**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION**
**DECISION MEMORANDUM**

**A. 510(k) Number:**

K150815

**B. Purpose for Submission:**

New assay and instrument

**C. Measurand:**

Lymphocyte CD4 absolute count, CD4 percentage of lymphocytes, and hemoglobin concentration

**D. Type of Test:**

Quantitative test for CD4% and CD4 absolute count by cytometry imaging and quantitative test for hemoglobin by absorbance spectrometer

**E. Applicant:**

BD Biosciences

**F. Proprietary and Established Names:**

BD FACSPresto™ System

BD FACSPresto™ CD4/Hb Cartridge

BD FACSPresto™ CD4/Hb Cartridge Kit

BD Multi-Check Control

BD Multi-Check CD4 Low Control

Eurotrol FACSPresto Hb Control (Levels 1−3)

**G. Regulatory Information:**

1. Regulation section:

   21 CFR §864.5220, Automated Differential Cell Counter
   21 CFR §864.8625, Hematology Quality Control Mixture

2. Classification:

Class II (assay)

Class II (controls)

3. Product code:
   PMG, Automated multicolor fluorescent imaging cytometric analysis system
   OYE, System, Test, Flow cytometric reagents and accessories
   GKL Hemoglobin assay
   JPK, Analyte Controls, Hematology Quality Control

4. Panel:

   Hematology (81)

   Immunology (82)

## H. Intended Use:

1. Intended use(s):

   Instrument
   BD FACSPresto™ System
   BD FACSPresto™ System is an automated multicolor fluorescent imaging cytometer and absorbance spectrometer to be used in conjunction with single use reagent cartridges in performing the direct cell enumeration and measurement of absorbance spectrums.

   - For use with the BD FACSPresto CD4/Hb Cartridge and BD FACSPresto CD4/Hb Cartridge Kit in the direct quantification and enumeration of CD4 absolute count, CD4 of lymphocyte, and determination of hemoglobin concentration in normal and HIV positive patients in conjunction with other laboratory and clinical findings.
   - For use in children, adolescents, and adults.
   - For use with human whole blood from fingerstick and/or venous collections in K2 EDTA or K3 EDTA blood collection tubes.
   - Not for point-of-care use.
   - For in vitro diagnostic use.

   Device
   BD FACSPresto CD4/Hb Cartridge and BD FACSPresto CD4/Hb Cartridge Kit
   BD FACSPresto CD4/Hb Cartridge is a single use reagent cartridge to be used with the BD FACSPresto System for performing the direct quantification and enumeration of CD4 absolute count, CD4 percentage of lymphocytes, and determination of hemoglobin concentration in normal and HIV positive patients in conjunction with other laboratory and clinical findings.
   - For use in children, adolescents, and adults.

- For use with human whole blood from fingerstick and/or venous collections in K2 EDTA or K3 EDTA blood collection tubes.
- Not for point-of-care use.
- For in vitro diagnostic use.

BD Multi-Check Control
The BD Multi-Check control is intended as a complete process control for immunophenotyping by flow cytometry. It is a control for antibody staining, red blood cell (RBC) lysis, instrument setup and performance, and data analysis.
The BD Multi-Check control is also intended as a CD4 and %CD4 process control for antibody staining, instrument performance, and data analysis on the BD FACSPresto™ system, an imaging cytometer.

BD Multi-Check CD4 Low Control
The BD Multi-Check CD4 low control is intended as a complete process control for immunophenotyping by flow cytometry. It is a control for antibody staining, red blood cell (RBC) lysis, instrument setup and performance, and data analysis.
The BD Multi-Check CD4 low control is also intended as a CD4 and %CD4 process control for antibody staining, instrument performance, and data analysis on the BD FACSPresto system, an imaging cytometer.

Eurotrol FACSPresto Hb Control
Eurotrol FACSPresto Hb Control is an assayed hemoglobin control intended for in vitro diagnostic use in the verification of the precision and accuracy of the FACSPresto System.

