BD Protocol #: CAS-LIBHIVREF

Protocol Title: Validation of the Reference Intervals for BD Multitest 4-Color and 6-Color reagents using EDTA anticoagulated venous blood on the Investigational BD FACSLyric 10-Color Clinical Flow Cytometer.

Current Version	Version 1.0/03Nov2015 (Original)
Version History	Version 1.0/03Nov2015 (Original)
Sponsor	BD – Biosciences 2350 Qume Drive San Jose, CA 95131
Sponsor Risk Assessment	☐ Significant Risk (SR) ☐ Non-significant Risk (NSR) ☒ Minimal Risk

The product information and data disclosed through this protocol are confidential and may not be disclosed without prior written consent of Becton, Dickinson and Company.

This study will be performed in accordance with all stipulations of the protocol and in compliance with all applicable BD Policies and Procedures. This study will be conducted in accordance with the ethical principles that originate from the Declaration of Helsinki and the Belmont Report. Study conduct will comply with US FDA Regulations, EU Directive 98/79/EC, applicable state and local regulations, and the Good Clinical Practice guidelines set forth by the International Conference on Harmonization (ICH-E6).

The performance characteristics of this product have not been established. Results must not be used as a diagnostic procedure without confirmation of the diagnosis by another, medically established diagnostic product or procedure.

All Study Product(s) used in this study should be considered to be for investigational use only.

SPONSOR PROTOCOL APPROVAL

Signature below indicates approval of the protocol as written.			
Individual or function	**Name**	**Signature**	**Date**
Medical Affairs Team Representative		*This document is signed electronically in the eTMF system*	
Study Statistician		*This document is signed electronically in the eTMF system*	
Regulatory		*This document is signed electronically in the eTMF system*	
Study Manager		*This document is signed electronically in the eTMF system*	
Business Unit Medical Director		*This document is signed electronically in the eTMF system*	

INVESTIGATOR SIGNATURE PAGE

Principal Investigator	{Name} {Address} (if different from site)
Investigational Site	{Site Name} {Address}

I have read this protocol and agree to conduct this study in accordance with all stipulations of the protocol and in compliance with all applicable Good Clinical Practices and regulations.

_____ _____

Signature of Principal Investigator Date

Table of Contents

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse event
BD	Becton Dickinson and Company
CFR	Code of Federal Regulations
CI	Confidence Interval
CRF	Case Report/Record Form
CS&T	Cytometer Setup and Tracking
DCF	Data Clarification Form
EDC	Electronic Data Capture
EDTA	Ethylene diamine tetra acetic acid
FDA	Food and Drug Administration
FDAAA	FDA Amendments Act of 2007
FTP	File Transfer Protocol
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonization
IFU	Instructions for Use
IRB/EC	Institutional or Independent Review Board/Ethics Committee
IUO	Investigational Use Only
IVD	In Vitro Diagnostics
IVDD	In Vitro Diagnostic Directive
µl	microliter
mL	Milliliter
mm	Millimeter
PEO	Performance Evaluation Only
PHI	Protected Health Information
PI	Package Insert
QNS	Quantity Not Sufficient
SAE	Serious Adverse Event
SD	Standard deviation
SOP	Standard Operating Procedure
WFN	Work Flow Notes
WHO	World Health Organization

1.0 INTRODUCTION

1.1 Statement of Compliance

This study is to be conducted in compliance with this protocol, Good Clinical Practices (GCP)[1], the Declaration of Helsinki, Title 21 of the Code of Federal Regulations §§ 50, 56, and 812, and the International Conference on Harmonization E6 and ISO Standards 14155-1:2003 and 14155-2:2003.

1.2 Proposed Intended Use

The BD FACSLyric flow cytometers (3-1, 4-2, 4-2-2 and 4-3-3 configurations) function as part of a system with BD FACSuite Clinical software intended for use with cleared or approved in vitro diagnostic (IVD) assays that are indicated for use with the instrument for identification and enumeration of human cell subsets. Only six fluorescence detection channels and two scatter 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 BD FACS™ Universal Loader.

1.3 Investigational Device System and Description

The investigational device system will be labeled for "Investigational Use Only (IUO)"/for "Performance Evaluation Only" (PEO) to satisfy FDA and IVDD requirements for products that are not cleared/approved. In this document, "IUO" will be used across the document to indicate the test product(s). The IUO system will consist of the following:

Instrument related:

- IUO BD FACSLyric 10 Color flow cytometer with universal sample loader
- Computer workstation with IUO FACSuite Clinical software.

IUO set-up beads:

- IUO BD CS&T beads
- IUO BD FC beads (7-Color Kit for BD FACSuite)

The IUO BD FACSLyric flow cytometer has four configurations (3-1, 4-2, 4-2-2 and 4-3-3). The 4-3-3 configuration will be tested in clinical trials. This configuration has three lasers, two light scatter detectors, 10 fluorescence detectors with optical filters.

The IUO FACSuite Clinical software, which runs on a companion workstation, is an integrated suite of applications and utilities that will enable clinical laboratories to easily run automatic setup and clinical assays using the IUO BD FACSLyric flow cytometer.

IUO BD CS&T beads and IUO BD FC (Fluorescence Control) beads are intended for use with IUO BD FACSLyric and IUO FACSuite Clinical software. The IUO BD CS&T beads are used to check cytometer performance and automatically make adjustments. They are run daily for the Performance QC and twice-a-year for characterization QC. The IUO BD FC beads allow the software to determine and automatically update spillover values for fluorescent compensation for clinical assays.

2.0 OBJECTIVES

2.1 Primary Objectives

To validate the reference intervals of a normal male and female adult cohort using the investigational-use-only (IUO)-labeled system (IUO BD FACSLyric with universal loader/IUO FACSuite Clinical software/IUO FC beads/IUO CS&T beads) in the following IVD cleared reagents

1. Multitest 4-color (4C) and 6-color (6C) TBNK reagents with Trucount tubes on determination of lymphocyte sub-populations [absolute CD3+ (**AbsCD3**), CD3+CD4+ (**AbsCD4**), CD3+CD8+ (**AbsCD8**), CD16+CD56+ (**AbsCD16+CD56**), and CD19+ (**AbsCD19**) cells counts, and percentage of CD3+ (**%CD3**), CD3+CD4+ (**%CD4**), CD3+CD8+(**%CD8**), CD16+CD56+ (**%CD16+CD56**), and CD19+ (**%CD19**)] using remnant/prospectively and de-linked donor specimens at one study site.

