510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
INSTRUMENT ONLY

I Background Information:

A 510(k) Number

K232600

B Applicant

Beckman Coulter, Inc

C Proprietary and Established Names

DxFLEX Flow Cytometer
ClearLLab 10C Panels (B, T, M1, M2)

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
OYE	Class II	21 CFR 864.5220 - Automated differential cell counter	HE - Hematology
PWD	Class II	21 CFR 864.7010 - Flow cytometric test system for hematopoietic neoplasms	HE - Hematology

II Submission/Device Overview:

A Purpose for Submission:

New instrument for previously cleared assay

B Type of Test:

Immunophenotyping, qualitative, flow cytometric instrument

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

DxFLEX Flow Cytometer
The DxFLEX Flow Cytometer is intended for use as an in vitro diagnostic device for immunophenotyping using up to ten fluorescent detection channels using three lasers (488 nm, 638 nm, and 405 nm) and two light scatter detection channels. It is intended for use with in vitro diagnostic (IVD) assays and software that are indicated for use with the instrument.

The ClearLLab 10C Panels
The ClearLLab 10C Panels are intended for in vitro diagnostic use for qualitative identification of various cell populations by multiparameter immunophenotyping on the Navios, Navios EX and DxFLEX flow cytometers. These reagents are used as an aid in the differential diagnosis of hematologically abnormal patients having, or suspected of having, the following hematopoietic neoplasms: chronic leukemia, acute leukemia, non-Hodgkin lymphoma, myeloma, myelodysplastic syndrome (MDS), and/or myeloproliferative neoplasms (MPN). The reagents can be used with peripheral whole blood (collected in K2EDTA, Acid Citrate Dextrose (ACD) or Heparin), bone marrow (collected in K2EDTA, ACD or Heparin) and lymph node specimens. Interpretation of the results should be confirmed by a pathologist or equivalent professional in conjunction with other clinical and laboratory findings. These reagents provide multiparameter, qualitative results for the surface antigens listed below:

- ClearLLab 10C B Cell Tube: Kappa, Lambda, CD10, CD5, CD200, CD34, CD38, CD20, CD19, CD45
- ClearLLab 10C T Cell Tube: TCRγδ, CD4, CD2, CD56, CD5, CD34, CD3, CD8, CD7, CD45
- ClearLLab 10C M1 Cell Tube: CD16, CD7, CD10, CD13, CD64, CD34, CD14, HLA-DR, CD11b, CD45
- ClearLLab 10C M2 Cell Tube: CD15, CD123, CD117, CD13, CD33, CD34, CD38, HLA-DR, CD19, CD45

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only
For in vitro diagnostic use.

IV Device/System Characteristics:

A Device Description:

1. Device Description:

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

The DxFlex Flow cytometer is being cleared for use with the ClearLLab 10C Reagent System which was cleared on the Navios EX Flow cytometer (K183592). The ClearLLab 10C Reagent System on the DxFLEX Flow Cytometer is comprised of the following:

- DxFLEX Flow Cytometer [3 laser/10 color configurations]
- DxFLEX Daily QC Fluorospheres
- ClearLLab 10C Panels [B, T, M1 and M2]
- ClearLLab Compensation Kit
- ClearLLab Compensation Beads
- ClearLLab Control Cells, normal and abnormal
- IOTest 3 Fixative Solution [Existing] • IOTest 3 Lysing Solution
- Kaluza C Software

The DxFLEX Flow Cytometer includes ten fluorescent detection channels (FL1-FL10) and three laser configurations (blue, red and violet).

The ClearLLab 10C Reagent System on the DxFLEX Flow Cytometer includes the CytExpert for DxFLEX Software (i.e., acquisition software) and the Kaluza C Analysis Software (i.e., analysis software) (cleared in K183592 (version 1.1)).

As with the workflow on the predicate system (K183592), listmode (LMD) data analysis is performed manually using the Kaluza C Analysis Software. This Analysis Software package is supplied separately from the DxFLEX and is installed on an independent computer workstation for off-line analysis of listmode files generated on the flow cytometer with the associated reagents and cytometer system software package, including Control Cell Quality Control (QC) data and sample data analysis. The CytExpert for DxFLEX software is used to run the DxFLEX Daily QC Fluorospheres and Compensation products, Kaluza C Software is a software tool designed to work with *.fcs and *.lmd files generated from flow cytometers. Preset Kaluza C analysis templates for the ClearLLab 10C reagent system will be provided. The total WBC gate is defined in the histogram of SS vs. CD45-KO525-A by the inclusion of all CD45+ events with low, medium, and high Side Scatter after the exclusion of the cells that were compromised and/or aggregated. A subsequent histogram defines specific leukocyte subsets such as lymphocytes, monocytes, granulocytes. The low SS/bright CD45+ population identifies lymphocytes. Applicable markers are then displayed in subsequent histograms gated on lymphocytes. This process is repeated for mid SS/medium CD45+ populations to identify monocytes and for high SS/medium CD45+ to identify granulocytes

As acquisition software, CytExpert.exe enables the user to acquire data from the DxFLEX flow cytometer and allows the user to analyze, display, print and export acquired data.
The major features of the software are as follows:

- System Startup
- Instrument Quality Control and Standardization
- Data Acquisition
- Sample Analysis
- Compensation
- Data Review
- Report Generation
- Daily Clean

Kaluza C software is used for post-acquisition analysis and reporting.

2. Principles of Operation:

The DxFLEX 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 Avalanche Photodiode detectors and transduced to an electronic pulse and counted by the instrument's computer. This test depends on the ability of a monoclonal or polyclonal antibody to bind to the surface of cells expressing discrete antigenic determinants. Specific cell staining is accomplished by incubating specimens prepared for staining with the appropriate antibody reagent. After sample preparation, the specimens are acquired on the QC controlled flow cytometer with manual gating.

B Instrument Description Information:

1. Instrument Name:

DxFLEX Flow Cytometer

2. Specimen Identification:

Barcode reader or manual entry

3. 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.

4. Calibration:

Fluorescence intensity is calibrated and confirmed using the DxFLEX Daily QC Fluorospheres with lot specific target values.

5. Quality Control:

The DxFLEX QC process verifies important system functions. The system verifies or automatically adjusts the following parameters:

- Hardware configuration
- Laser power of each individual laser
- Laser delays parameters
- Gain settings

Beckman Coulter recommends performing QC on a daily basis.

V Substantial Equivalence Information:

A Predicate Device Name(s):

ClearLLab 10C Panels (B, T, M1, M2)
Navios Flow Cytometer
Navios EX Flow Cytometer

B Predicate 510(k) Number(s):

K183592

C Comparison with Predicate(s):

Device & Predicate Device(s):	**K232600 (Candidate)**	**K183592 (Predicate)**
Device Trade Name	DxFLEX Flow Cytometer	Navios EX Flow Cytometer
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The DxFLEX Flow Cytometer is intended for use as an in vitro diagnostic device for immunophenotyping using up to ten fluorescent detection channels using three lasers (488 nm, 638 nm, and 405 nm) and two light scatter detection channels. It is intended for use with in vitro diagnostic (IVD) assays and software that are indicated for use with the instrument.	The Navios EX Flow Cytometer is intended for use as an in vitro diagnostic device for immunophenotyping using up to ten fluorescent detection channels using three lasers (488 nm, 638 nm, and 405 nm) and two light scatter detection channels. It is intended for use with in vitro diagnostic (IVD) assays and software that are indicated for use with the instrument.
Sample Preparation	Off-board sample preparation following instructions provided with cleared antibody reagent	Same
Sample Presentation	Sample prepared in dry reagent tube presented in the same tube	Same
Resuspension of Prepared Sample Prior to Introduction to System	Prepared sample is vortex mixed	Same
Sample Introduction	• Automated presentation with Multi-tube Carousel Loader (MCL) from 32 test tube capacity carousel. • Manual presentation into a tube location on a MCL	Same
Sample Aspiration Probe	Adjustable (both Beckman Coulter field service personnel and user)	Adjustable (by Beckman Coulter field service personnel)

Sample Identification	Bar-code reading of carousel position and labeled sample tube. User may also identify samples based on carousel location with a worklist.	Same
Lasers / Driver Boards	Blue (488 nm) • Diode Pumped Solid State (DPSS), 50mW • 488 nm laser driver board	Blue (488 nm) • Diode Pumped Solid State (DPSS), 55mW • 488 nm laser driver board
	Red (638 nm) • Diode Pumped Solid State (DPSS), 50 mW • 638 nm laser driver board	Same
	Violet (405 nm) • Diode Pumped Solid State (DPSS), 85 mW • 405 nm laser driver board	Violet (405 nm) • Diode Pumped Solid State (DPSS), 80 mW • 405 nm laser driver board
Forward Angle Light Scatter	• Forward scatter mask • Band-pass filter passes only 488nm light • Diode detector converts light into electrical signals	Same
Color Separation	Fluorescent signal is separated into desired components with dichroic filters.	Same
Reagents Used for Compensation and Sample Preparation	ClearLLab Compensation Kit and Compensation Beads will be used with the ClearLLab 10C application.	Same, except without labeling changes to include DxFLEX Flow Cytometer only
Analysis Software	Kaluza C data analysis software to be used with the ClearLLab 10C application and to be used off-line. Analysis protocols are specific for ClearLLab Reagents on DxFLEX Flow Cytometer	Same, except analysis protocols are instrument specific
Data Reporting	Report, Plots and Statistics printouts	Same
Cleaning Cycle Between Samples	Executed with Sheath Fluid ensuring carryover specification is met	Same
Quality Control Techniques	Daily Instrument Checks Supplied Controls for ClearLLab 10C application	Same
Cleanse Cycle	Cleaning cycle performed with FlowClean 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. (Additional cleaning cycle required after running	Same, except no additional cleaning cycle required after running Daily QC Fluorospheres