2. Indication(s) for use:

   Same as above

3. Special conditions for use statement(s):

   For Prescription Use Only

4. Special instrument requirements:

   BD FACSPresto™ System instrument

## I. Device Description:

The device consists of the BD FACSPresto system, the BD FACSPresto CD4/Hb Cartridge, and the BD FACSPresto CD4/Hb Cartridge Kit.

| | |
|---|---|
| BD FACSPresto System | This instrument is an automated multicolor fluorescent imaging cytometer and absorbance spectrometer with integrated BD FACSPresto System Software. |
| BD FACSPresto CD4/Hb Cartridge | BD FACSPresto CD4/Hb Cartridge contains antibody-fluorochrome conjugates, CD4 PE-Cy5, CD3 APC, CD45RA-APC, and CD 14-PE, dried on a reagent disc and is embedded with reagent quality controls.<br>Transfer Pipettes |
| BD FACSPresto CD4/Hb Cartridge Kit | BD FACSPresto CD4/Hb Cartridge<br>BD FACSPresto CD4/Hb Finger Stick Sample Collection Kit (including lancets, alcohol pads, sponges, and bandages) |

The BD FACSPresto System (instrument) includes a power supply, adapter cords, instrument cover, a sample incubation work station, printer paper and a USB flash drive.

| | |
|---|---|
| BD Multi-Check Control | Process control composed of human leukocytes and erythrocytes in a stabilizing medium |
| BD Multi-Check CD4 Low Control | Process control composed of human leukocytes and erythrocytes in a stabilizing medium |
| Eurotrol FACSPresto Hb Control Levels 1−3 | Process control composed of purified bovine hemolysate |

## J. Substantial Equivalence Information:

1. Predicate device name(s):
   a. BD FACSCalibur using BD Tritest CD3/CD4/CD45 with BD Trucount Tubes
   b. Sysmex Automated Hematology Analyzer KX-21N
   c. R&D Systems Whole Blood Flow Control, also known as StatusFlow
   d. StatusFlow Lo
   e. Eurotrol Hb 301 Control (Levels 1−3)

2. Predicate 510(k) number(s):
   a. K071141
   b. K981761
   c. K961610, BK990005
   d. K982231
   e. BK030067

3. Comparison with predicate:

Instrument

| Similarities and Differences | | |
|---|---|---|
| Item | Device<br>BD FACSPresto System | Predicate<br>BD FACSCalibur |
| Intended Use | BD FACSPresto™ System is an automated multicolor fluorescent imaging cytometer and absorbance spectrometer to be used in conjunction with single use reagent cartridges in performing the direct cell enumeration and /or measurement of absorbance spectrums. | For use with any flow cytometer equipped with a 488 nm laser and capable of detection in the ranges: 510−545 nm, 562−607 nm, and >650 nm<br>• For use in erythrocyte-lysed whole peripheral blood<br>• For use with or without isotype control<br>• To characterize and monitor some forms of autoimmune disease<br>• To characterize and monitor some forms of immunodeficiency disease, such as in HIV-infected individuals |
| Instrument Setup and Quality Control | Setup: Automated instrument setup.<br>Instrument QC: automated verification of instrument performance at power-on-self-test (POST) and during cartridge runs.<br>Cartridge QC: rat anti-mouse antibodies bound to polystyrene beads confirm presence of sample and reagent. | Setup: Semi-automated setup using BD FACSComp software with BD Calibrite beads for setting PMT voltages, fluorescence compensation, and checking instrument sensitivity. |
| Software | Integrated BD FACSPresto System Software | Integrated software on instrument and BD MultiSet Software on external computer |
| Optics | Fluorescence excitation of stained cells in microfluidic channel by LED illumination;<br>Fluorescence emission measured by CCD camera imaging | Fluorescence excitation of stained cells in flow stream by laser illumination;<br>Fluorescence emission measured by PMTs |
| Cytometry | Imaging | Flow |

Absolute CD4 Count and %CD4 Assays

| Similarities | | |
|---|---|---|
| Item | Device<br>BD FACSPresto System for use with BD FACSPresto CD4/Hb Cartridge and BD FACSPresto CD4/Hb Cartridge | Predicate<br>BD FACSCalibur using BD Tritest CD3/CD4/ CD45 with BD Trucount Tubes (K071141) |
| Results Reporting | • Absolute CD4 count (cells/µL)<br>• %CD4 (the percentage of CD4 positive lymphocytes counted within the total lymphocyte population count) | Same |
| Sample Type | Whole blood | Same |