3.0 STUDY DESIGN

3.1 Overall Study Design

This is a single site prospective validation of the BD Multitest 6-Color TBNK reagents for reference intervals on the investigational BD FACSLyric flow cytometer. Testing will be performed using remnant or prospectively procured EDTA venous blood specimens from healthy adult male and female subjects to satisfy age and gender binning requirements. A three tiered approach will be used, with a minimum of 20 specimens per assay. If results from the first tier of enrollment do not verify the reference intervals, then the second tier of enrollment will be required. Likewise, if results from the second tier of enrollment do not verify the reference intervals, enrollment will be extended to the third tier to re-establish the claims. Specifics concerning the tiers and binning are discussed in Section 4.0. Previously established reference intervals are listed in Appendix 18.2.

Specimens will be de-identified and delinked prior to enrollment into the study. If quantities and time are sufficient, specimens may be used to test multiple assays.

3.2 Specification of Study Endpoints

3.2.1 Primary Endpoints

Calculating and validating the reference interval for each lymphocyte subset absolute count and percentage of cells in the total lymphocyte population with the previously established reference interval for Multitest IMK kit (4C TBNK), and Multitest 6-Color TBNK reagents.

For each reagent, remnant or prospectively procured and de-identified patient specimens will be enrolled to satisfy age and gender binning requirements as indicated in Section 4.0.

3.2.1 Safety Endpoints

During study testing, occurrence and severity of any adverse events will be evaluated, recorded, and followed up as required by BD procedures (Corporate Clinical Development and BD Biosciences).

3.3 Acceptance Criteria

3.3.1 Primary Objective

If no more than 10% of the subjects (2 out of 20) fall outside the original reported limits, the original reference interval claims will be considered successfully validated to be transferred to the new BD FACSLyric system. If less than 20% but more than 10% of the subjects fall outside the original claims, then an additional 20 samples will be collected and all data will be re-examined. If less than 10% of

the 40 subjects are found outside the claim, the claim will be considered successfully validated. If more than 10% of the 40 subjects fall outside the claim, the reference intervals will be re-established, requiring data collection from 60 healthy subjects of each gender (total of 120 subjects). Non-parametric rank statistics will be used to establish the new reference intervals.

See Table 2 for the minimum number of evaluable specimens per tier and bin.

BD Reagent	Absolute Counts	Percentage
4C-Multitest 6C-Multitest	CD3+, CD3+CD4+, CD3+CD8+	CD3+, CD3+CD4+, CD3+CD8+
4C-Multitest 6C-Multitest	CD16+56+	CD16+56+
4C-Multitest 6C-Multitest	CD19+	CD19+

3.4 Treatment Allocation and Methods to Reduce Bias

3.4.1 Randomization

Remnant and/or prospectively procured patient specimens will be de-identified and delinked from patient protected health information and assigned a Study ID number prior to enrollment in the study.

3.4.1 Skill and Behavior of Persons Interacting with the Device (if applicable)

The operators conducting the study should be qualified and have experience with flow cytometric testing, including identifying appropriate specimens, staining samples, acquire data on a flow cytometer, and analyzing flow cytometry results.

3.5 Stopping Rules

No stopping rules for the study have been developed by the Sponsor. The Principal Investigator is responsible for suspending study enrollment for reasons of subject/clinician safety and well-being.

4.0 STUDY POPULATION

The study will be conducted at the MedLab at BD Biosciences in San Jose, CA. The study site will obtain specimens from healthy donors free of hematological abnormalities from the BD In-House donor program, Stanford Blood Bank, or other similar institutions. Specimens with evaluable results will be binned by subject age and gender. Specimens that meet the binning requirements, depicted in Table 2 below, must be tested by each reagent. The determination of the number of specimens required for testing is described in Section 3.3.1.

Table 2. Three Level Tier for Specimen Enrollment

Age	1st Tier		2nd Tier		3rd Tier	
	Female	Male	Female	Male	Female	Male
18-29	2-3	2-3	5	5	15	15

30-41	2-3	2-3	5	5	15	15
42-53	2-3	2-3	5	5	15	15
>54	2-3	2-3	5	5	15	15
Sub-total	10	10	20	20	60	60
Total	**20**		**40**		**120**	
See CLSI EP28-3c, section 11.						

Unless specified otherwise, these criteria apply at screening and throughout the study.

4.1 Inclusion Criteria

Peripheral whole blood specimens must satisfy all of the following conditions to be considered for participation:

a. Collected in a blood collection tube with EDTA anticoagulant and stored at room temperature (20-25°C) until enrollment

b. Provided with the specimen draw date and time

c. Provided with specimen age and gender

d. Of acceptable quality for flow cytometry testing (e.g., no hemolysis or clots and acceptable pre-analytical handling)

e. Of sufficient residual volume for the Study: approximately 1.5 mL

f. Drawn within an adequate time to perform post-enrollment staining for specific assay
BD Multitest IMK kit: within 48h
BD Multitest 6C-TBNK reagent: within 24h

4.2 Exclusion Criteria

ANY of the following is regarded as criterion for excluding a specimen from this study:

a. Clotted or hemolyzed specimens

b. Frozen or refrigerated samples

c. Fixed samples

Enrolled specimens may be subsequently excluded from study testing if unsuitable for testing, for example visual inspection prior to study acquisition show clotting or hemolysis. See Section 7.0: Discontinuation of Testing for further information.

5.0 DESCRIPTION OF STUDY PRODUCTS

5.1 Investigational Product(s)

- IUO labeled BD FACSLyric 10 color flow cytometer with 4-3-3 configuration (BD Catalogue # 659180)
- Universal Loader (BD Catalogue # 651166)
- BD FACSuite workstation
- IUO FACSuite Clinical software

- IUO Multitest assay module
- IUO 6C TBNK assay module
- IUO BD CS&T beads (BD Catalogue # 656504/656505)
- IUO BD FC beads (7-Color Kit for BD FACSuite)
- IUO FACSLyric Instructions for Use
- IUO FACSLyric Safety and Limitations Guide
- IUO Assay Setup Report
- IUO QC Report
- IUO Lab Report
- IUO Physician Report
- IUO Supplemental Report

5.2 Reference Products (or Methods)

- BD Multicheck Normal process controls (BD Catalogue # 340911, 340912, 340913)
- BD Multicheck CD4 Low process control (BD Catalogue # 340914, 340915, 340916)
- BD Trucount Tubes (BD Catalogue # 340334)
- BD Multitest IMK Kit (BD Catalogue # 340503)
- BD Multitest 6-color TBNK reagent (BD Catalogue # 644611)
- BD FACSFlow sheath fluid (BD Catalogue # 342003)
- BD FACS Lysing Solution (BD Catalogue # 349202)

5.3 Ancillary Products (if applicable)

Ancillary products are materials that are critical to the use of the study product or execution of the protocol, such as certain concomitant medications or critical device components which are used with the study device and must be used exactly as specified in this protocol.

