lyse Reagent:

AQUIOS Lyse Closed Vial Stability:

Three lots of each AQUIOS Lyse A and Lyse B reagents were tested for closed vial stability over six (6) months.

AQUIOS Lyse Closed Vial Stability with temperature stress:
To study the effects of shipping, one lot of each AQUIOS Lyse A and B was exposed to a summer (22 to 39°C) and winter (-20 to +22°C) shipping profile after the T0 time point testing, then returned to controlled room temperature storage and included in subsequent testing points.

AQUIOS Lyse Opened Vial Stability:
For open vial stability testing, new containers of each Lyse lot were opened towards the end of the products closed vial stability claim (>150 days). At each open vial stability test point, one container of each Lyse reagent lot was tested on the AQUIOS flow cytometer with IT and ITL gathering ten replicates for each control.

AQUIOS Lyse On-board Stability:
For on-board stability testing, containers of each Lyse lot were opened towards the end of the products closed vial stability claim (≥150 days) and held in controlled room temperature storage until they reached the open vial dating (≥23 days). Once opened, one container of each lot was subjected to thermal cycling and testing on the AQUIOS CL flow cytometer, with six to ten replicates at each time point.

Conclusions: All of the above AQUIOS Lyse stability studies passed the sponsor's acceptance criteria and so support the following closed, open, and onboard claims:

AQUIOS Lyse Stability Claims:

	Closed Vial	Open Vial	On Board	
			Cumulative	Continuous
Claim	180 days	30 days	72 hours	8 hours
Storage Temperature	18-26°C (Controlled Room Temperature)			

AQUIOS Immuno-Trol Reagent:

AQUIOS Immuno-Trol Closed Vial Reagent Stability:
Closed vial Immuno-Trol (IT) stability was assessed on four lots of AQUIOS Immuno-Trol (IT) and three lots of Immuno-Trol Low (ITL) per CLSI EP-25A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. Ten replicates were tested at each time point.

AQUIOS Immuno-Trol Temperature Stress Test:
To study the effects of shipping, one lot of IT and ITL controls was also exposed to a summer (22 to 39°C) and winter (-20 to +22°C) shipping profile after the T0 time point testing, then returned to 2-8°C storage and included in subsequent testing points.
All lots of IT and ITL and all analyses met the sponsor's acceptance criteria over the seven months tested and therefore supported the 210 day (2-8°C) stability claim.

AQUIOS Immuno-Trol Open Vial Stability:

For open vial stability testing, new vials of each control lot were opened towards the end of the products closed vial stability claim (>150 days) and tested at defined intervals over the course of 99 to 123 days. Ten replicates were tested at each time point. The acceptance criteria are depicted in the table above for closed vial stability. All lots of IT and ITL and all analyses met the sponsor's acceptance criteria and therefore support the 90 day stability claim when stored at 2-8°C.

All of the above AQUIOS Immuno-Trol stability studies passed the sponsor's acceptance criteria and so support the following closed, open, and onboard claims:

AQUIOS Immuno-Trol and Immuno-Trol Low Stability Claims

	Closed Vial	Open Vial
Claim	270 days	90 days
Temperature Storage	2-8°C	2-8°C

Fresh and Prepared Sample Stability:

Samples are automatically processed and run from within 0 to 3 minutes following staining and lysing. Specimens whose analyses are delayed beyond three minutes are flagged for rejection and reprocessing. Specimens are typically run at Time 0, however, analyses that fail are reaquired, providing the acquisition can occur within the 3 minute time frame.

In order to demonstrate sample stability, 73 specimens, including normal and clinical (HIV+) donors, spanning the analytical measuring range (AMR, CD3+/CD4+: 35-3000 cell/µL) were included in a stability study to compare performance on specimen processed within 8 hours of draw and analyzed at Time 0 following processing to specimens processed at 24 hours and analyzed 3 minutes post processing.

Sponsor's Acceptance Criteria

Marker, Units of Measure	**Stability Specifications**
CD3+/CD4+ count, cells/µL	±30 cells/µL or ±10%, whichever is greater
CD3+/CD4+, %	±2 percentage points
CD3+/CD8+ count, cells/µL	±45 cells/µL or ±15%, whichever is greater
CD3+/CD8+ %	±3 percentage points
Total CD3+ count, cells/µL	±45 cells/µL or ±15%, whichever is greater
Total CD3+, %	± 3 percentage points
CD3-/CD56+ and/or CD16+ count, cells/µL	±45 cells/µL or ±15%, whichever is greater
CD3-/CD56+ and/or CD16+, %	±2 percentage points