	DxFLEX Daily QC Fluorospheres)	
Process Controls	ClearLLab Control Cells (Normal and Abnormal) except with labeling changes to include DxFLEX Flow Cytometer and assay ranges specific for the DxFLEX Flow Cytometer	ClearLLab Control Cells (Normal and Abnormal)
Processor	PowerPC	Same
Workstation Interface	Ethernet	USB, Ethernet
Data Acquisition	Digital Pulse Processing	Same
Safety Features	Interlocks and mitigation of hazards via software and hardware controls	Same
Software Architecture	The acquisition software enables the user to acquire data from the instrument and to analyze, display, print and export acquired data. The embedded software resides in the 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.	Same
Software Functionality	Allows Patient and Control data management – storage, review, reporting and system configuration management	Same
Data Processing	• Region/gates evaluation • Statistics generation • Export of results to MS Excel/PDF file Incoming data stream formatted into per-parameter vectors for improved performance of lin/log data transformations and compensation calculations	• Region/gates evaluation • Statistics generation • Export of results to CSV/PDF file Incoming data stream formatted into per-parameter vectors for improved performance of lin/log data transformations and compensation calculations
User Interface	Plots and reports	Same
Report Generator	Panel reports & QC	Same

Device & Predicate Device(s):	K232600 (Candidate)	K183592 (Predicate)
Device Trade Name	DxFLEX Flow Cytometer	Navios EX Flow Cytometer
General Device Characteristic Differences		
Forward Angle Light Scatter	Ellipsoidal mirror to focus the light	Single lens collimates the light
Optics	Aspherical and cylindrical lenses coupled to the flow cell	Spherical and cylindrical lenses coupled to the flow cell
Flow Cell	• Channel Size: 180 x 430 microns • Sheath Flow Pressure: 18.8 kPa • Optics module temperature management hardware: two thermoelectric devices at the top and bottom of the optics module box	• Channel Size: 140 x 460 microns • Sheath Flow Pressure: 4 psi • Thin wall to allow for gel coupling distance • Optics module temperature management hardware: one thermoelectric device at the top and bottom of the optics module box
Aspiration Pathway	Aspiration pathway uses peristaltic pump for automatic presentation	Aspiration path uses compressor for automated and manual presentation
Fluorescence Collection	• Collection lens and light path • Spherical mirror and aspheric Schmidt lens • Gel coupled to flow cell • Light passes through optical fiber to WDM area • Light leaves the fiber and is collimated • Collimated light is separated by filters into desired bands APDs (Avalanche Photodiodes) detect light and convert to electrical signals	• Collection lens and light path: • Spherical mirror and aspheric Schmidt lens • No gel coupling, NA=1.2 • Light passes through optical fiber to PMT area • Light leaves the fiber and is collimated • Collimated light is separated by filters into desired bands PMTs detect light and convert to electrical signals
Electronics	25 MHz sampling	40 MHz sampling
Detectors/ Colors	ClearLLab 10C application will require the 10 APDs: • Standard 5 APDs (FITC – PC7 off of 488 nm laser (blue) • Standard 3 APDs (APC – APC-A750) off of 638 nm laser (red) • Standard 2 APDs (PB450 – KO525) off of 405 nm laser (violet)	• Standard 5 PMTs (FL1 – FL5) off of 488 nm laser (blue) • Standard 3 PMTs (FL6 – FL8) off of 638 nm laser (red) • Standard 2 PMTs (FL9 – FL10) off of 405 nm laser (violet)

Maximum Parameter Detectors	Twelve (Forward Scatter, Side Scatter and 10 fluorescence channels)	Twelve (Forward Scatter, Side Scatter and 10 fluorescence channels)
Optical Filters	Optical Filters (all band pass): • FITC 525/40 • PE 585/42 • ECD 610/20 • PC5.5 690/50 • PC7 780/60 • APC-A750 780/60 • APC 660/10 • APC-700 712/25 • PB450 450/45 • KO525 525/40	Optical Filters: (SP = Short Pass, BP = Band Pass, LP = Long Pass) • FITC 550 SP - 525 BP • PE 595 SP- 575/30 BP • ECD 655 SP - 614/20 BP • PC5.5 730 SP - 695/30 BP • PC7 755 LP • APC 710 SP – 660/20 BP • APC-A700 750 SP –725/20 BP • APC-A750 755 LP • PB 480 SP – 450/50 BP • KRO 550/40 BP
Assay Controls and Calibrators	DxFLEX Daily QC Fluorospheres have target values assigned to monitor optical alignment and fluidics and to aid in standardization.	Flow-Check Pro Fluorospheres Flow-Set Pro Fluorospheres
Programming Language	C# C++	C++
Real-time Operating System	N/A	VxWorks
Standardization	Automatic voltage/gain adjustments	Automatic gain calibration using target value
Control Software	CytExpert for DxFLEX software enables the user to acquire data from the DxFLEX flow cytometer instrument and to analyze, display, print and export acquired fcs file. CytExpert for DxFLEX controls the instrument functionality including the multi-carousel loader (MCL) for sample introduction. CytExpert for DxFLEX controls the instruments' lasers, acquisition system and fluidics. The instrument fluidics aspirates the sample and performs instrument maintenance functions such as startup/shutdown. CytExpert for DxFLEX also captures and provides the data to the workstation for processing. Communication protocol	The acquisition software enables the user to acquire data from the Navios EX Flow Cytometer and to analyze, display, print and export acquired listmode data. The embedded software resides in the Navios EX 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. Communication protocol
Data Processing	Region and Gate processing Statistics Generation Export to CSV	Region and Gate processing Statistics Generation Export to Excel

VI Standards/Guidance Documents Referenced:

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures
- CLSI EP09c 3rd Edition, Measurement Procedure Comparison and Bias Estimation Using Patient Samples
- CLSI EP12 3rd Edition, Evaluation of Qualitative, Binary Output Examination Performance
- CLSI EP43-A2, Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells
- CLSI H26-A2, Validation, Verification, and Quality Assurance of Automated Hematology Analyzers
- IEC 61326-1 Edition 3.0 2020-10, Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 1: General requirements
- IEC 61326-2-6 Edition 3.0 2020-10, Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 2-6: Particular requirements - In vitro diagnostic (IVD) medical equipment

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision studies were conducted according to the methodology presented in CLSI EP12-Ed3 and CLSI EP05-A3.

a. Precision (repeatability):

The repeatability of normal and abnormal clinical specimens representing clinical matrices and anticoagulants was evaluated with the ClearLLab 10C Panels on the DxFLEX Flow Cytometer at three sites. Sixty-four specimens across the three specimen types, i.e., whole blood (WB), bone marrow (BM), and lymph node (LN), were evaluated, with two excluded (due to specimen age and operator error). Each site employed a different anticoagulant for specimens, according to their current clinical practice: K2EDTA, acid citrate dextrose (ACD), or heparin.

Unless otherwise specified, a minimum of two specimens per specimen type per panel for each anticoagulant (site) was included. For LN specimens, there was no minimum requirement for the T panel (due to the low incidence of T lineage abnormalities), nor for M1 and M2 (due to the development property of myeloid cells and the pathogenesis of Myeloid abnormalities). Each specimen per panel was analyzed twice each day. There were six sample preparations in the morning and six sample preparations in the afternoon, resulting in a total of 12 replicates per specimen per panel. From 768 initial total replicates, 24 were excluded for reasons of specimen quality or operator error. Both quantitative and qualitative assessment were applied to the precision assessment where the presence or absence of an abnormal phenotype was reported.

All results showed 100% qualitative agreement across all sites, panels, anticoagulants, specimen types, and phenotypes.

For repeatability evaluation, pre-defined hematopoietic lineage populations were defined as in the table below:

Population definitions for evaluation of multi-site reproducibility of ClearLLab 10C				
Panel	Normal Population	Immunophenotype of Normal Population	Specimen Type	Abnormal Markers
B	Mature κ^+ B cells	$CD45^{bright}$ SS^{low} $CD19^+$ $CD20^+$ $Kappa^+$ $CD38^{dim}$ $CD200^+$ $CD10^-$ $CD5^-$ $CD34^-$	WB	CD5 CD10 CD34
			BM	
			LN	
	Mature λ^+ B cells	$CD45^{bright}$ SS^{low} $CD19^+$ $CD20^+$ $Lambda^+$ $CD38^{dim}$ $CD200^+$ $CD10^-$ $CD5^-$ $CD34^-$	WB	
			BM	
			LN	
T	Helper T cells	$CD45^{bright}$ SS^{low} $CD2^+$ $CD3^+$ $CD4^+$ $CD8^-$ $CD5^+$ $CD7^+$ $CD56^-$ $TCR\gamma\delta^-$ $CD34^-$	WB	CD34
			BM	
			LN	
	Cytotoxic T cells	$CD45^{bright}$ SS^{low} $CD2^+$ $CD3^+$ $CD8^+$ $CD4^-$ $CD5^+$ $CD7^+$ $CD56^-$ $TCR\gamma\delta^-$ $CD34^-$	WB	
			BM	
			LN	
	NK cells	$CD45^{bright}$ SSc^{low} $CD56^+$ $CD2^+$ $CD3^-$ $CD4^-$ $CD8^{+/-}$ $CD5^-$ $CD7^+$ $TCR\gamma\delta^-$ $CD34^-$	WB	
			BM	
			LN	
	γδ T cells	$CD45^{bright}$ SS^{low} $CD2^+$ $CD3^{bright}$ $TCR\gamma\delta^+$ $CD4^-$ $CD8^-$ $CD5^+$ $CD7^+$ $CD56^-$ $CD34^-$	WB	
			BM	
			LN	
M1	Neutrophils	$CD45^{dim}$ SS^{high} $CD16^+$ $CD10^+$ $CD13^+$ $CD11b^+$ $CD7^-$ $CD64^-$ $CD14^-$ $CD34^-$ $HLA\text{-}DR^-$	WB	CD7 CD34
			BM	
	Monocytes	$CD45^{med}$ SS^{med} $CD13^+$ $CD64^+$ $CD14^+$ $HLA\text{-}DR^+$ $CD11b^+$ $CD16^-$ $CD7^-$ $CD10^-$ $CD34^-$	WB	
			BM	
M2	Neutrophils	$CD45^{dim}$ SS^{high} $CD15^+$ $CD13^+$ $CD33^{+/-}$ $CD123^-$ $CD117^-$ $CD34^-$ $CD19^-$ $CD38^-$ $HLA\text{-}DR^-$ $CD34^-$	WB	CD19 CD34 CD117
			BM	
	Monocytes	$CD45^{med}$ *SSmed* $CD13^+$ $CD33^+$ $CD38^+$ $HLA\text{-}DR^+$ $CD15^-$ $CD123^-$ $CD19^-$ $CD34^-$ $CD117^-$	WB	
			BM	
	Basophils	$CD45^{dim}$ $CD123^{bright}$ $CD38^{bright}$ $CD13^+$ $CD33^+$ $CD15^-$ $CD117^-$ $CD34^-$ $HLA\text{-}DR^-$ $CD19^-$	WB	
			BM	