BD will provide the following as needed:

- Electronic pipette(s) (BD Catalogue # 646539, 658921, or similar)
- USB drives, CDs, or DVDs (to store study data)

Site should have access to the following:

- Calibrated pipettes and pipette tips (to dispense 20-450µl)
- Vortex mixer
- Lab timer
- clock
- bleach
- printer and related supplies
- refrigerator with controlled temperature range between 2-8˚C
- Disposable 12x75-mm BD Falcon capped polystyrene 5ml tubes (Catalogue # 352058 or similar)
- Di H_2O
- Internet connection (optional)

5.4 Product Labeling

Investigational products (including marketed products used off-label) and units shall be labeled in accordance with regulatory requirements, including the following statement, "For Investigational Use Only. The performance characteristics of this product have not been established."

For investigational products, labeling will also include the following at minimum, when applicable:

- Study Number
- Product Identification (Product name, catalog number, instrument plate, etc)
- Manufacturer name and location
- Batch/Lot #
- Use by/Expiration Date

Commercial products will be supplied as labeled by the manufacturer.

5.5 Maintenance and Storage of Study Products

The IUO BD FACSLyric system along with associated workstations and software, should be stored at 16-29˚C. Flow cytometer should be up-to-date on regular maintenance.

BD FACSLysing Solution and BD FACSFlow sheath fluid should be stored at 16-29˚C. BD Trucount Tubes should be stored at 2-25˚C. Unless otherwise indicated, all other controls and reagents should be stored at 2-8˚C. No study product shall be used beyond its expiration date.

6.0 STUDY METHODS

6.1 Summary of Daily Study Activities

Each day's work will include completing relevant CRFs during the process.

- Enroll specimens that meet criteria (quantity, time, age, gender, other inclusion/exclusion criteria), and re-label with Study ID number.
- Start-up instrument and perform instrument setup using appropriate beads. Confirm that instrument passes.
- Stain MultiCheck controls with each reagent and acquire on instrument. Analyze data to determine if they are within manufacturer's ranges.
- Stain and acquire study samples.
    - When testing the BD Multitest IMK kit, samples must be stained with both reagents in the kit and be acquired together.
- Review and analyze study specimen data. Store data on site and provide to BD.

6.2 Specimen Enrollment

BD Multitest testing requires whole blood anticoagulated with EDTA from healthy adult donors free of hematological abnormalities.

For all specimens,

- Confirm that specimen meets inclusion and exclusion criteria.
- De-identify specimen and assign a Study ID number. The site will maintain a coded list that associate the specimen identity with the Study ID number. This list will not be transferred to the sponsor.

6.3 Instrument Setup and Process Control

The steps for Instrument Setup are depicted as part of Figure 1. The steps for Preparation and Acquisition of Process Controls are depicted as part of Figure 2.

IUO BD FACSLyric:

- Start instrument and workstation. Allow instrument to warm up for 20 minutes prior to running samples or controls.
- In FACSuite Clinical software, run Performance QC with BD CS&T beads. Confirm that the instrument passed. Save report.
- Stain MultiCheck process controls (2 levels) with appropriate reagents. Complete CRF.
- Acquire data on controls. Review and analyze data to confirm measured values are within manufacturer's ranges.

Figure 1. Instrument Setup and Acquisition

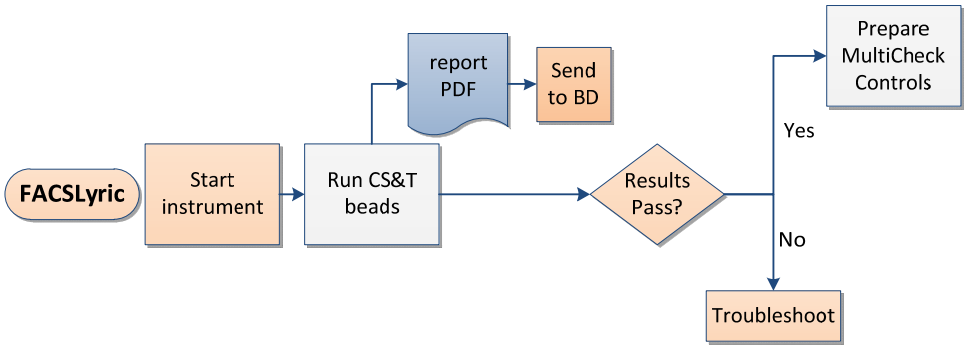
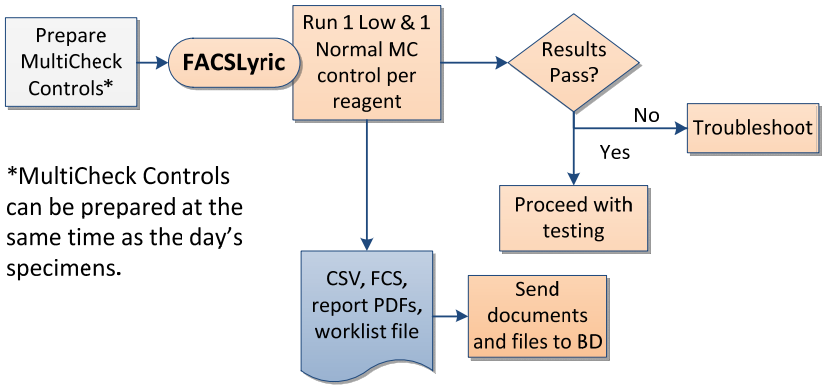


