510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K170974

B. Purpose for Submission:

New device

C. Manufacturer and Instrument Name:

BD Biosciences

BD FACSLyric Flow Cytometer (3−1, 4−2, 4−2−2 and 4−3−3 optical configurations) with BD FACSuite Clinical Software

D. Type of Test or Tests Performed:

Quantitative and Semi-quantitative Flow Cytometric Immunoassays

E. Systems Description:

1. Device Description:

The BD FACSLyric™ flow cytometer (3−1, 4−2, 4−2−2, and 4−3−3 optical configurations) systems consist of a flow cytometer, sheath tank, waste tank, and a computer workstation. System options include an automated FACS Universal Loader and a barcode reader.

The BD FACSLyric Flow Cytometer includes the 488 nm laser and 640 nm laser as part of four available manufactured instrument configurations.
BD FACSLyric Flow Cytometer, 3−1 configuration, 4-color/2-laser
BD FACSLyric Flow Cytometer, 4−2 configuration, 6-color/2-laser
BD FACSLyric Flow Cytometer, 4−2−2 configuration, 8-color/3-laser
BD FACSLyric Flow Cytometer, 4−3−3 configuration, 10-color/3-laser
The lower level configurations are upgradeable to higher level configurations by adding filters, photomultiplier tubes (PMTs), and a laser. Only the 488 nm laser and 640 nm lasers are utilized for cleared in vitro diagnostic (IVD) applications and only fluorescence channels 1 (FL1) through FL6 are the subject of this 510(k) submission. Seven to ten-color immunophenotyping is for research use only (RUO).

All optical configurations of the FACSLyric share the same dimensions: 22.8 inches in height by 24.93 inches in width by 22.8 inches in depth.

Accessory Reagents

BD™ FC beads 7-color kit: used to establish fluorescence compensation on the flow cytometer.

BD™ CS&T beads: used for the quality control of optics, electronics, and fluidics, and for adjusting detector voltages and fluorescence compensation on the flow cytometer.

BD Trucount™ tubes: used to determine absolute counts of leucocytes in erythrocyte-lysed whole blood.

Optional BD FACS™ Universal Loader

2. Principles of Operation:

Flow cytometers combine fluidics, optics, and electronics subsystems to measure and analyze signals emitted when particles in a liquid stream flow through a glass cuvette at which beams of laser light are directed. The scatter and fluorescence light signals from these particles provide information about cell size, internal complexity, and relative fluorescence intensity. The Instructions for Use for each BD FACSLyric instrument includes details on the system components and theory of operations.

The instruments are intended for use with cleared or approved in vitro diagnostic (IVD) assays for the identification and enumeration of human cell subsets that are indicated for use with the instrument.

3. 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 ____X____ or No _____

4. Specimen Identification:

Barcode reader or manual entry

5. Specimen Sampling and Handling:

The instruments may be used with or without the BD FACS Sample Prep Assistant III. Specimen handling should be performed according to the IVD assay intended for use

with the instruments.

6. Calibration:

   Calibration is performed with the IVD assay intended for use with the instruments.

7. Quality Control:

   Quality control is performed with the IVD assay intended for use with the instruments.

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

4. Panel:

   Hematology (81)

G. Intended Use:

1. Indication(s) for use:
   The BD FACSLyric™ flow cytometer is intended for use as an in vitro diagnostic device for immunophenotyping using up to six fluorescence detection channels and two light scatter channels using a blue (488-nm) and a red (640-nm) laser. It is intended for use with in vitro diagnostic (IVD) assays and software that are indicated for use with the instrument.

   BD Multitest™ 6-color TBNK, BD Multitest™ IMK kit, BD Multitest™ CD3/CD8/CD45/CD4, and BD Multitest™ CD3/CD16+CD56/CD45/CD19, all with

optional BD Trucount™ tubes, are intended for use on the BD FACSLyric flow cytometer with peripheral whole blood for immunophenotyping. These reagents are indicated for use in the immunological assessment of normal individuals, and patients having, or suspected of having, immune deficiency. These reagents determine the percentages and absolute counts of the following mature human lymphocyte subsets:

BD Multitest 6-color TBNK with optional BD Trucount tubes
• T lymphocytes (CD3+)
• B lymphocytes (CD19+)
• Natural killer (NK) lymphocytes (CD3–CD16+ and/or CD56+)
• Helper/inducer T lymphocytes (CD3+CD4+)
• Suppressor/cytotoxic T lymphocytes (CD3+CD8+)

BD Multitest IMK kit with optional BD Trucount tubes
• T lymphocytes (CD3+)
• B lymphocytes (CD19+)
• Natural killer (NK) lymphocytes (CD3–CD16+ and/or CD56+)
• Helper/inducer T lymphocytes (CD3+CD4+)
• Suppressor/cytotoxic T lymphocytes (CD3+CD8+)