CD45, cells/µL	±500 cells/µL
CD45+Low SS,%	± 10%
CD45+Low SS, cells/µL	±4 percentage points

The study results met the sponsor's acceptance criteria and supports controlled room temperature (18-26°C) stability of 24 hours specimen stability and up to three minutes of prepared sample stability for the AQUIOS CL Flow Cytometry System.

d. Detection limit:

This study established the Limit of Blank, Limit of Detection and Limit of Quantitation values for each AQUIOS Tetra marker when tested on the AQUIOS CL Flow Cytometry System. Studies were performed in accordance with CLSI EP17. The study design incorporated five (5) repetitions of each of the three (3) prepared blank samples which were collected each day over four (4) days yielding a total of 60 data collection points per measuring system. A total of 180 data points were collected for the entire study with three (3) measuring systems. The study incorporated two (2) lots of each AQUIOS Tetra reagent (Tetra-1, Tetra-2+), one lot of AQUIOS Lysing Reagents, three (3) Blank samples (prepared with one (1) lot of Red Cell Pool and three (3) lots of Immuno-Trol Storage Buffer and three (3) AQUIOS CL flow cytometers. The Limit of Blank was established for each marker as the upper 95% of the data as recommended in the EP17-A2 Guideline.

Each LoB value was derived by repeated measures and represents the highest absolute count (cells/µL) expected with the 95% confidence for the corresponding marker in a sample that contains no analyte. Each LoD value represents the lowest cell count for each marker that can be detected using the AQUIOS Tetra reagents on the AQUIOS instrument with 95% confidence.

Marker	**LOB**	**LOD**	**LOQ**	**Upper 95% Confidence Limit of LOQ**
Total CD3+	8	11	16	32
CD3+/CD4+	7	10	19	30
CD3+/CD8+	7	10	19	44
CD45+	56	67	67	78
CD45+ Low SS	8	11	14	32
Total CD3+	4	6	11	20
CD3-/CD19+	0	1	12	20
CD3- /CD56+ and/or CD16+	0	1	12	18

e. Analytical specificity:

Carryover

A carryover study was performed on three (3) AQUIOS CL 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 using SPHERO^{TM} AccuCount Ultra-Rainbow Fluorescent Particles. The study measured carryover on each of 3 instruments and in each case was found to be < 0.3%.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:
In accordance with CLSI EP09-A3, a prospective study was conducted at four sites comparing the accuracy of the AQUIOS CL Flow Cytometry System to the BD FACSCalibur with specimens targeting the medically important ranges for CD4. Evaluation included the use of multiple lots of AQUIOS reagents (AQUIOS Tetra-1, Tetra-2+, and Lyse A/B) across the clinical sites. A total of 443 samples that covered the CD4 analytical measuring range, with an emphasis on the medical decision points, were tested on the test system (AQUIOS CL) and the predicate systems: FACSCalibur for assessment of T cell, B cell and NK cell phenotypes, and Beckman Coulter UniCel DxH 800 for assessment of CD45+ and CD45+Low SS. Donor specimens were comprised of 78% clinical specimens and 22% normal specimens. Clinical specimens included specimens submitted for assessments associated with HIV infection, transplant, immunosuppressive therapy (plus other conditions), while the normal specimens consisted of specimens from healthy controls and clinic specimens that were demonstrated to be hematologically normal. SAS 9.3 statistical software was used for data analysis.

The sponsor's acceptance criteria are listed in the following table:

Marker / Units of Measure	Accuracy Limits
Total CD3+ cell/µL CD3+/CD4+ cell/µL CD3+/CD8+ cell/µL	≤ 300 ± 40 cell/µL > 300 ± 13%
CD3-/CD19+ cell/µL CD3-/CD56+ and/or CD16+ cell/µL	≤ 300 ± 65 cell/µL > 300 ± 22%
Total CD3+ % CD3+/CD4+ % CD3+/CD8+ % CD3-/CD19+ % CD3-/CD56+ and/or CD16+ %	± 2.5 percentage point bias
CD45+ cells/µL	± 13%
CD45+Low SS cells/µL	± 3 percentage point bias or ±10%, whichever is greater
CD45+Low SS %	

Summary Statistics for the method comparison versus the predicate device (all sites combined) are presented in the following table:

		Means			95% CI	
Marker	Unit	Reference	AQUIOS	Difference	Lower	Upper
CD3+ (Tetra-1)	%	71.6	71.8	0.1	-0.1	0.3
	cells/µL	1146	1118	-28	-42	-15
CD3+CD4+	%	31.1	31.3	0.2	0.0	0.3