The results based on qualitative agreement are summarized in the following tables:

Qualitative Agreement – by Specimen, by Site					
Site	Panel	Anticoagulant	Specimen Type	Phenotype	N
1	B	ACD	BM	Abnormal (+)	12
1	B	ACD	BM	Abnormal (+)	12
1	B	ACD	BM	Normal (−)	12
1	B	n/a	LN	Abnormal (+)	12
1	B	n/a	LN	Abnormal (+)	12
1	B	n/a	LN	Normal (−)	12
1	B	ACD	WB	Abnormal (+)	12
1	B	ACD	WB	Normal (−)	12
2	B	K_2EDTA	BM	Abnormal (+)	12
2	B	K_2EDTA	BM	Abnormal (+)	12
2	B	K_2EDTA	BM	Normal (−)	12
2	B	n/a	LN	Abnormal (+)	12
2	B	n/a	LN	Normal (−)	12
2	B	K_2EDTA	WB	Normal (−)	12
2	B	K_2EDTA	WB	Abnormal (+)	12
3	B	Heparin	BM	Abnormal (+)	12
3	B	Heparin	BM	Normal (−)	12
3	B	Heparin	WB	Abnormal (+)	12
3	B	Heparin	WB	Abnormal (+)	12
3	B	Heparin	WB	Normal (−)	12
3	B	Heparin	LN	Abnormal (+)	12
3	B	Heparin	LN	Abnormal (+)	12
1	M1	ACD	BM	Abnormal (+)	12
1	M1	ACD	BM	Normal (−)	12
1	M1	ACD	WB	Abnormal (+)	12
1	M1	ACD	WB	Normal (−)	12
2	M1	K_2EDTA	BM	Normal (−)	12
2	M1	K_2EDTA	BM	Abnormal (+)	12
2	M1	K_2EDTA	WB	Abnormal (+)	12
2	M1	K_2EDTA	WB	Abnormal (+)	12
2	M1	K_2EDTA	WB	Normal (−)	12
3	M1	Heparin	BM	Abnormal (+)	12
3	M1	Heparin	BM	Normal (−)	12
3	M1	Heparin	WB	Abnormal (+)	12
3	M1	Heparin	WB	Normal (−)	12
1	M2	ACD	BM	Abnormal (+)	12
1	M2	ACD	BM	Normal (−)	12
1	M2	ACD	WB	Abnormal (+)	12
1	M2	ACD	WB	Normal (−)	12
2	M2	K_2EDTA	BM	Abnormal (+)	12
2	M2	K_2EDTA	BM	Abnormal (+)	12
2	M2	K_2EDTA	BM	Normal (−)	12
2	M2	K_2EDTA	WB	Abnormal (+)	12
2	M2	K_2EDTA	WB	Normal (−)	12
2	M2	K_2EDTA	WB	Normal (−)	12

Qualitative Agreement – by Specimen, by Site					
Site	Panel	Anticoagulant	Specimen Type	Phenotype	N
3	M2	Heparin	BM	Abnormal (+)	12
3	M2	Heparin	BM	Abnormal (+)	12
3	M2	Heparin	WB	Normal (−)	12
3	M2	Heparin	WB	Abnormal (+)	12
1	T	ACD	BM	Abnormal (+)	12
1	T	ACD	BM	Normal (−)	12
1	T	ACD	WB	Normal (−)	12
1	T	ACD	WB	Normal (−)	12
2	T	K_2EDTA	BM	Normal (−)	12
2	T	K_2EDTA	BM	Normal (−)	12
2	T	K_2EDTA	WB	Abnormal (+)	12
2	T	K_2EDTA	WB	Normal (−)	12
2	T	n/a	LN	Normal (−)	12
2	T	n/a	LN	Normal (−)	12
3	T	Heparin	BM	Normal (−)	12
3	T	Heparin	BM	Abnormal (+)	12
3	T	Heparin	WB	Normal (−)	12
3	T	Heparin	WB	Normal (−)	12

Qualitative Agreement – by Anticoagulant and Specimen Type (Sites and Specimens combined)				
Panel	Anticoagulant	Specimen Type	Phenotype	N
B	ACD	BM	Normal (−)	12
B	ACD	BM	Abnormal (+)	12
B	ACD	BM	Both (+/−)	24
B	ACD	WB	Normal (−)	12
B	ACD	WB	Abnormal (+)	12
B	ACD	WB	Both (+/−)	24
B	Heparin	BM	Normal (−)	12
B	Heparin	BM	Abnormal (+)	12
B	Heparin	BM	Both (+/−)	24
B	Heparin	WB	Normal (−)	12
B	Heparin	WB	Abnormal (+)	24
B	Heparin	WB	Both (+/−)	36
B	K_2EDTA	BM	Normal (−)	12
B	K_2EDTA	BM	Abnormal (+)	24
B	K_2EDTA	BM	Both (+/−)	36
B	K_2EDTA	WB	Normal (−)	12
B	K_2EDTA	WB	Abnormal (+)	12
B	K_2EDTA	WB	Both (+/−)	24
B	n/a	LN	Normal (−)	24
B	n/a	LN	Abnormal (+)	60
B	n/a	LN	Both (+/−)	84
M1	ACD	BM	Normal (−)	12

Qualitative Agreement – by Anticoagulant and Specimen Type (Sites and Specimens combined)				
Panel	Anticoagulant	Specimen Type	Phenotype	N
M1	ACD	BM	Abnormal (+)	12
M1	ACD	BM	Both (+/−)	24
M1	ACD	WB	Normal (−)	12
M1	ACD	WB	Abnormal (+)	12
M1	ACD	WB	Both (+/−)	24
M1	Heparin	BM	Normal (−)	12
M1	Heparin	BM	Abnormal (+)	12
M1	Heparin	BM	Both (+/−)	24
M1	Heparin	WB	Normal (−)	12
M1	Heparin	WB	Abnormal (+)	12
M1	Heparin	WB	Both (+/−)	24
M1	K_2EDTA	BM	Normal (−)	12
M1	K_2EDTA	BM	Abnormal (+)	12
M1	K_2EDTA	BM	Both (+/−)	24
M1	K_2EDTA	WB	Normal (−)	12
M1	K_2EDTA	WB	Abnormal (+)	24
M1	K_2EDTA	WB	Both (+/−)	36
M2	ACD	BM	Normal (−)	12
M2	ACD	BM	Abnormal (+)	12
M2	ACD	BM	Both (+/−)	24
M2	ACD	WB	Normal (−)	12
M2	ACD	WB	Abnormal (+)	12
M2	ACD	WB	Both (+/−)	24
M2	Heparin	BM	Normal (−)	12
M2	Heparin	BM	Abnormal (+)	12
M2	Heparin	BM	Both (+/−)	24
M2	Heparin	WB	Normal (−)	12
M2	Heparin	WB	Abnormal (+)	12
M2	Heparin	WB	Both (+/−)	24
M2	K_2EDTA	BM	Normal (−)	12
M2	K_2EDTA	BM	Abnormal (+)	24
M2	K_2EDTA	BM	Both (+/−)	36
M2	K_2EDTA	WB	Normal (−)	24
M2	K_2EDTA	WB	Abnormal (+)	12
M2	K_2EDTA	WB	Both (+/−)	36
T	ACD	BM	Normal (−)	12
T	ACD	BM	Abnormal (+)	12
T	ACD	BM	Both (+/−)	24
T	ACD	WB	Normal (−)	24
T	Heparin	BM	Normal (−)	12
T	Heparin	BM	Abnormal (+)	12
T	Heparin	BM	Both (+/−)	24
T	Heparin	WB	Normal (−)	24
T	K_2EDTA	BM	Normal (−)	24

Qualitative Agreement – by Anticoagulant and Specimen Type (Sites and Specimens combined)				
Panel	Anticoagulant	Specimen Type	Phenotype	N
T	K_2EDTA	WB	Normal (−)	12
T	K_2EDTA	WB	Abnormal (+)	12
T	K_2EDTA	WB	Both (+/−)	24
T	n/a	LN	Normal (−)	24

Results based on quantitative analysis are summarized in the table below:

Quantitative Results – by Specimen, by Site												
Panel	Site	Specimen Type	Anti-coagulant	Population	N	Mean (%)	Repeatability		Between-Run		Within-Sample	
							SD	%CV	SD	%CV	SD	%CV
B	1	LN	N/A	CD19+CD5+	12	91.96	0.62	0.67	0.82	0.89	1.02	1.11
B	1	LN	N/A	CD19+CD5+	12	32.64	1.37	4.18	1.40	4.30	1.96	6.00
B	1	LN	N/A	Mature κ^+ B cells	12	66.93	1.82	2.72	0.00	0.00	1.82	2.72
B	1	LN	N/A	Mature λ^+ B cells	12	30.19	1.23	4.07	0.64	2.13	1.39	4.6
B	1	BM	ACD	Mature κ^+ B cells	12	42.04	1.45	3.46	0.00	0.00	1.45	3.46
B	1	BM	ACD	Mature λ^+ B cells	12	27.88	1.06	3.80	0.00	0.00	1.06	3.80
B	1	WB	ACD	Mature κ^+ B cells	12	64.03	0.61	0.95	0.00	0.00	0.61	0.95
B	1	WB	ACD	Mature λ^+ B cells	12	35.54	0.71	1.99	0.00	0.00	0.71	1.99
B	1	WB	ACD	CD19+CD5+	12	98.66	0.38	0.39	0.00	0.00	0.38	0.39
B	1	BM	ACD	CD19+CD5+	12	95.01	0.81	0.86	1.14	1.20	1.40	1.48
B	2	WB	Heparin	CD19+CD5+	12	98.81	0.09	0.09	0.00	0.00	0.03	0.04
B	2	BM	Heparin	CD19+CD5+	12	99.37	0.03	0.04	0.00	0.00	0.02	0.02
B	2	WB	Heparin	Mature λ^+ B cells	12	99.81	0.02	0.02	0.00	0.00	2.64	4.38
B	2	WB	Heparin	Mature κ^+ B cells	12	60.28	2.64	4.38	0.00	0.00	2.57	6.52
B	2	WB	Heparin	Mature λ^+ B cells	12	39.49	2.57	6.52	0.00	0.00	0.79	1.92
B	2	BM	Heparin	Mature κ^+ B cells	12	41.46	0.79	1.92	0.12	0.41	0.81	2.70
B	2	BM	Heparin	Mature λ^+ B cells	12	30.03	0.80	2.67	0.12	0.41	0.81	2.70
B	2	LN	N/A	CD19+CD5+	12	7.59	0.51	6.78	0.00	0.00	0.51	6.78
B	2	LN	N/A	CD19+CD10+	12	39.65	1.14	2.87	0.48	1.20	1.23	3.11
B	2	LN	N/A	CD19+CD5+	12	39.90	1.39	3.50	1.20	3.00	1.84	4.61
B	3	WB	K_2EDTA	Mature κ^+ B cells	12	71.02	0.63	0.89	0.00	0.00	0.63	0.89
B	3	WB	K_2EDTA	Mature λ^+ B cells	12	28.83	0.63	2.19	0.00	0.00	0.63	2.19
B	3	BM	K_2EDTA	Mature κ^+ B cells	12	67.86	0.82	1.22	2.06	3.04	2.22	3.27
B	3	BM	K_2EDTA	Mature λ^+ B cells	12	31.78	0.87	2.73	2.07	6.53	2.25	7.08
B	3	LN	NA	Mature κ^+ B cells	12	48.60	1.40	2.88	0.00	0.00	1.40	2.88
B	3	LN	NA	Mature λ^+ B cells	12	50.15	1.44	2.88	0.00	0.00	1.44	2.88
B	3	BM	K_2EDTA	CD19+CD5+	12	99.42	0.09	0.09	0.06	0.06	0.11	0.11
B	3	WB	K_2EDTA	CD19+CD10+	12	98.49	0.12	0.12	0.31	0.31	0.33	0.34
B	3	LN	N/A	CD19+CD5+	12	78.61	1.36	1.73	0.00	0.00	1.36	1.73
B	3	BM	K_2EDTA	CD19+CD5+	12	99.51	0.03	0.03	0.00	0.00	0.03	0.03
M1	1	BM	ACD	CD16+Neutrophiles	12	19.39	0.58	2.99	0.74	3.82	0.94	4.85
M1	1	BM	ACD	CD64+Monocytes	12	7.24	0.17	2.32	0.12	1.63	0.21	2.84
M1	1	WB	ACD	CD16+Neutrophiles	12	28.62	1.57	5.49	1.15	4.02	1.95	6.81
M1	1	WB	ACD	CD64+Monocytes	12	2.82	0.18	6.51	0.09	3.11	0.20	7.21
M1	1	WB	ACD	CD34-CD7+	12	2.21	0.08	3.55	0.00	0.00	0.08	3.55

Quantitative Results – by Specimen, by Site												
Panel	Site	Specimen Type	Anti-coagulant	Population	N	Mean (%)	Repeatability		Between-Run		Within-Sample	
							SD	%CV	SD	%CV	SD	%CV
M1	1	BM	ACD	CD34-CD7+	12	40.89	0.67	1.63	1.03	2.53	1.23	3.01
M1	2	BM	Heparin	CD34-CD7+	12	11.94	0.38	3.22	0.48	4.00	0.61	5.14
M1	2	WB	Heparin	CD16+Neutrophiles	12	70.20	1.31	1.87	0.60	0.85	1.44	2.05
M1	2	WB	Heparin	CD64+Monocytes	12	3.72	0.16	4.18	0.16	4.33	0.22	6.02
M1	2	BM	Heparin	CD16+Neutrophiles	12	31.90	1.21	3.78	0.81	2.53	1.45	4.55
M1	2	BM	Heparin	CD64+Monocytes	12	37.78	1.22	3.22	1.35	3.58	1.82	4.82
M1	2	WB	Heparin	CD34-CD7+	12	36.44	0.32	0.87	0.13	0.36	0.34	0.94
M1	3	BM	K_2EDTA	CD16+Neutrophiles	12	23.01	0.24	1.02	0.11	0.46	0.26	1.12
M1	3	BM	K_2EDTA	CD64+Monocytes	12	4.20	0.15	3.54	0.26	6.17	0.30	7.11
M1	3	WB	K_2EDTA	CD34-CD7+	12	12.43	0.24	1.91	0.00	0.00	0.24	1.91
M1	3	BM	K_2EDTA	CD16+Neutrophiles	12	64.05	0.41	0.63	0.36	0.56	0.54	0.85
M1	3	WB	K_2EDTA	CD64+Monocytes	12	8.02	0.17	2.10	0.52	6.44	0.54	6.77
M1	3	WB	K_2EDTA	CD34-CD7+	12	69.35	0.36	0.52	1.42	2.04	1.46	2.11
M1	3	BM	K_2EDTA	CD34-CD7+	12	12.23	0.58	4.77	0.00	0.00	0.58	4.77
M2	1	BM	ACD	CD117+	12	65.10	0.73	1.12	0.51	0.78	0.89	1.37
M2	1	BM	ACD	CD123+	12	64.57	0.98	1.52	0.56	0.87	1.13	1.75
M2	1	BM	ACD	CD15+Neutrophile	12	57.41	1.02	1.78	0.25	0.44	1.05	1.83
M2	1	BM	ACD	CD33+ Monocytes	12	10.80	0.16	1.51	0.00	0.00	0.16	1.51
M2	1	BM	ACD	CD15+Neutrophile	12	34.68	2.19	6.32	0.00	0.00	2.19	6.32
M2	1	BM	ACD	CD33+ Monocytes	12	3.46	0.30	8.68	0.25	7.21	0.39	11.28
M2	1	BM	ACD	CD117+	12	9.52	0.18	1.87	0.13	1.33	0.22	2.30
M2	1	BM	ACD	CD34+CD123+	12	6.63	0.12	1.76	0.29	4.40	0.31	4.74
M2	2	BM	Heparin	CD117+	12	10.79	0.26	2.38	0.55	5.12	0.61	5.65
M2	2	BM	Heparin	CD34+CD123+	12	5.83	0.27	4.57	0.30	5.18	0.40	6.91
M2	2	WB	Heparin	CD15+Neutrophile	12	80.51	0.84	1.04	1.02	1.27	1.33	1.65
M2	2	WB	Heparin	CD33+ Monocytes	12	2.11	0.12	5.90	0.38	17.96	0.40	18.90*
M2	2	BM	Heparin	CD15+Neutrophile	12	81.53	0.49	0.60	0.18	0.22	0.52	0.64
M2	2	BM	Heparin	CD33+ Monocytes	12	4.59	0.17	3.75	0.28	6.05	0.33	7.12
M2	2	WB	Heparin	CD117+	12	65.13	0.90	1.38	0.58	0.89	1.07	1.64
M2	2	WB	Heparin	CD34+CD123+	12	78.57	0.53	0.68	0.00	0.00	0.53	0.68
M2	3	BM	K_2EDTA	CD15+Neutrophile	12	67.45	0.78	1.16	2.00	2.96	2.15	3.18
M2	3	BM	K_2EDTA	CD33+ Monocytes	12	5.50	0.18	3.27	0.24	4.41	0.30	5.49
M2	3	WB	K_2EDTA	CD117+	12	14.31	0.23	1.58	0.31	2.15	0.38	2.67
M2	3	WB	K_2EDTA	CD34+CD123+	12	11.80	0.25	2.09	0.13	1.13	0.28	2.37
M2	3	BM	K_2EDTA	CD34+CD123+	12	94.61	0.28	0.29	0.00	0.00	0.28	0.29
M2	3	WB	K_2EDTA	CD15+Neutrophile	12	47.82	0.41	0.86	0.25	0.52	0.48	1.00
M2	3	WB	K_2EDTA	CD33+ Monocytes	12	2.25	0.19	8.56	0.70	31.09	0.73	32.24^
M2	3	BM	K_2EDTA	CD117+	12	70.74	0.44	0.62	1.19	1.68	1.27	1.80
M2	3	BM	K_2EDTA	CD34+CD123+	12	69.12	0.43	0.62	1.78	2.58	1.83	2.65
M2	3	WB	K_2EDTA	CD15+Neutrophile	12	73.72	0.71	0.97	0.00	0.00	0.71	0.97
M2	3	WB	K_2EDTA	CD33+ Monocytes	12	7.06	0.20	2.77	0.16	2.25	0.25	3.57
T	1	BM	ACD	NK	12	4.94	0.29	5.97	12	0.25	5.00	0.38
T	1	BM	ACD	γδ T cells	12	28.54	0.54	1.90	12	0.00	0.00	0.54
T	1	BM	ACD	T Helper %Gated	12	39.49	0.45	1.15	12	0.19	0.48	0.49
T	1	BM	ACD	Cytotoxic T cells	12	30.52	0.50	1.65	12	0.37	1.22	0.63
T	1	WB	ACD	NK	12	6.36	0.10	1.52	12	0.05	0.71	0.11