BD Multitest CD3/CD8/CD45/CD4 with optional BD Trucount tubes
• T lymphocytes (CD3+)
• Suppressor/cytotoxic T lymphocytes (CD3+CD8+)
• Helper/inducer T lymphocytes (CD3+CD4+)

BD Multitest CD3/CD16+CD56/CD45/CD19 with optional BD Trucount tubes
• T lymphocytes (CD3+)
• Natural killer (NK) lymphocytes (CD3–CD16+ and/or CD56+)
• B lymphocytes (CD3–CD19+)

2. Special conditions for use statement(s):

   For Prescription Use Only

H. Substantial Equivalence Information:

1. Predicate device names:

   BD FACSCanto II (4-2-2 and 5-3 configurations); BD FACSCanto II (4-2 configuration); BD Multitest CD3/CD16+56/CD45/CD19 and BD Multitest IMK Kit; BD Multitest CD3/CD8/CD45/CD4; BD Multitest 6-color TBNK; BD FACS 7-Color Setup Beads; BD Multi-Check Control; BD Multi-Check CD4 Low Control; BD Trucount Tubes

2. Predicate 510(k) numbers:

   K141468; K062087; K980858; K974360; K090967; K040026; K961610; K982231; K970836

3. Comparison with predicate:

Similarities		
Item	New device BD FACSLyric 3-1, 4-2, 4-2-2 and 4-3-3 Configurations	Predicate BD FACSCanto II 4-2-2 Configuration
Intended Use	The BD FACSLyric™ flow cytometer is intended for use as an in vitro diagnostic device for immunophenotyping using up to six fluorescence detection channels and two light scatter channels using a blue (488-nm) and a red (640-nm) laser. It is intended for use with in vitro diagnostic (IVD) assays and software that are indicated for use with the instrument.	The BD FACSCanto™ II flow cytometers (4-2-2 and 5-3 configurations) function as part of a system with dedicated clinical software intended for use with cleared or approved in vitro diagnostic (IVD) assays that are indicated for use with the instrument for the identification and enumeration of human cell subsets. Only six detection channels using a blue (488 nm) and a red (633 nm) laser have been cleared for in vitro diagnostic use. For use with or without the BD FACS Sample Prep Assistant III.
Forward Scatter Detection	Photodiode with built-in 488/10 bandpass filter	Same
IVD Lasers/Excitation	Blue Laser: Blue/488 nm, 20mW	Same
Fluorescence and Side Scatter Detection	Side scatter and fluorescence • Reflective optics with single transmission bandpass filter in front of each PMT • High performance PMT modules for all fluorescence and side scatter channels • Light collected by objective lens is delivered by fiber optics to specially designed detector arrays • The cuvette flow cell is gel-coupled by refractive index-matching optical gel to the fluorescence objective lens (1.2 NA) for optimal collection efficiency	Same
Sample Preparation	Manual pipetting for the lyse/wash or lyse/no-wash methods	Same
Sample Analysis	Automated gating of cellular populations by the software and manual adjustment by the user	Same
Sample Type**	Assay-dependent	Same
**Sample Types are assay-dependent; refer to FDA cleared or approved assays for designated sample types. For use with Multitest 6-color TBNK and IMK kit, whole blood is the indicated sample type.		
Note: Seven to ten-color immunophenotyping is for research use only (RUO).		