Figure 2. Process Control Preparation and Acquisition

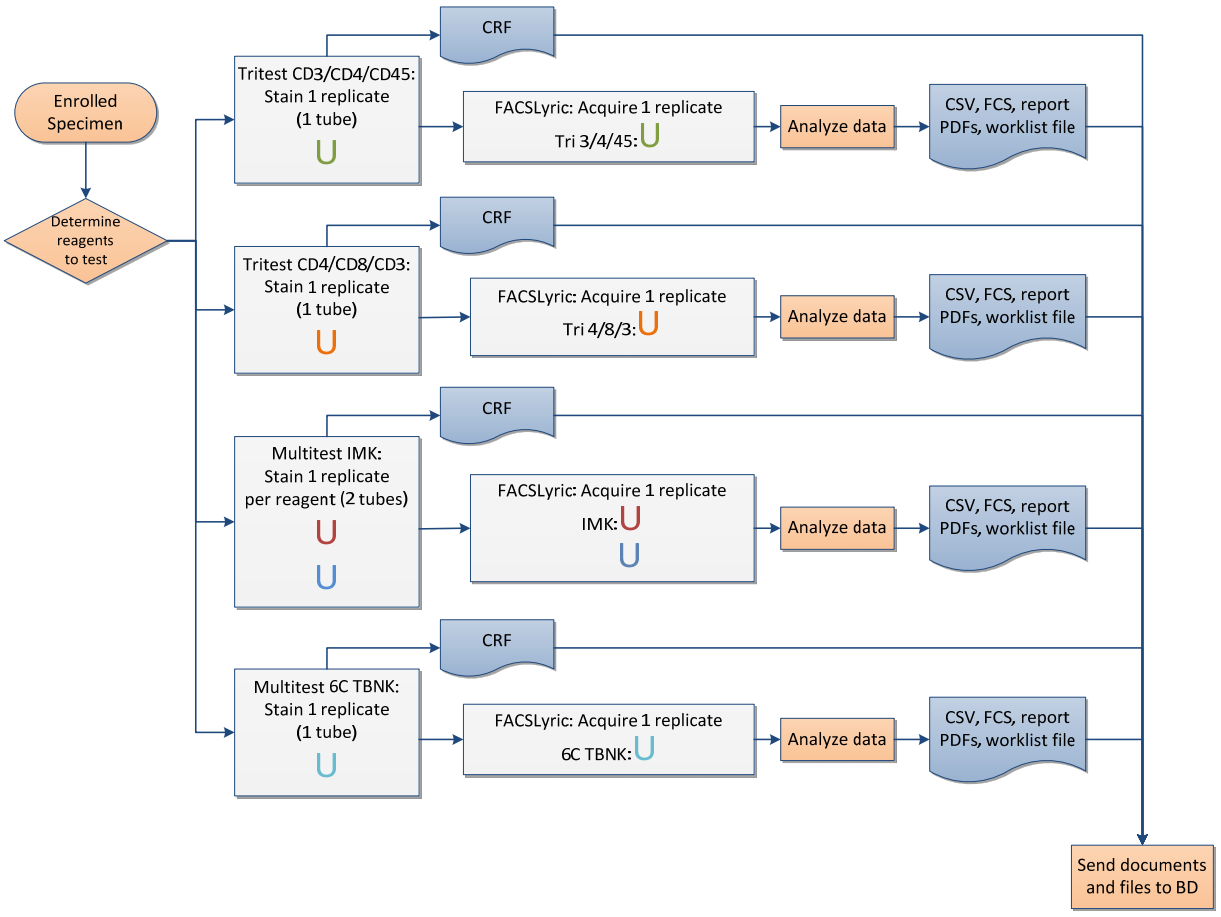


6.4 Sample Staining and Acquisition

Initially, an enrolled specimen should have enough volume to be used for each reagent in tier 1. The sponsor will update the site with the bin filling status. After analysis of a tier's evaluable data, the sponsor will inform the site when testing is no longer necessary for specific reagent or assay. The steps for Reference Interval testing are depicted in Figure 3.

- Specimen should be stained with study reagents (both reagents, BD Multitest IMK kit and Multitest 6-color TBNK reagents).
- For each enrolled specimen, stain the specimen in a singlicate tube and complete appropriate CRFs:
  - Stain one tube with each reagent in the BD Multitest IMK kit using Trucount Tubes. (Total of 2 tubes.)
  - Stain one tube with BD Multitest 6-color TBNK reagent using a Trucount Tube.
- If there is an issue with the data acquisition of a tube, rerun the tube. If there is still an issue, stain a new tube and acquire data from it. Appropriate CRF(s) must be completed.

Figure 3. Reference Interval Testing



6.5 Data Review and Analysis

- After data acquisition, all data from the investigational instrument should be reviewed and gates adjusted, as necessary. Store electronically or print all reports. Export CSV files.
- Review documentation and prepare data packet, to include electronic instrument data files, PDFs, completed CRFs, etc.
- Store data on provided electronic devices and in data binders.

- For submission of data to BD, scan print outs and CRFs into electronic files. Provide data package to BD using the BD format transfer protocol (FTP) site or, if required, by mail.

6.6 Required Training and Proficiency Testing

All study staff participating in the study will be trained in the study design and study procedures. Training will be documented. Each operator must also pass proficiency evaluation. Proficiency testing will involve performing the study workflow, including instrument startup, processing and running of controls and specimens, completing all appropriate paperwork, and submission of data. The data will be compared to expected criteria, and paperwork (CRFs, etc.) will be evaluated, for determination of the operator passing. Instructions for proficiency testing will be provided prior to study execution.

6.7 Additional Site Responsibilities

6.7.1 IUO BD FACSLyric Monthly Maintenance

The site will be responsible for normal monthly maintenance of the IUO BD FACSLyric flow cytometer. On a monthly basis, the "monthly clean procedure" needs to be performed. Instructions are in the Instructions for Use. Documentation of cleaning needs to be sent to BD.

Also, BD FC beads need to be run every 30 days to set compensation controls in order for study data to be evaluable. Documentation of running the BD FC beads needs to be sent to BD.

6.7.2 Communication with BD

As necessary, the site will be required to respond to DCFs and other study related questions in a timely fashion. Delay in responding could result in data being considered non-evaluable.

7.0 INTERRUPTION OR DISCONTINUATION OF PARTICIPATION/TESTING

7.1 Discontinuation of specimen testing

Post-enrollment, instances may occur that will require the discontinuation of the specimen testing such as:

- If further visual inspection of an enrolled specimen reveals clotting and/or hemolysis that was not initially apparent, this specimen would be unsuitable for study testing or, if results have been generated, these results would not be included in analysis.
- If an enrolled specimen (of sufficient quantity) is broken/spilled, this "quantity not sufficient (QNS)" specimen would require discontinuation of study testing.
- If an enrolled specimen (with adequate time post-draw to enroll for study testing) cannot be stained within the allotted time due to unanticipated testing difficulties, this specimen would require discontinuation of study testing.