Quantitative Results – by Specimen, by Site												
Panel	Site	Specimen Type	Anti-coagulant	Population	N	Mean (%)	Repeatability		Between-Run		Within-Sample	
							SD	%CV	SD	%CV	SD	%CV
T	1	WB	ACD	γδ T cells	12	2.21	0.16	7.09	12	0.17	7.52	0.23
T	1	WB	ACD	T Helper %Gated	12	68.34	0.59	0.86	12	0.22	0.32	0.63
T	1	WB	ACD	Cytotoxic T cells	12	23.80	0.49	2.06	12	0.44	1.83	0.66
T	1	BM	ACD	NK	12	8.78	0.43	4.87	12	0.26	3.00	0.50
T	1	BM	ACD	γδ T cells	12	6.99	0.21	3.02	12	0.12	1.69	0.24
T	1	BM	ACD	T Helper %Gated	12	38.92	0.59	1.51	12	0.36	0.92	0.69
T	1	BM	ACD	Cytotoxic T cells	12	49.85	0.64	1.28	12	0.00	0.00	0.64
T	1	WB	ACD	NK	12	20.43	0.48	2.34	12	0.19	0.94	0.52
T	1	WB	ACD	T Helper %Gated	12	39.15	0.48	1.23	12	0.00	0.00	0.48
T	1	WB	ACD	Cytotoxic T cells	12	55.24	0.48	0.87	12	0.49	0.89	0.69
T	2	WB	Heparin	NK	12	18.60	0.83	4.46	0.68	3.64	1.07	5.76
T	2	WB	Heparin	T Helper %Gated	12	83.71	1.34	1.60	0.98	1.17	1.66	1.98
T	2	WB	Heparin	Cytotoxic T cells	12	11.97	1.05	8.75	0.00	0.00	1.05	8.75
T	2	BM	Heparin	NK	12	12.27	0.46	3.73	0.19	1.57	0.50	4.05
T	2	BM	Heparin	T Helper %Gated	12	58.43	1.80	3.07	0.00	0.00	1.80	3.07
T	2	BM	Heparin	Cytotoxic T cells	12	36.64	1.48	4.03	0.00	0.00	1.48	4.03
T	2	WB	Heparin	NK	12	27.41	0.31	1.14	0.69	2.51	0.76	2.76
T	2	WB	Heparin	γδ T cells	12	3.11	0.17	5.39	0.00	0.00	0.17	5.39
T	2	WB	Heparin	T Helper %Gated	12	50.26	0.73	1.46	0.35	0.70	0.81	1.62
T	2	WB	Heparin	Cytotoxic T cells	12	44.48	0.69	1.54	0.00	0.00	0.69	1.54
T	2	BM	Heparin	NK	12	11.83	0.47	3.94	0.71	5.97	0.85	7.15
T	2	BM	Heparin	γδ T cells	12	2.62	0.21	7.98	0.21	8.18	0.30	11.43
T	2	BM	Heparin	T Helper %Gated	12	14.37	0.59	4.10	0.00	0.00	0.59	4.10
T	2	BM	Heparin	Cytotoxic T cells	12	77.33	0.79	1.02	1.82	2.35	1.98	2.56
T	3	LN	NA	T Helper %Gated	12	73.64	0.34	0.46	0.00	0.00	0.34	0.46
T	3	LN	NA	Cytotoxic T cells	12	22.85	0.23	0.99	0.20	0.89	0.30	1.33
T	3	BM	K_2EDTA	NK	12	7.75	0.20	2.63	0.00	0.00	0.20	2.63
T	3	BM	K_2EDTA	T Helper %Gated	12	72.87	0.49	0.68	0.00	0.00	0.49	0.68
T	3	BM	K_2EDTA	Cytotoxic T cells	12	22.76	0.52	2.30	0.00	0.00	0.52	2.30
T	3	WB	K_2EDTA	NK	12	27.78	0.91	3.28	0.26	0.95	0.95	3.42
T	3	WB	K_2EDTA	T Helper %Gated	12	70.07	0.50	0.72	0.00	0.00	0.50	0.72
T	3	WB	K_2EDTA	Cytotoxic T cells	12	19.93	0.80	4.04	0.00	0.00	0.80	4.04
T	3	LN	NA	γδ T cells	12	1.17	0.07	6.39	0.00	0.00	0.07	6.39
T	3	LN	NA	T Helper %Gated	12	93.44	0.16	0.17	0.00	0.00	0.16	0.17
T	3	LN	NA	Cytotoxic T cells	12	4.50	0.11	2.54	0.09	2.06	0.15	3.27
T	3	WB	K_2EDTA	NK	10	5.90	0.24	4.15	0.00	0.00	0.24	4.15
T	3	WB	K_2EDTA	γδ T cells	10	1.15	0.07	6.35	0.05	4.19	0.09	7.61
T	3	WB	K_2EDTA	T Helper %Gated	10	65.58	0.53	0.81	0.57	0.87	0.78	1.19
T	3	WB	K_2EDTA	Cytotoxic T cells	10	31.11	0.44	1.41	0.23	0.74	0.50	1.60
T	3	BM	K_2EDTA	NK	12	24.47	0.95	3.89	0.00	0.00	0.95	3.89
T	3	BM	K_2EDTA	γδ T cells	12	2.24	0.26	11.58	0.38	17.07	0.46	20.63[&]
T	3	BM	K_2EDTA	T Helper %Gated	12	55.28	1.03	1.86	0.00	0.00	1.03	1.86
T	3	BM	K_2EDTA	Cytotoxic T cells	12	38.60	0.92	2.39	0.37	0.97	1.00	2.58

***** Sample is a whole blood specimen collected in K2EDTA anticoagulant that demonstrated %CV of 17.96% for between-runs and 18.90% for within-sample for CD33 marked Monocytes, as the CD33 marked Monocytes recovery in this specimen was 2.11% of total CD45+ cells. The data analysis indicated that imprecision was

likely due to variations from morning preparation to afternoon preparation within the sample. The high imprecision for this sample has no clinical impact.

^ Samples is a phenotypically normal WB specimen collected in K2EDTA anticoagulant that demonstrated %CV of 31.09% for between-runs and 32.24% for within-sample for CD33 marked Monocytes, as the CD33 marked Monocytes recovery in this specimen was 2.25% of total CD45+ cells. The data analysis indicated that imprecision was likely due to variations from morning preparation to afternoon preparation within the sample. The high imprecision for this sample has no clinical impact.

& Sample is a phenotypically normal BM specimen collected in K2EDTA anticoagulant that demonstrated %CV of 20.63% for the TCRg/d cells, as the TCR g/d cells recovery in this specimen was extremely low with only an average of 73 events collected, representing only ~0.12% of total CD45+ cells. The high imprecision for this sample has no clinical impact.

b. Operator-to-operator and instrument-to-instrument imprecision:

The imprecision of between-operator and between-instrument was evaluated by testing four normal and four abnormal clinical specimens with the ClearLLab 10C Panels at one site, with three operators analyzing three replicates on each of two DxFLEX instruments on each of two runs. Each operator performed separate sample preparations in triplicate in both the morning and afternoon. Samples were acquired on each of two instruments, the order of which was randomized. Following acquisition, each operator analyzed listmode datafiles (LMD), using Kaluza C software. A reviewer assessed each LMD analysis as normal or abnormal, and further described the immunophenotype of abnormal results. The study included 288 analyses (three operators/replicate x three replicates/run x two runs/instrument x two instruments = 36 replicates/sample x eight samples).

All results showed 100% qualitative agreement across Instruments and Operators.

The number of samples tested is depicted in the tables below.

Qualitative Agreement across Instruments and Operators		
Panel	Phenotype	N
B	Normal (−)	36
B	Abnormal (+)	72
M1	Normal (−)	36
M1	Abnormal (+)	36
M2	Normal (−)	36
M2	Abnormal (+)	36
T	Normal (−)	36

Results based on quantitative analysis are summarized in the table below.