Differences						
Item	New device BD FACSLyric 3-1, 4-2, 4-2-2 and 4-3-3 Configurations			Predicate BD FACSCanto II 4-2-2 Configuration		
Flow Cytometer Dimension	Cytometer (W x D x H) 63.3 x 57.9 x 57.9 cm (24.93 x 22.8 x 22.8 in.) Cytometer with Standard Tanks (W x D x H) 85.2 x 57.9 x 57.9 cm (33.5 x 22.8 x 22.8 in.)			Cytometer (W x D x H) 91 x 61 x 64 cm (35.7 x 24 x 25.2 in.) Fluidics Cart (W x D x H) 79 x 61 x 64 cm (31.1 x 24 x 25.2 in.)		
IVD Lasers/Excitation	Red Laser: Red/640nm, 40mW			Red Laser: Red/633nm, 17mW HeNe		
Band pass filters (BP) and Long pass mirrors (LP)	Channel	BP Filter	LP Mirror	Channel	BP Filter	LP Mirror
	SSC	488/15	ND 10	SSC	488/10	N/A
	FITC	527/32	507LP	FITC	530/30	502LP
	PE	586/42	560LP	PE	585/42	556LP
	PerCP	700/54	655LP	PerCP	670/30	655LP
	Pe-Cy7	783/56	752LP	Pe-Cy7	780/60	735LP
	APC	660/10	660/10	APC	660/20	N/A
	APC Cy-7	783/56	752LP	APC Cy-7	780/60	735LP
Fluidics	• Utilizes a vacuum driven fluidic system which draws the sheath and samples into the system • Uses FACSFlow as the sheath fluid • Uses 10% bleach solution for system cleaning • System waste products are collected in the waste container (5 L standard size)			• Utilizes positive pressure to push the sheath and samples into the system • Uses FACSFlow as the sheath fluid, together with FACS shutdown solution and FACS cleaning solution • System waste products are collected in the waste container (10 L standard size)		
Fluidics Cart	No separate wet cart for fluidics			BD FACSCanto™ fluidics cart with the incorporation of a manifold assembly and improved chemical compatibility of valve material		
Sample Introduction	Manual loading onto the tube port of the flow cytometer Automated loading through a multi-tube FACS Universal Loader (holds one 30- or 40-tube rack)			Manual loading onto the tube port of the flow cytometer Automated loading through a multi-tube FACS Loader (carousel to hold up to 40 tubes)		

Differences		
Item	New device BD FACSLyric 3-1, 4-2, 4-2-2 and 4-3-3 Configurations	Predicate BD FACSCanto II 4-2-2 Configuration
Automated Sample Presentation	BD FACS™ Universal Loader	BD FACSLoader with updated motor, pneumatic actuation, and sliding access doors
Software	BD FACSuite Clinical Software with 4- and 6-color Assay Modules	FACSCanto Clinical software with Multitest 6-color and Multitest 4-color assays included as panels within the software
Instrument Setup and Quality Control	FC Beads 7-color Kit CS&T Beads	Automated setup using BD FACSCanto™ clinical software and BD FACS 7-color setup beads
RUO Lasers (4-2-2;4-3-3)	Violet laser: Violet/405 nm, 40mW	Violet laser: Violet/405 nm, 30mW
Electronics	Two PCBAs (Printed Circuit Board Assembly). The PCBAs are responsible for converting the analog output from the detectors to electronic and ultimately serial data that get passed on to the host computer.	One consolidated data acquisition electronics board with improved preamplifier circuitry

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

CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Methods; Approved Guideline – Third Edition

CLSI EP6-A Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

CLSI EP09-A3 Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Second Edition

CLSI EP25-A Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

CLSI H26-A2 Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard - Second Edition

CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition

CLSI EP28-A3c Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition

IEC 60825-1:2007: Safety of laser products - Part 1: Equipment classification and requirements

IEC 61010-1:2010 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use - Part 1: General Requirements

J. Performance Characteristics:

All results below met the manufacturer's pre-specified acceptance criteria.

For all population subsets referred to below, the following abbreviations are used:

- $CD3^+$ = cells that that positively express CD45 and CD3
- $CD4^+$ = cells that positively express CD45 and CD3 and CD4
- $CD8^+$ = cells that positively express CD45 and CD3 and CD8
- $CD19^+$ = cells that positively express CD45 and CD19 and negatively express CD3
- $CD16^+56^+$ = cells that positively express CD45 and both CD16 and CD56 and negatively express CD3

Cleared IVD assays and reagents used to demonstrate equivalence across instrument configurations:

- BD Multitest IMK kit (IMK; cleared K980858)
- BD Multitest 6-color TBNK reagent (TBNK; cleared K090967)

1. Analytical performance:

   a. Accuracy:

   The Accuracy study was performed based on recommendations in the CLSI document *CLSI EP9-A3*, *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline- Third Edition*. The accuracy study demonstrates equivalence between the test system BD FACSLyric™ with BD FACSuite™ clinical software and the BD FACSCantoII™ (predicate) with BD FACSCanto™ clinical software. Method comparison with the predicate instrument was used to determine accuracy through comparative evaluation of the same specimen stained with the BD Multitest IMK Kit (4-color; IMK) and the BD Multitest 6-color TBNK reagent (TBNK) and analyzed in parallel with the new instrument and the predicate device. A total of 297 specimens were tested using Multitest 6-Color TBNK and 336 specimens from apparently healthy normals and HIV patients were tested with Multitest IMK kit at five clinical sites. Seven manipulated specimens were included in the analysis. Results are presented in the tables below:

Agreement Analysis CD4+ at clinical decision point of 200 cells/µL
BD Multitest 6-color TBNK Reagents

New Device FACSLyric	Predicate FACSCanto II		
	Positive (≤200)	**Negative (>200)**	**Total**
Positive(≤200)	66	0	66
Negative(>200)	3	228	231
Total	69	228	297