7.1.1 Replacement of Discontinued Subjects/Specimens

Discontinued samples will be replaced by additional enrolled specimens.

7.1.2 Retention of Data from Discontinued Subjects/Specimens

No data will be collected from subjects/specimens after the point of discontinuation except as needed to follow ongoing adverse events. All study data collected from the subject up to the point of discontinuation will be recorded on the Case Report Form, entered into the study database, and included in subsequent analyses, as appropriate.

8.0 RISK / BENEFIT ASSESSMENT

8.1 Potential Risks

Sites will use only remnant and/or prospectively procured specimens for the execution of this protocol. There are no risks to the patient/subject other than those involved with the collection of a blood draw. In addition, the results produced for this study:

- Will not be used for diagnosis or treatment of the patient/subject.

To reduce the likelihood of any risks to site staff participating in this investigation, all processing, and testing of potentially infectious Specimens must always be performed according to Standard Precautions, CDC Guidelines, Standard Guidelines, and the participating sites' standard operating procedures and policies.

8.2 Potential Benefits

There are no direct benefits to the subject for participation in this study.

9.0 ASSESSMENT OF SAFETY AND ADVERSE EVENTS

This study is being conducted with remnant and/or prospectively procured specimens and does not include any human subjects. Refer to Study Incidents (section 10.0) for procedures in the event of injury to study site personnel.

10.0 INCIDENTS

A Clinical Study Incident is defined as any problem or issue involving the investigational product(s), reference methods, associated procedures or equipment, or represents a product-related injury (or potential for injury) to study subjects or personnel as a result of execution of this protocol. Clinical Study Incidents may adversely (or potentially adversely) affect human safety, the integrity of the evaluation data, or the operation of devices or systems, and warrant prompt attention.

Incidents involving injury to study subjects will also be reported as Adverse Events (refer to Section 9). Examples of Clinical Study Incidents that are not Adverse Events might be **mislabeling or adulteration of the investigational device, equipment or device malfunctions, errors in the device instructions, damage to devices caused by shipping or handling or improper storage, or injury to study personnel due to execution of the protocol**. If appropriate, an Incident may also be documented and reported as a protocol deviation.

Study-specific procedures for reporting Incidents, as well as adverse events and protocol deviations, will be provided to the study site prior to study execution. The Monitor should be contacted immediately when site becomes aware of or suspects any defective or malfunctioning product. This includes:

- Products that are involved in Study Incidents,
- Products that are found to be expired, damaged or defective,
- Products that are possibly the cause of an adverse effect, regardless of whether the product was believed to be damaged, defective or malfunctioning.

Such products (whether investigational or marketed) should be segregated and returned with appropriate documentation to the BD address below, unless instructed otherwise by BD. The Study Monitor should be contacted with any questions regarding return of study products. BD will supply mailing kits specifically intended for product contaminated with potentially bio-hazardous material.

11.0 RETURN OR DESTRUCTION OF STUDY PRODUCT

All disposable, used products not failed, damaged or otherwise involved in an Incident or Adverse Event are to be discarded into appropriate waste containers at the investigational site.

Unless instructed otherwise by BD, the Investigator will return all remaining unused or unopened test, reference, and ancillary study products to BD. At the conclusion of the study, and as appropriate during the course of the study, any products, supplies or BD equipment that are required to be returned will be shipped to BD at the address below, unless instructed otherwise:

Study Manager
2350 Qume Drive
San Jose, CA 95131
(408) 954-2384

12.0 DATA COLLECTION AND MANAGEMENT

12.1 Source Documents

Source data includes all information in original records (and certified copies of original records) of clinical findings, observations, or other activities (in a clinical study) used for the reconstruction and evaluation of the study. Source data are contained in source documents (original records or certified copies) and are used to verify the authenticity of information recorded on the Case Report Form (CRF). Typical source documents include the hospital chart, medical office file, laboratory report, clinician notes, patient record, recorded data from automated instruments or other documentation prepared and maintained by the investigator/staff or ancillary services which contains a record of all observations and other data pertinent to the investigation on a study subject.

The investigator is required to maintain original source documents at the site. Should an original source document (e.g., an instrument printout, direct entry CRF) need to be forwarded to BD for data entry, the site must retain a clearly designated certified copy. The Study Monitor will confirm that procedures for copy certification have been established at the site prior to transmittal of any original source documents.

12.2 Case Report Forms (CRF)

The case report forms (CRF) will be provided by the Sponsor. The term “CRF” as used in this protocol may refer to traditional paper CRFs, or electronic case report forms for electronic data capture (EDC), as determined by the Sponsor.

The Investigator may delegate CRF completion to study personnel. However, the Sponsor must be apprised in writing of the name of such persons and the scope of their authority. The Principal Investigator or designee is obligated to review each CRF page and sign or initial the indicated pages using ink or for EDC, an electronic signature.

All entries to a paper CRF should be made clearly in black or dark blue indelible ballpoint pen to ensure the legibility of self-copying or photocopied pages. Corrections are made by placing a single horizontal line through the incorrect entry, so that the original entry can still be seen, and placing the revised entry beside it. The revised entry must be initialed and dated by a member of the Investigator's research team authorized to make CRF entries. Correction fluid must not be used.

CRF entries will be compared to source documents by the study monitor or designated personnel. Unless specified otherwise, all information on the CRFs must be traceable to original source documents.

12.2.1 CRF as Source Document

Some study data will be collected directly on CRFs and there will be no matching source from which to verify the entry. As a result, these CRFs serve as original source documents.

12.2.2 CRF/Data Transmittal

CRF and other data should be transmitted to the sponsor within one week of the testing date. Instructions for CRF Transmittal will be provided to the Investigator at Study Initiation. Specific procedures may be described in a study-specific Monitoring Plan.

12.3 Electronic Source Data

Electronic files generated from study instruments contain source data. The site must maintain electronic files of all these data on storage devices (CDs, USB drives, etc.) provided by the Sponsor.

Some electronic source data will need to be transmitted to the Sponsor within one week of the testing date. Instructions for Data Transmittal will be provided to the Investigator at Study Initiation.