Quantitative Results – by Instrument, by Operator													
Panel	Population	N	Mean (%)	Repeatability		Between-Run		Between-Instruments		Between-Operators		Within-Specimen	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
B	CD19+CD5+	36	91.55	0.67	0.73	0.40	0.44	0.64	0.70	0.00	0.00	1.01	1.10
B	Mature κ^+ B cells	36	90.63	0.68	0.75	0.17	0.19	0.00	0.00	0.00	0.00	0.70	0.77
B	Mature λ^+ B cells	36	8.84	0.58	6.56	0.19	2.20	0.00	0.00	0.00	0.00	0.61	6.92
B	Mature κ^+ B cells	36	63.62	1.99	3.12	0.00	0.00	0.00	0.00	0.41	0.65	2.03	3.19

Quantitative Results – by Instrument, by Operator													
Panel	Population	N	Mean (%)	Repeatability		Between-Run		Between-Instruments		Between-Operators		Within-Specimen	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
B	Mature λ^+ B cells	36	35.75	1.87	5.22	0.26	0.73	0.00	0.00	0.77	2.16	2.04	5.70
M1	CD34-CD7+	36	6.22	0.19	3.01	0.09	1.39	0.00	0.00	0.26	4.11	0.33	5.28
M1	CD16+Neutrophiles	36	57.46	2.66	4.63	4.11	7.14	0.00	0.00	3.61	6.29	6.08	10.59
M1	CD64+Monocytes	36	6.91	0.47	6.86	0.72	10.37	0.00	0.00	0.00	0.00	0.86	12.43
M2	CD15+Neutrophile	36	62.79	0.39	0.62	0.89	1.42	0.00	0.00	0.15	0.24	0.98	1.57
M2	CD33+ Monocytes	36	4.79	0.09	1.92	0.07	1.54	0.02	0.41	0.00	0.00	0.12	2.49
M2	CD117+	36	7.06	0.28	4.03	0.32	4.47	0.67	9.55	0.00	0.00	0.80	11.29
M2	CD34+CD123+	36	2.85	0.10	3.41	0.16	5.75	0.00	0.00	0.03	0.99	0.19	6.76
T	NK cells	36	17.65	0.46	2.62	0.36	2.03	0.14	0.80	0.19	1.07	0.63	3.57
T	γδ T cells	36	2.20	0.17	7.95	0.00	0.00	0.04	1.68	0.00	0.00	0.18	8.12
T	Helper T cells	36	80.62	0.55	0.68	0.00	0.00	0.00	0.00	0.26	0.32	0.61	0.75
T	Cytotoxic T cells	36	15.43	0.44	2.88	0.00	0.00	0.15	0.98	0.25	1.62	0.53	3.45

c. *Site-to-Site Reproducibility with Control Material*:

The reproducibility of the ClearLLab 10C Reagent System on DxFLEX Flow Cytometer was evaluated utilizing one lot each of control cell materials: ClearLLab Normal Control Cells and ClearLLab Abnormal Control Cells. These control materials express all surface markers comprising the ClearLLab 10C panels. For multi-site testing, control materials allow samples to be shared between sites in a manner not available for native samples. For each panel, the two controls were tested on three instruments, across three total sites using one ClearLLab 10C reagent lot. At Site 1, testing was performed for 20 days × two runs/day × two replicates/run × three sites × one instrument/site. At sites 2 and 3, testing was performed for five days × two runs/day × three replicates/run × three sites × one instrument/site. Across all three sites, a total of 1133 analyses were generated; 13 were excluded due to operator error. The results are summarized in the tables below.

Normal Control Cells – across sites													
Cell Population	Panel	N	Mean (%)	Repeatability		Between-Run		Between-Day		Between-Site		Reproducibility	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
WBC	B	140	97.76	0.43	0.44	1.39	1.46	4.14	4.35	1.59	1.67	4.68	4.91
lymphocyte	B	140	30.70	0.76	2.47	1.32	1.35	2.87	2.94	1.93	1.97	3.72	3.81
monocyte	B	140	9.29	0.39	4.17	0.60	1.96	0.54	1.76	0.69	2.26	1.31	4.26
granulocyte	B	140	57.41	0.93	1.62	0.18	1.92	0.33	3.54	1.20	12.88	1.31	14.12
$CD5^+$	B	140	74.62	0.55	0.74	0.82	1.43	0.63	1.09	2.11	3.68	2.53	4.40
$CD10^+$	B	140	57.00	0.91	1.60	0.25	0.34	0.41	0.55	0.32	0.42	0.80	1.07
$CD19^+CD20^+$	B	140	10.09	0.28	2.77	0.80	1.41	0.57	1.00	2.04	3.59	2.44	4.29
$CD38^+$	B	140	8.83	0.36	4.03	0.14	1.41	0.17	1.71	1.06	10.48	1.12	11.06
$CD200^+$	B	140	9.22	0.25	2.76	0.25	2.84	0.16	1.87	1.42	16.09	1.49	16.93*
$Kappa^+$	B	140	59.62	1.32	2.22	0.16	1.75	0.14	1.53	0.94	10.15	0.99	10.78
$Lambda^+$	B	140	40.34	1.33	3.30	0.19	0.32	0.44	0.73	0.56	0.94	1.51	2.54
WBC	M1	140	97.80	0.23	0.24	1.31	1.34	2.77	2.84	1.91	1.95	3.62	3.70
lymphocyte	M1	140	30.53	0.73	2.38	0.62	2.02	0.68	2.24	1.95	6.37	2.27	7.44
monocyte	M1	140	9.10	0.39	4.31	0.27	2.95	0.27	2.96	0.51	5.61	0.75	8.22

Normal Control Cells – across sites													
Cell Population	Panel	N	Mean (%)	Repeatability		Between-Run		Between-Day		Between-Site		Reproducibility	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
granulocyte	M1	140	57.91	0.90	1.56	0.76	1.32	0.70	1.21	2.88	4.97	3.19	5.51
$CD10^+$	M1	140	57.46	0.90	1.56	0.83	1.45	0.71	1.24	2.92	5.09	3.25	5.65
$CD7^+$	M1	140	80.54	0.51	0.63	0.21	0.26	0.82	1.01	4.83	6.00	4.93	6.12
$CD14^+CD11b^+$	M1	140	7.89	0.32	4.08	0.25	3.13	0.00	0.00	1.02	12.90	1.10	13.89
$CD13^+$	M1	140	57.54	0.93	1.62	0.83	1.44	0.71	1.24	2.81	4.88	3.16	5.48
$CD16^+$	M1	140	54.36	0.86	1.59	0.75	1.38	0.83	1.52	2.86	5.26	3.19	5.87
$CD14^+CD64^+$	M1	140	7.80	0.32	4.13	0.26	3.38	0.10	1.33	0.98	12.63	1.07	13.77
$HLA\text{-}DR^+$	M1	140	8.44	0.33	3.93	0.27	3.15	0.22	2.61	0.42	4.98	0.64	7.55
WBC	M2	140	97.90	0.38	0.38	0.67	0.68	2.50	2.55	1.87	1.91	3.21	3.28
lymphocyte	M2	140	30.38	0.85	2.79	0.82	2.71	0.67	2.21	1.98	6.53	2.40	7.91
monocyte	M2	140	9.12	0.48	5.23	0.00	0.00	0.39	4.32	0.74	8.11	0.96	10.57
granulocyte	M2	140	58.06	1.22	2.10	0.98	1.70	0.86	1.47	2.79	4.81	3.32	5.71
$CD38^+$	M2	140	8.69	0.42	4.84	0.13	1.50	0.38	4.40	0.83	9.59	1.02	11.71
$CD13^+$	M2	140	57.57	1.24	2.15	1.07	1.85	0.87	1.50	2.47	4.29	3.08	5.36
$HLA\text{-}DR^+$	M2	140	8.43	0.44	5.25	0.00	0.00	0.28	3.37	0.58	6.92	0.79	9.32
$CD15^+$	M2	140	57.44	1.24	2.17	1.03	1.80	0.94	1.64	2.71	4.72	3.29	5.74
$CD19^+$	M2	140	9.82	0.32	3.23	0.21	2.12	0.33	3.33	0.50	5.14	0.71	7.24
$CD33^+$	M2	140	8.90	0.49	5.56	0.20	2.24	0.47	5.29	1.31	14.75	1.49	16.78*
WBC	T	140	97.72	0.50	0.51	0.95	0.97	2.88	2.95	1.95	2.00	3.64	3.72
lymphocyte	T	140	30.59	0.66	2.17	0.59	1.94	0.63	2.04	1.41	4.59	1.78	5.81
monocyte	T	140	9.17	0.29	3.18	0.37	4.04	0.27	2.99	1.09	11.87	1.22	13.28
granulocyte	T	140	57.56	0.81	1.40	0.82	1.43	0.77	1.33	2.58	4.49	2.93	5.09
$CD5^+$	T	140	75.54	0.46	0.61	0.40	0.53	0.49	0.65	0.60	0.80	0.99	1.31
$CD2^+$	T	140	84.68	0.37	0.44	0.26	0.31	0.28	0.33	1.04	1.23	1.17	1.38
$CD3^+$	T	140	76.05	0.43	0.57	0.29	0.39	0.42	0.56	0.49	0.64	0.83	1.10
$CD4^+CD8^-$	T	140	65.08	0.68	1.05	0.38	0.59	0.00	0.00	1.12	1.72	1.36	2.10
$CD4^-CD8^+$	T	140	29.06	0.71	2.44	0.53	1.84	0.00	0.00	2.32	7.98	2.48	8.54
$CD7^+$	T	140	79.47	0.46	0.58	0.70	0.88	1.04	1.31	3.15	3.97	3.43	4.31
$CD3^-CD56^+$	T	140	11.33	0.31	2.70	0.24	2.08	0.35	3.09	1.84	16.28	1.92	16.91*
$TCR\gamma\delta^+$	T	140	2.77	0.13	4.59	0.00	0.00	0.07	2.63	0.15	5.48	0.21	7.62