Positive Percent Agreement 95.7% (95%CI: 88.0−98.5)
Negative Percent Agreement 100.0% (95%CI: 98.3−100.0)

Agreement Analysis CD4+ at clinical decision point of 200 cells/µL
BD Multitest IMK kit Reagents

New Device FACSLyric	Predicate FACSCanto II		
	Positive (≤200)	**Negative (>200)**	**Total**
Positive(≤200)	78	2	80
Negative(>200)	2	254	256
Total	80	256	336

Positive Percent Agreement 97.5% (95%CI: 91.3−99.3)
Negative Percent Agreement 99.2% (95%CI: 97.2−99.8)

Predicted Bias Interval at CD4 Clinical Cut-off

CD4 Cut-off	Reagent	% Bias	SE	95% CI
200 cells/µL	TBNK	3.18	0.61	1.99–4.38
	IMK	2.43	0.52	1.41–3.44

All results for both the IMK kit and TBNK kit were determined by assay, result type (absolute count or subset percentages), and subset in both Bland Altman (bias) plots and Deming Regression scatter plots comparing the FACSLyric with the predicate device. All summary results for the FACSLyric on the TBNK kit for all outcomes and regression results are shown below:

BD FACSLyric− Lymphocyte Absolute Count and %Lymphocytes Results for BD Multitest 6-color TBNK Reagents:

Lymphocyte Subset		N	Absolute counts (cells/µL)	Median
Absolute Counts (Cells/µL)	AbsCD3$^+$	297	4−6422	1549
	AbsCD4$^+$		0−2823	606
	AbsCD8$^+$		1−5638	769
	AbsCD19$^+$		0−2060	207
	AbsCD16$^+$CD56$^+$		0−1528	221
Percentage (%) of Lymphocytes	% CD3+		0.8−94.2	75.4
	% CD4+		0.1−81.2	29.2
	% CD8+		0.2−82.5	41.5
	% CD19+		0−84.8	10.4
	% CD16+CD56+		0.2−91.9	11.4

BD FACSLyric −Summary of the Deming regression absolute count results using BD Multitest 6-color TBNK Reagents:

Subset	N	Slope (95% CI)	Intercept	R^2
AbsCD3$^+$	297	1.04 (1.03−1.04)	−1.26	0.99
AbsCD4$^+$		1.04 (1.02−1.05)	−0.82	0.99
AbsCD8$^+$		1.03 (1.02−1.05)	0.18	0.99
AbsCD19$^+$		1.01 (1.00−1.03)	−0.20	0.99
AbsCD16$^+$CD56$^+$		0.94 (0.90−0.99)	-0.22	0.98

BD FACSLyric-−Summary of the Deming regression subset percentage results for BD Multitest 6-color TBNK Reagents:

Subset	N	Slope (95% CI)	Intercept	R^2
% CD3$^+$	297	1.00 (0.98−1.01)	1.23	0.99
% CD4$^+$		1.00 (0.97−1.02)	0.35	1.00
% CD8$^+$		1.01 (1.00−1.02)	0.12	1.00
% CD19$^+$		1.02 (1.01−1.04)	−0.28	0.99
% CD16$^+$CD56$^+$		0.99 (0.97−1.03)	−0.76	0.99

BD FACSLyric− Lymphocyte Absolute Count and % Lymphocytes Results for BD Multitest IMK kit Reagents:

Lymphocyte Subset		N	Absolute counts (cells/µL)	Median
Absolute Counts (Cells/µL)	Average $AbsCD3^+$	336	6−6499	1539
	AbsCD3+ Tube A		6−6553	1544
	AbsCD3+Tube B		6−6445	1519
	$AbsCD4^+$		1−3194	600
	$AbsCD8^+$		1−5774	762
	$AbsCD19^+$		0−2770	207
	$AbsCD16^+CD56^+$		14−1502	217
Percentage (%) of Lymphocytes	Average % CD3+		1.3−95.8	74.6
	% CD3+ Tube A		1.3−94.8	74.3
	% CD3+ Tube B		1.4−96.7	74.9
	% CD4+		0.1−84.7	28.4
	% CD8+		0.3−82.9	41.3
	% CD19+		0−92.4	10.4
	% CD16+CD56+		1.5−87.7	11.4

BD FACSLyric −Summary of the Deming regression absolute count results using BD Multitest IMK kit Reagents:

Subset	N	Slope (95% CI)	Intercept	R^2
Average $AbsCD3^+$	336	1.04 (1.03−1.05)	1.43	0.99
AbsCD3+Tube A		1.03 (1.02−1.05)	3.96	0.99
AbsCD3+Tube B		1.04 (1.03−1.05)	−0.58	0.99
$AbsCD4^+$		1.02 (1.01−1.04)	−0.04	0.99
$AbsCD8^+$		1.02 (1.00−1.04)	−0.59	0.99
$AbsCD19^+$		1.02 (1.00−1.04)	−0.16	1.00
$AbsCD16^+CD56^+$		0.96 (0.94−0.98)	−3.95	0.99

BD FACSLyric-−Summary of the Deming regression subset percentage results for BD Multitest IMK kit Reagents:

Subset	N	Slope (95% CI)	Intercept	R^2
Average % $CD3^+$	336	1.00 (0.99−1.01)	0.47	0.99
% CD3+ Tube A		1.00 (0.99−1.01)	0.50	0.99
% CD3+ Tube B		1.00 (0.99−1.01)	0.44	0.99
% $CD4^+$		1.01 (1.00−1.02)	−0.26	1.00
% $CD8^+$		1.00 (0.98−1.01)	−0.08	0.99
% $CD19^+$		1.02 (1.01−1.04)	−0.24	1.00
% $CD16^+CD56^+$		1.00 (0.98−1.02)	−0.85	0.99

Instrument Configuration Comparison was performed with 44 specimens comprised of HIV+ donors and normal donors. Four of the normal donor samples were manipulated in order to provide for absolute CD4 count across the AMR. Manipulations were done by diluting the whole blood specimen using cell free plasma and PBS to yield the desired CD4+ absolute count. The comparison demonstrated the performance equivalency of the six IVD channels between the 3-1, 4-2, 4-2-2 and 4-3-3 optical configurations of the FACSLyric flow cytometer.

b. *Precision/Reproducibility:* The precision study was performed based on recommendations in the CLSI document *CLSI EP5-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline-Third Edition.*

Repeatability:

Within-Site Repeatability was performed for the enumeration of the lymphocyte subset percentages and absolute counts across four FACSLyric flow cytometers, each with a different optical configuration. Testing was performed by four operators, two runs per day for 21 days using Streck CD-Chex Plus (K960894) and Streck CD-Chex Plus CD4 Low (K931825) as the control samples with four lots of 6-color TBNK reagent and four lots of IMK reagent. Multi-Check and Multi-Check CD4 Low Controls were used as the process controls. Each sample was stained in duplicate in Trucount tubes.

Within-Site BD Multitest 6-color TBNK absolute counts

	Subset	Mean (cells/µL)	Within Run %CV	Total %CV
Streck CD-Chex Plus	CD3+	1728.97	4.40	4.47
	CD4+	1081.02	5.17	5.67
	CD8+	488.98	5.91	6.00
	CD19+	271.23	7.19	7.29
	CD16+CD56+	232.54	7.86	7.95
Streck CD-Chex Plus CD4 Low	CD3+	866.44	5.23	5.58
	CD4+	164.18	8.03	8.44
	CD8+	593.38	5.42	5.87
	CD19+	329.94	6.32	6.59
	CD16+CD56+	291.36	6.82	7.00

Within-Site BD Multitest IMK kit absolute counts

	Subset	Mean (cells/µL)	Within Run %CV	Total %CV
Streck CD-Chex Plus	CD3+(Average)	1733.81	3.05	3.21
	CD3+ (Tube A)	1729.61	3.85	4.03
	CD3+ (Tube B)	1738.01	4.00	4.12
	CD4+	1142.52	4.04	4.18
	CD8+	500.42	5.56	5.67
	CD19+	273.84	6.02	6.16
	CD16+CD56+	234.38	7.41	7.52
Streck CD-Chex Plus CD4 Low	CD3+ (Average)	870.51	3.15	3.29
	CD3+ (Tube A)	869.06	4.24	4.32
	CD3+ (Tube B)	871.97	3.82	3.97
	CD4+	176.91	6.59	6.67
	CD8+	612.12	4.55	4.65
	CD19+	330.87	5.22	5.35
	CD16+CD56+	295.88	6.03	6.21

Whole Blood Repeatability performance was determined by enumeration of CD3+, CD4+, CD8+, CD19+ and CD16+56+ lymphocyte subset percentages and absolute counts across all configurations of BD FACSLyric cytometers, using the BD Multitest 4-Color TBNK reagents (BD Multitest CD3/CD8/CD45/CD4 and BD Multitest CD3/CD16+CD56/CD45/CD19) and BD Multitest 6-Color TBNK reagents in Trucount tubes. A total of 12 instruments were used with the 4-color assay and 9 instruments were used with the 6-color assay. Patient specimens were run in duplicate per instrument x three instruments x four configurations for 4-color reagents and 3 configurations for 6-color reagents. Specimens were tested with representation of values for CD4 across the AMR.