		Means			95% CI	
Marker	Unit	Reference	AQUIOS	Difference	Lower	Upper
	cells/μL	514	499	-15	-22	-8
CD3+CD8+	%	38.6	38.2	-0.4	-0.6	-0.3
	cells/μL	603	582	-20	-28	-12
CD3+ (Tetra-2+)	%	71.7	71.9	0.3	0.1	0.5
	cells/μL	1166	1106	-60	-75	-45
CD3-CD19+	%	14.0	13.6	-0.4	-0.5	-0.3
	cells/μL	226	207	-19	-23	-15
CD3-56+ and/or CD16+	%	12.9	13.2	0.3	0.1	0.4
	cells/μL	190	181	-8	-11	-5
CD45+	cells/μL	6131	5991	-140	-171	-109
CD45+ Low SS	%	27.6	27.5	-0.1	-0.2	0.0
	cells/μL	1583	1537	-46	-56	-36

Regression Statistics for the method comparison versus the predicate device (all sites combined) are presented in the following table:

			95% CI			95% CI		
Marker	**Unit**	**Slope**	Lower	Upper	**Intercept**	Lower	Upper	**Correlation**
CD3+ (Tetra-1)	%	0.96	0.95	0.98	2.70	1.52	4.05	0.99
	cells/μL	0.98	0.97	0.99	10.45	5.58	15.32	0.98
CD3+CD4+	%	1.00	0.99	1.01	0.23	-0.02	0.48	1.00
	cells/μL	0.98	0.97	1.00	2.66	-0.12	5.43	0.99
CD3+CD8+	%	0.98	0.97	0.99	0.21	-0.14	0.56	1.00
	cells/μL	0.97	0.95	0.98	4.69	1.94	7.44	0.98
CD3+ (Tetra-2+)	%	0.97	0.95	0.99	2.43	1.09	3.77	0.99
	cells/μL	0.95	0.94	0.96	9.64	5.00	14.29	0.97
CD3-CD19+	%	0.99	0.97	1.015	-0.26	-0.52	0.00	0.99
	cells/μL	0.87	0.80	0.90	9.67	-5.01	24.34	0.98
CD3-56+ and/or CD16+	%	1.00	0.97	1.03	0.28	0.08	0.63	0.99
	cells/μL	0.97	0.93	1.00	2.76	-0.04	5.55	0.98
CD45+	cells/μL	0.99	0.97	1.00	-51.43	-110.1	7.19	0.99
CD45+ Low SS	%	1.00	0.98	1.02	-0.08	-0.61	0.44	1.00
	cells/μL	0.98	0.97	0.99	-6.16	-18.7	6.34	0.99

Bias for percent positives comparisons was clinically insignificant. A negative bias for the comparison of AQUIOS to the BD FACSCalibur was observed in the absolute count data for all four (4) sites combined. This negative trend was observed for all markers, with smaller differences observed for CD3+/CD4+. This bias was clinically insignificant at the medical decision points for CD3+/CD4+ (e.g., <1 cell/μL bias at 200 cells/μL and < 2 cells/μL bias at 50 cells/μL). The bias observed for all CD markers was within the sponsor's acceptance limits.

CD45+ Low SS percentage comparisons had clinically insignificant to no bias. A negative bias was observed in the comparison of CD45+ and CD45+ Low SS absolute counts obtained from AQUIOS vs. the DxH 800 from all four (4) sites combined. The bias observed for all parameters was within the sponsor's acceptance limits.

In addition, bias was calculated at the medical decision points for CD3+CD4+ cells and the results for the combined sites for AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 can be found in the following table:

Marker	Unit	Level	Bias	95% CI	Acceptance Limit	Conclusion
CD3+CD4+	cells/ µL	50	1.8	-0.5 to 4.1	40	Pass
		100	1.0	-1.1 to 3.0	40	Pass
		200	-0.7	-3.1 to 1.7	40	Pass
		500	-5.6	-11.9 to 7.8	65	Pass

b. Matrix comparison:

K_2EDTA vs. K_3EDTA

Site selection was based on targeting sites with large HIV populations and transplant centers to cover the main screening populations for the AQUIOS Tetra tests. A total of 90 samples, covering the CD4 analytical measuring range, with emphasis on the medical decision ranges were collected in K_2EDTA and K_3EDTA anticoagulants and then analyzed on the AQUIOS CL flow cytometer in the autoloader presentation mode. Testing was performed at two sites (two instruments).