Specific procedures will be described in a study-specific Data Management Plan.

12.4 Data Management and Storage

Data Management will be performed by the Sponsor. Data from completed CRFs will be entered into a controlled database and the database verified for accuracy against the CRFs, when applicable. If electronic data capture is utilized, the electronic records entered at the site will be entered directly into the controlled database. Data security is ensured through password protection, limited access, audit trails, and regular backups of the data. Upon completion of the study and verification of data, data will be screened for accuracy and completeness, after which the database will be locked from any additional changes. A copy of the locked database will be provided to the BD Corporate Statistics Department for statistical analysis.

13.0 STATISTICAL METHODS

13.1 Sample Size Determination

For tier 1 requirements, each reagent will have evaluable data from 10 females and 10 males, distributed by age.

If, upon data analysis, the reference interval of a reagent cannot be verified, the site will collect evaluable data from an additional 20 specimens (total of 40 specimens) to meet tier 2 requirements for that reagent.

If, upon data analysis, the reference interval of a reagent cannot be verified, the site will collect data to re-establish the claim ranges. Evaluable data from an additional 80 specimens (minimum of 120 specimens) will be collected to meet tier 3 requirements for that reagent.

Distribution and binning of specimens is depicted in Table 2.

13.2 Data Evaluability

Data will be considered Evaluable if all the following is met:

- Adequate controls have been run and are acceptable.
- Data has been produced from testing of an evaluable specimen (has met inclusion and exclusion criteria).
- Specimen testing has occurred within the allotted time for the assay (Age of Stain).
- Results are within the reportable range of the assay.
- Data packets are complete and all forms are complete.
- Documentation is complete.

Discrepancies in source documents can result in the data being considered non-evaluable.

13.3 Statistical Methods

13.3.1 General Statistical Considerations

All statistical analyses will be performed using commercially available statistical software. Adequate source document verification and/or audit activities will be utilized to assure the validity of performance evaluation conclusions. Analysis shall be performed based on methods described in the Statistical Analysis Plan. All related and resulting reports, documents and data shall be produced and maintained in such a way as to ensure their control and the protection of subject privacy as far as is reasonably practicable. Data files and analytic reports will be archived according to requisite regulatory standards.

13.3.2 Reference Interval Statistical Methods

The minimum number of EDTA whole blood specimens that provide valid study results (i.e., meeting all inclusion criteria and protocol requirements) is 20 from adult subjects that satisfied eligibility for study enrollment by age and gender. For each assay (, 4C-Multitest and 6C-Multitest), the desired minimum number of enrollment by tier and bins is shown in Table 2.

Data from a single replicate from each specimen will be used for determination of the reference intervals analysis. Statistical significance testing will be performed for gender according to CLSI Guideline EP28-A3c. The partition criteria will be applied only in instances where the subclasses are both, statistically significant and clinically justifiable.

The following parameters will be evaluated:

Multitest 6Color TBNK: AbsCD3, AbsCD4, AbsCD8, AbsCD19, AbsCD16+CD56, %CD3, %CD4, %CD8, %CD19, %CD16+CD56.

Multitest IMK kit: AbsCD3, AbsCD4, AbsCD8, AbsCD19, AbsCD16+CD56, %CD3, %CD4, %CD8, %CD19, %CD16+CD56. (AbsCD3 and %CD3 values from tubes for reagents A and B will be averaged.)

13.4 Demographics/Other Descriptive Information

Specimens will be obtained from male and female donors who are 18 years and older. As depicted in Table 2, data from specimens enrolled will be binned by gender and in one of four age groups.

BD will track bin enrollment during the course of the study and will notify the site when a target for any bin has been reached, so that the site can preferentially enroll specimens for other bins.

13.5 Interim Analysis

Analysis will be performed after data acquisition for tier 1 and tier 2, if needed, to determine if specimens for the next tier need to be tested.

Other interim analysis of the study data may be performed as needed.

14.0 QUALITY CONTROL AND ASSURANCE

14.1 Accountability of Study Products

Investigational study products will be only be released for use to Investigators who have obtained written IRB/EC approval (as required) for participation in this study, who have completed all required study

documentation, and who have been qualified by the Sponsor. Investigators must maintain control over all study products, and ensure they are used in accordance with this protocol. Failure to do so may result in the Sponsor suspending or terminating the study at the Investigator's site.

The Investigator will ensure that study products are only dispensed to subjects (or used for specimens) properly enrolled in the study. The Investigator must maintain records of receipt, disposition, return and/or destruction of all study products. All investigational study products released to the site must be accounted for at the unit level prior to study close out, regardless of disposition. The Study Monitor will regularly review all records regarding study product accountability.

The Sponsor will maintain records that document the shipment, receipt, disposition, return and/or destruction of study products.

14.2 Monitoring

BD, the study sponsor, will designate trained and qualified personnel to monitor the progress of this clinical study in accordance with BD Monitoring SOPs and the study-specific Monitoring Plan. A pre-study site qualification visit will be conducted to assess the adequacy of the site facilities and staff with respect to study requirements.

Prior to study start, a study initiation visit will be conducted to provide training to site staff with regard to the protocol, the completion of study documentation and Case Report Forms (CRFs), the monitoring schedule, and all regulatory requirements. During the study, routine monitoring visits will be conducted to assure the site continues to adhere to the protocol, the investigator agreement, and regulations regarding conduct of clinical studies. Assessments will be made regarding the subjects' protection and safety, when relevant, as well as the quality, completeness, and integrity of the data.. The Study Monitor will assist the investigative site with query resolution and will perform site close-out activities once all queries have been resolved.

Additional visits may be carried out depending upon site activity and performance. The Investigator must agree to the inspection of all study related records and give direct access to source documents for verification of data on CRFs.

The Investigator is responsible for ensuring that any site-owned equipment required for use in the study is properly installed and maintained (e.g., inspected, calibrated, alarmed). Documentation of equipment maintenance procedures must be available for review by the Monitor.

14.3 Audits and Inspections

If the study is selected for audit by the Sponsor or if there is an inspection by the appropriate Health Authorities, then the Investigator and his team will make themselves available during the visit. The Investigator must agree to the inspection of all study related records and give the auditor/inspector direct access to source documents for verification of data on CRFs. The subject's anonymity must be safeguarded and data checked during the audit remain confidential.