Whole Blood Repeatability was also performed for absolute CD4counts. The study was performed with 44 samples including normal and HIV+ patient samples. Specimens spanned the analytical measuring range (AMR) and closely bracketed the medical decision points (very low <50 CD4 cells/µL and low 50 to <200 CD4 cells/µL).

Whole Blood Repeatability: absolute CD4counts

	CD4 Count	Mean (cells/µL)	Within Run %CV	Total %CV
TBNK	CD4 < 200	98.08	7.66	7.66
	200 ≤ CD4 <500	344.83	5.18	5.27
	500 ≤ CD4 <1000	830.57	4.63	4.70
	1000 ≤ CD4 < 4500	1194.28	4.22	4.31
IMK	CD4 < 200	97.28	7.13	7.13
	200 ≤ CD4 <500	329.33	5.46	5.46
	500 ≤ CD4 <1000	821.09	4.30	4.46
	1000 ≤ CD4 < 4500	1214.03	4.20	4.23

Whole Blood Repeatability for absolute counts all subsets

	Subset	Mean (cells/µL)	Within Run %CV	Total %CV
6-color TBNK	CD3+	1402.64	4.31	4.35
	CD4+	634.13	5.22	5.24
	CD8+	722.11	5.37	5.42
	CD19+	218.10	7.54	7.57
	CD16+CD56+	233.55	7.01	7.06
IMK	CD3+ (Average)	1398.45	3.21	3.35
	CD3+ (Tube A)	1400.10	4.49	4.61
	CD3+ (Tube B)	1396.78	4.17	4.26
	CD4+	633.59	5.32	5.40
	CD8+	726.59	5.42	5.53
	CD19+	215.01	7.84	7.94
	CD16+CD56+	229.21	7.32	7.47

Reproducibility:

Inter-laboratory reproducibility was carried out at four clinical sites using Streck CD-Chex Plus (K960894) process control materials tested for a minimum of 5 non-consecutive days with one operator per site and one instrument per site. Three replicates of each level (normal and low) of the control material were tested during two runs per day on the FACSLyric system. The variability for within-run, between-run, between-day, between-site and total precision per reagent and lymphocyte subset was evaluated.

Precision study using control materials Streck CD-Chex Plus (All sites combined)
BD Multitest 6-Color TBNK absolute counts

Control Level	Unit	Mean	Within Run %CV	Between Runs %CV	Between Days %CV	Between Site %CV	Total %CV
Low	AbsCD3+ Cells/µL	875.81	4.63	0.32	1.21	0.81	4.86
	AbsCD4+ Cells/µL	185.79	6.84	0.0	2.46	0.31	7.28
	Abs CD8+ Cells/µL	616.88	5.00	0.0	1.19	0.02	5.14
	AbsCD19+ Cells/µL	335.03	6.05	0.0	0.66	1.87	6.37
	AbsCD16+CD56+Cells/µL	294.49	6.49	0.24	0.0	2.07	6.82
Normal	CD3+ Cells/µL	1742.38	5.08	0.0	1.58	1.31	5.48
	AbsCD4+ Cells/µL	1175.19	5.50	0.0	1.88	1.30	5.95
	Abs CD8+ Cells/µL	557.86	6.18	0.0	3.08	4.64	8.32
	AbsCD19+ Cells/µL	276.52	7.31	0.48	2.43	0.0	7.72
	AbsCD16+CD56+Cells/µL	231.03	8.79	0.0	2.27	2.70	9.48

Precision study using control materials Streck CD-Chex Plus (All sites combined)
BD Multitest 6-Color TBNK percentages

Control Level	Unit	Mean	Within Run SD	Between Runs SD	Between Days SD	Between Site SD	Total SD
Low	CD3+ %	57.46	1.09	0.13	0.0	0.32	1.14
	CD4+ %	12.19	0.71	0.0	0.15	0.0	0.73
	CD8+ %	40.47	1.01	0.19	0.0	0.22	1.05
	CD19+%	21.97	0.82	0.10	0.00	0.14	0.84
	CD16+CD56+%	19.31	0.80	0.06	0.0	0.22	0.84
Normal	CD3+ %	76.98	0.94	0.0	0.0	0.39	1.02
	CD4+ %	51.91	1.08	0.14	0.0	0.12	1.09
	CD8+ %	24.64	0.91	0.0	0.59	1.16	1.59
	CD19+%	12.21	0.59	0.15	0.0	0.20	0.65
	CD16+CD56+%	10.2	0.69	0.0	0.14	0.27	0.75

Precision study using control materials Streck CD-Chex Plus (All sites combined)
BD Multitest IMK kit absolute counts