Acceptance Criteria

Marker, Units of Measure	Accuracy Limits
Total CD3+, cell/µL CD3+/CD4+, cell/µL CD3+/CD8+, cell/µL CD3-/CD19+, cell/µL CD3-/CD56+CD16+, cell/µL	≤ 300 ± 40 cell/µL > 300 ± 13% cell/µL
CD45+, cell/µL	≤ 2000 ± 100 cell/µL or 10%, whichever is greater > 2000 ± 200 cell/µL or 3%, whichever is greater
CD45+ Low SS, cell/µL	± 10 percentage point bias
Total CD3+, % CD3+/CD4+, % CD3+/CD8+, % CD3-/CD19+, % CD3-/CD56+CD16+, %	0 – 40% : ± 1.5 percentage point bias >40% : ± 2.5 percentage point bias
CD45+ Low SS, %	± 3 percentage point bias or ± 10%, whichever is greater

Regression analysis for comparison between K_2EDTA vs. K_3EDTA:

Marker	Unit	Slope	95% CI	Intercept	95% CI	r^2
CD3+ (Tetra-1)	%	0.98	0.95 to 1.01	1.87	-0.27 to 4.00	0.99
	cells/µL	0.96	0.93 to 0.99	14.81	-17.89 to 47.52	0.99
CD3+/CD4+	%	1.00	0.98 to 1.02	0.33	-0.034 to 0.70	1.00
	cells/µL	0.97	0.93 to 1.00	3.45	-4.29 to 11.20	1.00
CD3+/CD8+	%	1.00	0.99 to 1.02	0.14	-0.64 to 0.93	1.00
	cells/µL	0.95	0.92 to 0.98	14.67	-5.52 to 34.86	0.99
CD3+ (Tetra-2+)	%	0.99	0.96 to 1.02	1.00	-1.11 to 3.10	0.99
	cells/µL	0.96	0.94 to 0.99	16.21	-8.63 to 41.05	0.99
CD3-/CD19+	%	1.01	0.98 to 1.04	-0.17	-0.59 to 0.24	0.99
	cells/µL	0.97	0.94 to 0.99	0.08	-2.94 to 3.10	0.99
CD3-/CD56+ and/or CD16+	%	0.99	0.94 to 1.04	-0.12	-0.65 to 0.41	0.99
	cells/µL	0.97	0.93 to 1.02	-3.17	-8.94 to 2.59	0.99
CD45+	cells/µL	0.96	0.80 to 1.12	32.27	-727 to 791	0.99
CD45+ Low SS	%	1.00	0.75 to 1.24	0.02	-7.88 to 7.92	0.93
	cells/µL	0.97	0.95 to 1.00	-10.00	-45.03 to 23.04	0.99

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):

4. Clinical cut-off:

   Not applicable

5. Expected values/Reference range:
   The reference intervals were established according to CLSI EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory, Approved Guideline – Third Edition. One hundred sixty one (161) specimens from apparently healthy male and female subjects between 18-65 years of age, with hematologically normal CBC and differential and with no known hematological disease were selected from three (3) distinct geographical areas. Results are consistent with published values for T, B, and NK lymphocyte subsets.

	Reference Range
CD3+ cells/µL	856 - 2237
CD3+ %	58 - 84
CD3+/CD4+ cells/µL	518 - 1472

CD3+/CD4+ %	34 - 65
CD3+/CD8+ cells/µL	205 - 294
CD3+/CD8+ %	13 - 38
CD3-/CD19+ cells/µL	87 - 507
CD3-/CD19+ %	6 - 25
CD3-CD56+ and/or CD16+ %	74 - 562
CD45+ cells/µL	3897 - 9997
CD45+ Low SS %	18 – 43
CD45+ Low SS cells/µL	1198 - 2856
CD3-CD56+ and/or CD16+ cells/µL	4 - 27

N. Instrument Name:

AQUIOS CL Flow Cytometry System

O. System Descriptions:

1. Modes of Operation:
   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?
   Yes _x__ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?
   Yes _____ or No ___x___

2. Software:
   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:
   Yes ___x___ or No _____

3. Specimen Identification:
   Specimen identification can be entered either manually or with a barcode reader.

4. Specimen Sampling and Handling:
   Sample introduction is routinely accomplished by placing specimen tubes in a cassette and then loading the cassette in the autoloader. A single tube may be presented for analysis using the single-tube loader.

5. Calibration:
   Performed by manufacturer

6. Quality Control:
   AQUIOS Immuno-Trol and Immuno-Trol Low Cells are assayed, lysable whole blood quality control product for immunophenotyping analysis using monoclonal antibody reagents and flow cytometry. It provides a positive cell control that is processed in the same manner as a whole blood sample. This allows verification of instrument and reagent performance. It also verifies

the methods used for staining targeted cells, lysing erythrocytes, and analyzing samples by the AQUIOS CL Flow Cytometer.

P. Proposed Labeling:

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

Q. Conclusion:

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