Abnormal Control Cells – across sites													
Cell Population	Panel	N	Mean (%)	Repeatability		Between-Run		Between-Days		Between-Site		Reproducibility	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
WBC	B	140	97.92	0.61	0.62	0.58	0.59	2.49	2.54	1.73	1.76	3.14	3.21
lymphocyte	B	140	30.34	0.88	2.89	0.70	2.31	0.27	0.88	1.30	4.27	1.73	5.72
monocyte	B	140	9.09	0.35	3.85	0.19	2.04	0.25	2.72	0.87	9.56	0.99	10.85
granulocyte	B	140	57.80	1.15	1.98	0.96	1.66	0.38	0.66	2.45	4.24	2.90	5.01
$CD5^+$	B	140	74.89	0.48	0.64	0.36	0.48	0.41	0.55	0.16	0.21	0.75	1.00
$CD10^+$	B	140	56.99	1.19	2.09	0.89	1.57	0.37	0.66	2.71	4.75	3.11	5.46
$CD19^+CD20^+$	B	140	10.05	0.32	3.20	0.16	1.61	0.00	0.00	1.17	11.62	1.22	12.16
$CD34^+$	B	140	7.99	0.26	3.23	0.20	2.55	0.35	4.43	0.50	6.25	0.69	8.70
$CD38^+$	B	140	8.65	0.31	3.56	0.28	3.25	0.29	3.33	1.15	13.29	1.26	14.52

Abnormal Control Cells – across sites													
Cell Population	Panel	N	Mean (%)	Repeatability		Between-Run		Between-Days		Between-Site		Reproducibility	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
$CD200^+$	B	140	9.16	0.31	3.42	0.16	1.72	0.09	0.99	1.06	11.59	1.12	12.25
$Kappa^+$	B	140	59.51	1.38	2.32	0.47	0.80	0.54	0.91	0.39	0.65	1.61	2.70
$Lambda^+$	B	140	40.45	1.37	3.38	0.48	1.19	0.56	1.39	0.23	0.56	1.57	3.88
WBC	M1	140	97.88	0.95	0.98	0.71	0.72	2.38	2.43	1.88	1.92	3.26	3.33
lymphocyte	M1	140	30.12	1.04	3.44	0.75	2.49	0.42	1.39	1.97	6.53	2.38	7.91
monocyte	M1	140	8.89	0.30	3.37	0.17	1.86	0.37	4.20	0.55	6.19	0.75	8.42
granulocyte	M1	140	58.38	1.27	2.18	0.94	1.61	0.63	1.08	2.98	5.11	3.43	5.88
$CD10^+$	M1	140	57.62	1.29	2.24	1.00	1.74	0.72	1.25	3.20	5.55	3.67	6.36
$CD34^+$	M1	140	8.04	0.28	3.51	0.23	2.92	0.28	3.44	0.71	8.84	0.85	10.53
$CD7^+$	M1	140	80.95	0.44	0.55	0.34	0.42	0.52	0.64	4.00	4.94	4.07	5.03
$CD14^+CD11b^+$	M1	140	7.77	0.36	4.61	0.20	2.54	0.20	2.58	0.94	12.04	1.04	13.40
$CD13^+$	M1	140	58.14	1.31	2.25	0.90	1.54	0.80	1.38	2.85	4.91	3.36	5.79
$CD16^+$	M1	140	54.33	1.22	2.25	0.96	1.76	0.65	1.20	3.19	5.87	3.61	6.64
$CD14^+CD64^+$	M1	140	7.70	0.35	4.58	0.21	2.72	0.23	3.00	0.89	11.51	1.00	13.03
$HLA\text{-}DR^+$	M1	140	8.45	0.35	4.08	0.20	2.36	0.39	4.67	0.37	4.40	0.67	7.96
WBC	M2	140	98.03	0.65	0.66	0.30	0.31	2.19	2.23	1.74	1.78	2.89	2.95
lymphocyte	M2	140	29.97	0.83	2.76	0.83	2.76	0.63	2.09	2.48	8.26	2.81	9.37
monocyte	M2	140	8.78	0.35	3.97	0.14	1.65	0.25	2.82	0.64	7.32	0.79	8.95
granulocyte	M2	140	58.61	1.03	1.76	0.98	1.67	0.81	1.38	3.44	5.87	3.81	6.50
$CD34^+$	M2	140	7.96	0.31	3.85	0.27	3.34	0.32	4.07	0.63	7.93	0.82	10.26
$CD38^{++}$	M2	140	8.31	0.32	3.81	0.25	3.03	0.23	2.81	0.85	10.28	0.97	11.72
$CD13^+$	M2	140	58.01	1.05	1.80	0.90	1.55	0.86	1.48	3.78	6.52	4.11	7.09
$HLA\text{-}DR^+$	M2	140	8.35	0.34	4.02	0.21	2.51	0.23	2.76	0.60	7.21	0.76	9.06
CD15	M2	140	57.80	1.04	1.80	1.05	1.82	0.82	1.42	3.62	6.26	3.99	6.91
$CD19^+$	M2	140	9.69	0.33	3.45	0.06	0.63	0.18	1.88	0.65	6.74	0.76	7.83
$CD33^+$	M2	140	8.62	0.36	4.19	0.19	2.26	0.25	2.94	1.11	12.88	1.21	14.04
$CD117^+$	M2	140	8.06	0.31	3.84	0.25	3.14	0.34	4.23	0.48	5.90	0.71	8.79
$CD123^+$	M2	140	8.16	0.31	3.79	0.28	3.46	0.34	4.12	0.47	5.81	0.72	8.78
WBC	T	140	97.83	1.22	1.25	0.62	0.64	2.31	2.36	1.90	1.94	3.29	3.36
lymphocyte	T	140	30.34	0.86	2.84	1.10	3.62	0.41	1.34	2.02	6.65	2.49	8.20
monocyte	T	140	8.91	0.34	3.84	0.33	3.66	0.29	3.27	0.98	10.95	1.12	12.60
granulocyte	T	140	58.08	1.09	1.88	1.38	2.38	0.51	0.88	3.25	5.59	3.73	6.42
$CD5^+$	T	140	75.50	0.55	0.73	0.19	0.25	0.58	0.76	0.94	1.25	1.25	1.65
$CD34^+$	T	140	8.15	0.44	5.46	0.00	0.00	0.00	0.00	0.50	6.11	0.67	8.19
$CD2^{+e}$	T	140	84.61	0.48	0.57	0.13	0.15	0.48	0.56	1.47	1.73	1.62	1.92
$CD3^+$	T	140	76.08	0.51	0.67	0.29	0.38	0.44	0.58	0.33	0.43	0.81	1.06
$CD4^+CD8^-$	T	140	65.11	0.70	1.08	0.67	1.03	0.00	0.00	1.22	1.87	1.56	2.39
$CD4^-CD8^+$	T	140	29.10	0.63	2.15	0.67	2.31	0.00	0.00	2.39	8.20	2.56	8.78
$CD7^+$	T	140	79.02	0.50	0.63	0.55	0.69	0.77	0.98	3.92	4.96	4.06	5.14
$CD3^-CD56^+$	T	140	11.16	0.33	2.95	0.18	1.57	0.22	1.93	1.81	16.17	1.86	16.62*
$TCR\gamma\delta^+$	T	140	2.79	0.11	4.09	0.09	3.22	0.04	1.44	0.15	5.30	0.21	7.57

* %CV of reproductivity for the sample is >15%. As the repeatability of individual CD measurements includes unique variability sources, sources of variability include the frequency of cells with a particular phenotype, the level of the marker expression on the specific population being tested (in relation to the expression on other populations that

are present), as well as the fluorescence intensity of the fluorochrome (quantum efficiency) of the specific conjugate used for staining. These differences in cell number, target analyte level, distribution on non-target populations and fluorochrome intensity affect the ability of the instrument/cytometrist to distinguish specific populations. These additional sources of variability contribute to the increased imprecision in several of the phenotypes tested.

d. *ClearLLab 10C Reagents Lot-to-Lot Reproducibility:*

Refer to K183592

2. Linearity (Fluorescence Detection):

The study was performed to evaluate the linearity and dynamic range of the DxFLEX Flow Cytometers' acquisition systems, from the single-photon detection APD transfer to the electronics, independent of the application and gating methodology using SpheroTECH Rainbow particles (RCP-30-5A) analyzed at six different gain settings covering the dynamic range of the DxFLEX Flow Cytometer. The study was conducted on three DxFLEX Flow Cytometers. The results indicated the constancy (<5% deviation) of the ratio of adjacent bead fluorescence, across a series of gain settings. Representative data from one DxFLEX Flow Cytometer is presented in the following table.

Gain Setting	**Median-A Ratio of P7/P6**									
	FITC	**PE**	**ECD**	**PC5.5**	**PC7**	**APC**	**APC700**	**APC750**	**PB450**	**KO525**
100	2.88	2.51	3.02	3.19	3.28	2.82	2.95	2.97	2.79	2.96
500	2.88	2.51	3.04	3.21	3.20	2.82	2.95	2.94	2.81	2.98
1000	2.86	2.52	3.03	3.19	3.16	2.81	2.94	2.94	2.79	2.96
1500	2.87	2.52	3.03	3.19	3.19	2.82	2.93	2.92	2.80	2.97
2000	2.88	2.51	3.03	3.20	3.20	2.83	2.93	2.94	2.81	2.98
3000	2.88	2.51	3.03	3.19	3.19	2.81	2.95	2.96	2.78	2.96
Lower Limit	2.73	2.39	2.88	3.04	3.05	2.68	2.79	2.80	2.66	2.82
Upper Limit	3.02	2.64	3.18	3.36	3.37	2.96	3.09	3.09	2.94	3.12

3. Analytical Specificity/Interference:

a. Interfering Substances:

Not applicable. The test system requires washing with phosphate buffered saline (PBS) before staining with reagents to remove residual plasma and interfering substances.

b. *Carryover:*

Specimen Carryover

Specimen carryover was evaluated by following the protocol from CLSI H26-A2 on three DxFLEX Flow Cytometers. Samples with high concentration (high target value; HTv)

and low concentration (low target value; LTv) were run in multiple replicates on the DxFLEX Flow Cytometer. CD34 marker analysis was performed using a CD34+ clinical specimen. Three high concentration samples (HTv1-HTv2-HTv3) targeting to 20,000 cells /µL white blood cells (WBC) were prepared from one specimen containing a high level of white blood cells Low concentration samples (LTv1-LTv2-LTv3) counts were prepared by staining a diluted (1:10,000) specimen with a blank Red Cell Pool (a red blood cell pool which has been depleted of white blood cells diluted 4:1 with buffer). This resulted in a specimen composition similar to whole blood regarding the red blood cell concentration with residual concentrations of WBCs. On each of three DxFLEX Flow Cytometers, three HTv samples (HTv1, HTv2, HTv3) were analyzed consecutively followed immediately by the three LTv samples (LTv1, LTv2, LTv3) using the four ClearLLab 10C Panels. A total of 54 specimen carryover runs were generated for each panel. The % carryover, which is calculated as % Carryover = (LTV1-LTV3)/average (HTv1, HTv2, HTv3), demonstrated to be < 1%, for each of the 37 phenotypes measured across the four ClearLLab 10C tubes.