Control Level	Unit	Mean	Within Run %CV	Between Runs %CV	Between Days %CV	Between Site %CV	Total %CV
Low	Average AbsCD3+ Cells/µL	883.06	3.90	0.0	0.44	1.42	4.18
	AbsCD3+ Tube A Cells/µL	881.62	4.72	0.0	1.20	1.26	5.03
	AbsCD3+ Tube B Cells/µL	884.57	4.60	0.0	0.0	1.44	4.82
	AbsCD4+ Cells/µL	187.01	6.68	0.0	2.55	1.47	7.30
	Abs CD8+ Cells/µL	628.51	5.04	0.0	0.61	1.26	5.23
	AbsCD19+ Cells/µL	336.79	5.47	0.0	0.0	1.66	5.71
	AbsCD16+CD56+Cells/µL	301.25	6.98	0.0	1.77	1.69	7.40
Normal	Average AbsCD3+ Cells/µL	1747.70	3.53	0.27	1.32	1.26	3.98
	AbsCD3+ Tube A Cells/µL	1746.97	4.22	0.0	1.45	1.30	4.65
	AbsCD3+ Tube B Cells/µL	1748.31	4.55	0.25	1.04	1.08	4.80
	AbsCD4+ Cells/µL	1177.59	4.88	0.25	1.45	0.87	5.17
	Abs CD8+ Cells/µL	529.63	5.14	0.0	2.41	2.08	6.05
	AbsCD19+ Cells/µL	273.58	7.03	0.0	0.0	1.69	7.23
	AbsCD16+CD56+Cells/µL	234.28	7.24	1.07	1.66	0.0	7.50

Precision study using control materials Streck CD-Chex Plus (All sites combined)
BD Multitest IMK kit percentages

Control Level	Unit	Mean	Within Run SD	Between Runs SD	Between Days SD	Between Site SD	Total SD
Low	Average CD3+ %	57.12	0.92	0.0	0.27	0.28	1.00
	CD3+% Tube A	57.14	1.16	0.0	0.12	0.31	1.21
	CD3+% Tube B	57.10	1.22	0.0	0.41	0.19	1.30
	CD4+ %	12.12	0.57	0.0	0.18	0.12	0.61
	CD8+ %	40.74	1.09	0.0	0.0	0.25	1.12
	CD19+%	21.74	0.79	0.12	0.21	0.0	0.83
	CD16+CD56+%	19.44	0.93	0.0	0.22	0.47	1.06
Normal	Average CD3+ %	76.74	0.68	0.07	0.34	0.10	0.77
	CD3+% Tube A	76.64	0.85	0.12	0.31	0.0	0.91
	CD3+% Tube B	76.84	1.00	0.0	0.23	0.22	1.05
	CD4+ %	51.67	1.39	0.0	0.74	0.0	1.58
	CD8+ %	23.23	0.83	0.0	0.0	0.19	0.85
	CD19+%	12.02	0.64	0.07	0.0	0.11	0.66
	CD16+CD56+%	10.30	0.57	0.03	0.16	0.15	0.62

c. *Linearity/assay reportable range:* The linearity study was performed based on recommendations in the CLSI document *CLSI EP6-A6, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.*

Linearity was evaluated using triplicate measurements of 11 concentrations of lymphocyte subsets across a range approximately 20 to 30% wider than the anticipated linear range. In addition, seven supplemental concentration levels for CD4 were used to evaluate linearity near the medical decision point at 50 cells/µL and 200 cells/µL for CD4 absolute counts. Linearity studies were performed on each configuration to verify that the relationship between the observed values and the true concentrations of the analyte was linear for each configuration. The objective of each linearity study was to estimate the linearity of the BD FACSLyric with BD FACSuite™ clinical software using the BD Multitest IMK Kit (4-color) and the BD Multitest 6-color TBNK reagent for the lyse/no-wash method of sample preparation.

Whole blood samples collected in EDTA tubes were fractionated by centrifugation to isolate PBMCs, plasma, and RBCs. A low concentration pool was created using autologous plasma reconstituted with RBCs; a high concentration pool was created using concentrated PBMCs in autologous plasma and RBCs. Intermediate concentration levels were created by proportionally mixing high and low pools. Replicates of each dilution were stained with the BD Multitest IMK Kit (4-color) and BD Multitest 6-color TBNK Reagent in Trucount tubes.