As soon as the Investigator is aware of an upcoming inspection/audit by the Health Authorities, he/she will promptly inform BD. As agreed with the Investigator, BD personnel may be present at the site during the inspection.

14.4 Protocol Deviations

Protocol deviations are not permitted and should be implemented prospectively as a protocol amendment whenever practical or appropriate, unless required to protect the safety and well-being of the subject. The Investigator must notify the Sponsor immediately of any such deviation resulting from the need to protect a subject.

Protocol deviations (other than those required to protect the safety and well-being of a subject) may impact the evaluability of study data, and may place subjects at risk. If the Investigator or their staff inadvertently deviates from the study plan, the Investigator should implement appropriate corrective and preventive procedures, and should notify the Sponsor at their earliest convenience. Significant deviations may also need to be reported to the IRB/EC and local health authority.

The Study Monitor will evaluate records of study conduct at the site to identify any deviations, and will also report them to the Sponsor. Upon evaluation by the Sponsor, actions may be required to prevent additional deviations, such as retraining of the site, implementation of additional site procedures, and more frequent monitoring. If these steps fail, more serious measures, up to and including termination of the site and withdrawal of study product may be necessary.

15.0 ETHICAL AND REGULATORY STANDARDS

15.1 IRB/EC

IRB/EC oversight is not required for this minimal risk study.

BD In-House Blood Collection Program, Stanford Blood Center, and other similar institutions will conduct their prospective procurement clinical studies with Informed Consent under the oversight of their respective IRB/EC and will provide these specimens to BD MedLab as coded specimens.

15.2 Informed Consent

For this study, BD In-House Blood Collection Program, Stanford Blood Center, and other similar institutions will conduct their prospective procurement clinical studies with Informed Consent under the oversight of their respective IRB/EC and will provide these specimens to BD MedLab as coded specimens.

For remnant specimens: Leftover de-linked specimens from routine laboratory testing may be enrolled in the study, and all subject's personal health information will be removed from the specimen prior to enrollment. Therefore, Informed Consent may not be required by the IRB/EC.

15.3 Confidentiality of Data

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and BD and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating subjects. Subject confidentiality and anonymity will be maintained at all times by removal of all identifiers from any data, clinical samples or documentation submitted for this study.

Any data collected meeting the definition of PHI will be collected and maintained using the designated authorizations and following all privacy procedures as specified in the applicable health authority regulations.

BD will maintain the security and confidentiality of all clinical study data sent to BD. BD clinical study databases will not be shared with any third party without the express written consent of the Principal Investigator and/or Site.

The Study Monitor or other authorized representatives of BD may inspect all documents and records required to be maintained by the Investigator. The Site will permit access to such records. BD and the Site may be required to provide regulatory agencies access to clinical study data and records, as well as source documents.

All other agreements as to confidentiality by BD, the Principal Investigator, and the Site may be found in the Confidential Disclosure Agreement and the Clinical Trial Agreement.

15.3.1 De-identification of Remnant/Prospecitively Procured Specimens

Personal information will be maintained confidential in accordance with the site's procedures and the Health Insurance Portability and Accountability Act (HIPAA) (if applicable). Donors/specimens will be assigned a coded identification number according to the site policy for delinked specimens. The coded list will remain with the clinical site or principal investigator or the site-specific policy will be followed.

For *in vitro* diagnostic device studies using leftover specimens, the FDA Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens must be followed when Informed Consent is not obtained. The specimens must be provided to the investigator(s) without identifiers and the supplier of the specimens must have established policies and procedures to prevent the release of personal information.

15.4 Protocol Modifications

Amendments to the protocol will not be implemented without agreement from the Sponsor and prior submission to and written approval from the governing IRB/EC, except when necessary to eliminate an immediate hazard to the subject. Notice of an emergency modification shall be given to the Sponsor and the reviewing IRB/EC as soon as possible, but in no event later than 5 working days after the emergency occurred. Protocol amendments may affect Informed Consent Forms for current and future subjects.

Minor changes to the protocol, such as correction of typographical errors or changes in personnel names (other than the PI) or contact information will be processed as administrative changes. Administrative changes will be submitted to the governing IRB/EC but implementation of the administrative change may proceed without prior IRB/EC approval, unless so required by the IRB/EC or site SOPs.

15.5 Study Discontinuation

BD reserves the right to temporarily suspend or prematurely discontinue the study at a single site or at all sites at any time and for any reason. If such action is taken, BD will discuss the reasons with all Investigators (the Investigator). If the study is terminated or suspended due to safety reasons, the sponsor will inform the health authorities as required, and provide the reason(s) for the action. Investigator(s) must inform their IRB/EC promptly and provide the reason(s) for the suspension or termination.

15.6 Clinical Study Registration

In compliance with Title VIII of Public Law 110-85, known as FDA Amendments Act of 2007 (FDAAA), BD will register all applicable studies and disclose study results in a publicly accessible database, e.g. the ClinicalTrials.gov web site. Applicable studies will be registered no later than 21 days after commencing enrollment. Study results for applicable studies will be posted to the website within 12 months of the last subject visit for collection of primary outcome data, or after health authority approval for previously unapproved devices. BD has responsibility for determining whether this study qualifies as an "applicable" study under the law, and if so, will take responsibility for registration and disclosure as required by law.

15.7 Publication of Results

BD believes that results of applicable clinical studies of our products should be published in peer-reviewed literature in a timely, accurate, complete and balanced manner, regardless of study outcomes. BD is committed to making information public whenever it relates to the safety and efficacy of its marketed products.

Should this study be considered an "applicable study," any formal presentation or publication of data collected from this study will be considered as a joint publication by the investigator(s) and the appropriate personnel of BD. Authorship will be based on generally accepted criteria of the ICMJE (International

Committee of Medical Journal Editors) and determined by mutual agreement. For multi-center studies, it is mandatory that the first publication is based on data from all centers, analyzed as stipulated in the protocol by BD statisticians, and not based on data from single sites or a subset of sites. Investigators participating in multi-center studies agree not to present data gathered from one center or a small group of centers before the full, initial publication, unless formally agreed to by all other investigators and BD (the sole exception being an unanticipated adverse event that is product-related and which might have clinically significant safety implications for a marketed product or a class of products).

BD must receive copies of any intended communication in advance of publication as specified in the Clinical Study Agreement. In a timely manner, BD will review the communications for accuracy (thus avoiding potential discrepancies with submissions to health authorities), verify that confidential information is not being inadvertently divulged and to provide any relevant supplementary information to the investigators.