Reagent Carryover

Reagent carryover was evaluated by following the protocol from CLSI H26-A2 on three DxFLEX Flow Cytometers. Samples with high concentration (high target value; HTv) and low concentration (low target value; LTv) were run in multiple replicates on the DxFLEX flow cytometer. Clinical bone marrow specimens stained with all four ClearLLab 10C Panels were used as the high concentration samples. Low concentration samples (LTv1, LTv2, LTv3) were prepared from the same clinical bone marrow specimens as in the high concentration samples, that were processed in triplicate using antibody dilution buffer (RD1), in place of the ClearLLab 10C Panels, to produce an unstained sample. On each of three DxFLEX Flow Cytometers and three HTv samples (HTv1, HTv2, HTv3) were analyzed consecutively followed immediately by the three LTv samples (LTv1, LTv2, LTv3). A total of 54 specimen carryover runs were generated for each panel. The % carryover, which is calculated as % Carryover = (LTV1-LTV3)/average (HTv1, HTv2, HTv3), demonstrated to be < 1%, for each of the 37 phenotypes measured across the four ClearLLab 10C tubes.

4. Accuracy (Instrument):

Method Comparison with Predicate Device:

A method comparison study was performed to demonstrate qualitative agreement for the presence of abnormal cells between ClearLLab 10C Reagent System on the DxFLEX Flow Cytometer vs Navios EX Flow Cytometer. The study was based on the data generated from a multi-site retrospective clinical study conducted at five sites in which the test results from the ClearLLab 10C Reagents used to detect the presence or absence of an abnormal phenotype were compared to the clinical outcome of “malignant” or “non-malignant” based on the clinical sites’ final patient diagnosis.

Testing was conducted on the DxFLEX and Navios EX flow cytometers utilizing de-identified residual specimens, covering both hematologically abnormal malignant patients and hematologically abnormal non-malignant patients in line with the intended use of the ClearLLab 10C panels. The study included specimens of whole blood (WB) and bone

marrow (BM) collected in anticoagulant K2EDTA, Acid Citrate Dextrose (ACD) or Heparin, as well as specimens of lymph node (LN), which overall reflect the three major specimen types distributed in leukemia and lymphoma (L&L) diseases and clinical indications encountered in the L&L population. Patients with abnormal B-cell, T-cell, Natural Killer (NK) cell, and myeloid cell populations were tested. Specimen types tested reflected the distribution of the diseases and/or clinical indications encountered in the population of patients that are routinely encountered in evaluations of patients suspected of having hematopoietic neoplasia by flow cytometry. The study design was purposive, and specimens were enrolled consecutively.

Samples were prepared with all four ClearLLab 10C Panels, except LN where only B and T tubes were required. The same prepared tube was randomized for acquisition once on the Navios EX and once on the DxFLEX flow cytometer. Immunophenotyping was performed with provided analysis protocols using Kaluza C software. The phenotype assessments of the data generated from DxFLEX-10C system and Navios EX-10C system were independently assessed from each other. If clinical diagnosis was provided in the subject's case report form, the Principal Investigator at each site was blinded to such diagnosis to further assure the review was exclusively phenotype based. The assessment for presence or absence of an abnormal phenotype was based on Bethesda and WHO guidelines as follows:

- A 3% (in total leukocytes) clinical cutoff will be applied to BM blast population which is normally undetected in peripheral WB (<1%) or LN (<1%)
- 1% (in total leukocytes) clinical cutoff will be applied to all the other abnormal populations detected in BM, WB, or LN specimens

Of the 550 samples enrolled, 38 were excluded due to failing the inclusion criteria, preanalytical requirements or quality control or due to instrument, process or operator errors. A total of 512 specimens were qualified and included in the final analysis for evaluating method agreement per CLSI EP12-Ed3. The distribution of specimens is presented in the following tables.

Distribution of Subjects - by Gender and Age			
Gender	Male		268
	Female		239
	Not Provided		5
Age	Infant (1 mon-2 yrs)	Mean 4 months	1
	Child (>2-12 yrs)	Mean 6.3 yrs	12
	Adolescent (>12-21 yrs)	Mean 17.6 yrs	14
	Adult (>21-65 yrs)	Mean 50.6 yrs	230
	Geriatric (>65-89 yrs)	Mean 75.6 yrs	250
	>89 yrs	Mean N/A	5
	Total		**512**

Distribution of Subjects - by Category							
	Abnormal Hematology No Malignancy	Abnormal Hematology- Malignancy					**Total**
		Acute Leukemia	Chronic Leukemia	Lymphoma	Plasma Cell Neoplasm	Others: MDS MPN	
Site 1	46	12	14	26	7	10	115
Site 2	41	10	12	14	0	8	85
Site 3	60	14	30	18	5	2	129
Site 4	21	2	3	26	11	3	66
Site 5	28	23	18	29	8	11	117
Total	**196**	**61**	**77**	**113**	**31**	**34**	**512**

Distribution of Subjects - by Specimen Type	
WB	291
BM	167
LN	54
Total	**512**

The qualitative immunophenotype agreement (presence or absence of an abnormal population) was evaluated by comparing the assessment from the ClearLLab 10C on DxFLEX Flow Cytometer (Test Method) to that from the ClearLLab 10C on the Navios EX Flow Cytometer (Predicate Method). % The results are shown in the following tables:

Method Agreement – All Specimens Combined			
	ClearLLab 10C on Navios EX		
ClearLLab 10C on DxFLEX	**Presence of Abnormal cells (+)**	**Absence of Abnormal cells (-)**	**Total**
Presence of Abnormal cells (+)	231	0	231
Absence of Abnormal cells (-)	0	281	281
Total	246	281	512

PPA: 100% (95% CI: 98.46% – 100%)
NPA: 100% (95% CI: 99.28% – 100%)

Method Agreement – Whole Blood Specimens Only			
	ClearLLab 10C on Navios EX		
ClearLLab 10C on DxFLEX	**Presence of Abnormal cells (+)**	**Absence of Abnormal cells (-)**	**Total**
Presence of Abnormal cells (+)	139	0	139
Absence of Abnormal cells (-)	0	152	152
Total	139	152	291

PPA: 100% (95% CI: 97.47% – 100%)
NPA: 100% (95% CI: 97.54% – 100%)

Clinical performance (sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV)) of the ClearLLab 10C Reagent on DxFLEX Flow Cytometer was also assessed by measuring the agreement of the flow cytometric results with the clinical diagnosis. The results of this evaluation are presented below.

Diagnostic Accuracy – All Specimen Types Combined			
	Clinical Outcome		
ClearLLab 10C on DxFLEX	**Malignant**	**Non-Malignant**	**Total**
Malignant (+)	220	11	231
Non-Malignant (-)	96	185	281
Total	316	196	512

Sensitivity: 70% (95% CI: 64% – 74%)
Specificity: 94% (95% CI: 90% – 97%)
PPV: 95% (95% CI: 92% – 97%)
NPV: 66% (95% CI: 60% – 71%)

Diagnostic Accuracy – Whole Blood Only			
	Clinical Outcome		
ClearLLab 10C on DxFLEX	**Malignant**	**Non-Malignant**	**Total**
Malignant (+)	133	6	139
Non-Malignant (-)	27	125	152
Total	160	131	291

Sensitivity: 83% (95% CI: 77%– 88%)
Specificity: 95% (95% CI: 90% – 98%)
PPV: 96% (95% CI: 91% – 98%)
NPV: 82% (95% CI: 75% – 88%)

The clinical performance was also assessed with the predicate device. The results demonstrated 100% phenotypic agreement between the candidate and predicate devices. For both methods, when compared to clinical diagnostic outcome, there were a total of 107 discordant cases including 11 non-concordant to non-malignancy (false positive) and 96 non-concordant to malignancy (false negative) cases. The false negative was categorized as being due to specimen type limitation, flow cytometry limitation, or ClearLLab reagents limitation. These limitations occur when the phenotypically abnormal population is not found in a particular specimen, the clinical condition is not typically detected through flow cytometry, or the reagents did not have all the antibodies required to detect a particular clinical condition, respectively. False positive cases were primarily due to increases in populations that could represent reactive responses to viral or bacterial infection or autoimmunity, where lymphoid proliferation is common.

B Other Supportive Instrument Performance Characteristics Data:

The detection capability of the DxFLEX flow cytometer with the ClearLLab 10C Reagent, was assessed for each reagent tube using one normal specimen (Blank) that was spiked with samples having abnormal populations (CLL, T-ALL, Myelomonocytic Leukemia and AML), to target 0.5%, 1% and 2% of the abnormal population, each in five replicates. The results demonstrated

that 100% of the replicates showed the correct populations, down to a level of 0.5% and verified that the Limit of Detection of 1% was confirmed on the new instrument.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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