Regression statistics were provided for all each cellular subset. The instrument system using the FACSuiteClinical software for the IMK and TBNK assay was found to be linear for each parameter (absolute counts) for each configuration.

d. *Carryover:* The carryover studies were performed based on *CLSI H26-A2 Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard- Second Edition.*

Sample Carryover:

Specimen carry over studies were performed on the BD FACSLyric™ to determine whether results were affected by contamination from neighboring samples. System carryover was evaluated by estimating the percent carryover of abnormally high leucocyte count samples to abnormally low leucocyte count samples. Three high leucocyte concentration samples were acquired sequentially, immediately followed by the sequential acquisition of three low leucocyte concentration samples. Carryover of both BD FACS Universal Loader (UL) and manual acquisition was evaluated on three FACSLyric instruments (one 3-1 and two 4-3-3) using two different sample volumes, 500 uL and 1500 uL and calculating the percent difference according to the formula % Carryover = [(L1-L3)/H3-L3)]*100.

Carryover from one specimen to another was demonstrated to be leucocyte carryover less than or equal to 0.1% for low carryover and less than or equal to 0.5% for

standard carryover on all three FACSLyric instruments.

Reagent Carryover:

Percent volume carryover was measured by enumeration of Trucount Control beads in known concentrations and sample volumes. Percent reagent carryover was then calculated using the volume carryover because the amount of reagent is consistent throughout the sample. Three replicates of Trucount tubes with Trucount High Control beads were run first, followed by three replicates of Trucount tubes without Trucount Control beads. The volume carryover was quantified by the number of Trucount High Control beads present in the first tube without Trucount Control beads (L1) after subtracting the number of events present in the third tube without Trucount Control beads (L3), this tube is considered background. This procedure was repeated three times on each instrument and each run was evaluated individually. The volume carryover experiment was carried out on three FACSLyric instruments using UL acquisition on two instruments and manual acquisition on one instrument. The amount of reagent being carried over is found to be well below the amount of reagent required for effective staining of the sample.

e. Interfering Substances:

Not applicable

2. Other Supportive Instrument Performance Data Not Covered Above:

Limit of Blank (LoB); Limit of Detection (LoD); Limit of Quantitation (LoQ):
The study was performed in accordance with *CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline-Second Edition*.

Twenty four samples with sixty total replicates were evaluated for each blank for LoB and low concentration sample for LoD per reagent lot, across four test days for each instrument configuration. Three lots of the reagent were collected for a total of 180 data points per assay and configuration combination. LoB and LoD for all reagents tested was less than 15 cells/µL. For CD4 Lymphocyte subset absolute counts of each of the reagents, the established LoB and LoD were less than 50 cells/µL.

For LoQ determination, four low concentration pools were created by diluting normal whole blood with cell free plasma to achieve CD4 absolute counts of 10, 20, 30 and 50 cells/µL. Forty replicates were prepared from each of the concentration pools and were stained with two different lots of each reagent. Ten replicates from each concentration pool were run on each FACSLyric instrument configuration. The remaining ten replicates were run on the predicate device, FACSCanto II. The LoQ of the FACSLyric system with Multitest reagents was established for the CD3+, CD3+CD4+, CD3+CD8+, CD19+, CD16+CD56+ and CD45+ lymphocyte subsets. The LoQ is less than 50 cells/µL for CD4 absolute count.

Expected values/Reference range:

The reference interval study was performed based on recommendations in the CLSI document *CLSI EP28-A3c, How to Define and Determine Reference Intervals in the Clinical Laboratory, Approved Guideline-Second Edition*. A total of 134 samples for Multitest 6-Color TBNK and 130 samples for Multitest IMK kit from one clinical site were evaluated. Reference Intervals for all lymphocyte subsets in the Multitest 6-Color TBNK and Multitest IMK kit were established for the FACSLyric system. Testing was performed using prospectively procured EDTA venous blood specimens from apparently healthy adult male and female subjects free of hematological abnormalities to satisfy age.

Reference Intervals Analysis Results for Multitest 6-Color TBNK

Parameters	Reference Range
AbsCD3+	856–2669
AbsCD4+	491–1734
AbsCD8+	162–1074
AbsCD19+	73–562
AbsCD16+CD56+	108–680
%CD3+	57.5–83.1
%CD4+	31.5–62.4
%CD8+	9.6–38.3
%CD19+	5.9–24.2
%CD16+CD56+	5.2–30.4

Reference Intervals Analysis Results for Multitest IMK kit

Parameters	Reference Range
Average AbsCD3+	827–2547
AbsCD3+ Tube A	840−2641
AbsCD3+ Tube B	812−2655
AbsCD4+	488–1711
AbsCD8+	154–1097
AbsCD19+	60–551
AbsCD16+CD56+	102–617
Average %CD3+	56.9–82.5
%CD3+ Tube A	56.7−83.4
%CD3+ Tube B	56.7−82.5
%CD4+	32.4–63.2
%CD8+	9.0–39.0
%CD19+	5.1–23.0
%CD16+CD56+	5.4–30.0

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.