15.8 Record Retention

If the Principal Investigator or Clinical Center withdraws from the responsibility of keeping the study records, custody must be transferred to a person or entity who will accept the responsibility. BD must be notified in writing of the name and address of the new custodian.

Federal regulations require that a copy of all essential study documents (e.g., IRB/EC approvals, signed informed consent forms, source documents, CRF copies, safety reports, test article dispensing records, etc.), must be retained in the files of the responsible Investigator for a minimum of 2 years following notification by BD that all investigations are completed, terminated, or discontinued, or that the FDA has approved the application (21 CFR 812.140).

16.0 BIBLIOGRAPHY/REFERENCES

1. E6, International Conference on Harmonization: Good Clinical Practice: Consolidated (Published in the Federal Register May 9, 1997).
2. CLSI EP28-A3c Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition. October 2010.

17.0 PROTOCOL REVISION HISTORY

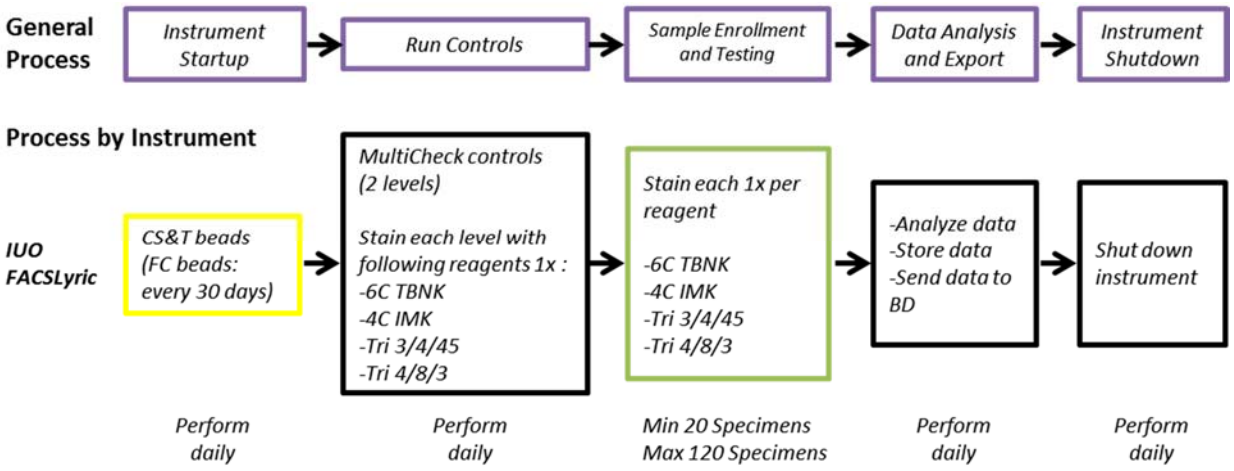
Version #	Rationale for Change	Section or Page affected	Description of change
1.0	New Protocol		

18.0 APPENDICES

The following are included in the appendices:

- Study Design
- Previously Established Reference Interval Claims

18.1 Study Design



18.2 Previously Established Reference Interval Claims

18.2.1 Multitest 6-Color TBNK reagent

Reference: version 1/2014

Lymhocyte Subset	n	Mean (%)	95% Range
CD3+CD4+	123	46.4	28.2-62.8
CD3+CD8+	123	24.0	10.2-40.1
Total CD3+	123	71.1	49.1-83.6
CD3-CD19+	123	14.9	6.5-27.0
CD3-CD19+CD56+	123	11.7	4.2-25.2
Lymhocyte Subset	**n**	**Mean (cells/µl)**	**95% Range**
CD3+CD4+	123	1106	441-2156
CD3+CD8+	123	583	125-1312
Total CD3+	123	1705	603-2990
CD3-CD19+	123	351	107-698
CD3-CD19+CD56+	123	266	95-640

18.2.2 Multitest IMK kit

Reference: version 2/2011

Subset	n	Mean (%)	95% Range
CD3+ (Total T lymphs)	164	72	56-86
Subset	**n**	**Mean (cells/µl)**	**95% Range**
CD3+ (Total T lymphs)	164	1510	742-2750

Multitest CD CD3/CD8/CD45/CD4 reagent

Subset	n	Mean (%)	95% Range
CD3+CD4+ (Helper/inducer T lymphs)	164	45	33-58
CD3+CD8+ (Suppressor/cytotoxic T lymphs)	164	24	13-39
CD3+ (Total T lymphs)	164	72	56-86
Subset	**n**	**Mean (cells/µl)**	**95% Range**
CD3+CD4+ (Helper/inducer T lymphs)	164	941	404-1612
CD3+CD8+ (Suppressor/cytotoxic T lymphs)	164	511	220-1129
CD3+ (Total T lymphs)	164	1513	723-2737

Multitest CD CD3/CD16+56/CD45/CD19 reagent

Subset	n	Mean (%)	95% Range
CD3-CD16+ or CD56+ (NK lymphs)	164	13	5-26
CD19+ (B lymphs)	164	14	5-22
CD3+ (T lymphs)	164	72	56-86

Subset	n	Mean (cells/µl)	95% Range
CD3-CD16+ or CD56+ (NK lymphs)	164	267	84-724
CD19+ (B lymphs)	164	293	80-616
CD3+ (T lymphs)	164	1507	754-2764

18.2.3 CD3/CD4/CD45 reagent

Reference: version 5/2014

Subset	n	Mean (%)	Lower 2.5 percentile	Upper 97.5 percentile
CD3+CD4+ (Helper/inducer T lymphs)	523	45	31	60
CD3+ (Total T lymphs)	516	72	55	84
Subset	**n**	**Mean (cells/µl)**	**Lower 2.5 percentile**	**Upper 97.5 percentile**
CD3+CD4+ (Helper/inducer T lymphs)	523	880	410	1590
CD3+ (Total T lymphs)	516	1410	690	2540

18.2.4 CD4/CD8/CD3 reagent

Reference: version 5/2014

Subset	n	Mean (cells/µl)	Lower 2.5 percentile	Upper 97.5 percentile
CD3+CD4+ (Helper/inducer T lymphs)	523	880	410	1590
CD3+CD8+ (Suppressor/cytotoxic T lymphs)	523	490	190	1140
CD3+CD4+ (Helper/inducer T lymphs)	516	1410	690	2540