| Differences | | |
|---|---|---|
| Item | Device<br>BD FACSPresto System for use with BD FACSPresto CD4/Hb Cartridge and BD FACSPresto CD4/Hb Cartridge Kit | Predicate<br>BD FACSCalibur using BD Tritest CD3/CD4/ CD45 with BD Trucount Tubes (K071141) |
| Intended Use/ Indications for Use | BD FACSPrestoCD4/Hb Cartridge is a single use reagent cartridge to be used with the BD FACSPresto™ System for performing the direct quantification and enumeration of CD4 absolute count, CD4 percentage of lymphocytes, and determination of hemoglobin concentration in normal and HIV positive patients in conjunction with other laboratory and clinical findings.<br>•For use in children, adolescents, and adults.<br>•For use with human whole blood from fingerstick and/or venous collections in K2EDTA or K3 EDTA blood collection tubes.<br>•Not for point-of-care use.<br>•For in vitro diagnostic use. | • For use with any flow cytometer equipped with a 488 nm laser and capable of detection in the ranges: 510−545 nm, 562−607 nm, and >650 nm<br>• For use in erythrocyte-lysed whole peripheral blood<br>• For use with or without isotype control<br>• To characterize and monitor some forms of autoimmune disease<br>• To characterize and monitor some forms of immunodeficiency disease, such as in HIV-infected individuals |
| Assay Methodology | Cytometry (imaging) | Cytometry (flow) |
| Sample Volume | 1–2 drops venous or capillary whole blood | Minimum 100 µL whole blood |
| Sample Preparation | Manual introduction of venous or capillary blood onto BD FACSPresto CD4/Hb Cartridge | Manual pipetting for the lyse/wash or lyse/no-wash methods, or automated with the BD FACS Sample Prep Assistant (SPA) for the lyse/no-wash method |
| Sample Analysis | • Capillary chamber height is precisely measured in manufacturing for each cartridge and encoded in the cartridge barcode. The size of the analysis image areas is determined by the instrument. The two are used to calculate the volume of | • A controlled quantity of fluorescent beads is included in the sample through preparation in BD TruCount tubes to determine the volume of sample analyzed.<br>• Fluorescence intensity of beads and |

| Differences | | |
|---|---|---|
| Item | Device<br>BD FACSPresto System for use with BD FACSPresto CD4/Hb Cartridge and BD FACSPresto CD4/Hb Cartridge Kit | Predicate<br>BD FACSCalibur using BD Tritest CD3/CD4/ CD45 with BD Trucount Tubes (K071141) |
| | analyzed sample.<br>• Fluorescence intensity of cells of interest labeled by specific fluorescent antibodies is quantitatively measured.<br>• Cells are algorithmically classified based on these signal intensities.<br>• The number of cells in each classification and the volume of sample analyzed are used to calculate the reported assay results. | of cells of interest labeled by specific fluorescent antibodies is quantitatively measured.<br>• Cells and fluorescent beads are algorithmically classified based on these signal intensities.<br>• The number of cells in each classification and the volume of sample analyzed are used to calculate the reported assay results. |
| Assay Principles | CD4 and %CD4 imaging cytometry assays using a 3-color direct immunofluorescent reagent to identify cell subset populations in whole blood with automated analysis. Precise dimensions of microfluidic channel and image area are used for volumetric determination. | CD4 and %CD4 flow cytometry assays using a 3-color direct immunofluorescent reagent to identify cell subset populations in lysed blood with automated analysis. Trucount beads are used for volume determination. |
| Optics Principles - CD4 and %CD4 | Fluorescence excitation of stained cells in microfluidic channel by LED illumination; Fluorescence emission measured by CCD camera imaging | Fluorescence excitation of stained cells in flow stream by laser illumination; Fluorescence emission measured by PMTs |
| Fluidics | Cartridge contains a microfluidic channel through which the sample fills by capillary action. After filling completes, sample is static during data acquisition. | Consists of a pinch valve assembly which controls the flow of sample, saline sheath fluid, and waste fluids during data acquisition. |

Total Hemoglobin assay

| Similarities | | |
|---|---|---|
| Item | Device<br>BD FACSPresto System for use with BD FACSPresto CD4/Hb Cartridge and BD FACSPresto CD4/Hb Cartridge Kit | Predicate<br>Sysmex Automated Hematology Analyzer KX-21N (K981761) |
| Assay Methodology | Absorbance spectrophotometry | Same |
| Results Reporting | Total Hb concentration | Same |
| Sample Type | Whole blood | Same |

| Differences | | |
|---|---|---|
| Item | Device<br>BD FACSPresto System for use with BD FACSPresto CD4/Hb Cartridge and BD FACSPresto CD4/Hb Cartridge Kit | Predicate<br>Sysmex Automated Hematology Analyzer KX-21N (K981761) |
| Intended Use/ Indications for Use | BD FACSPresto™ System is an automated multicolor fluorescent imaging cytometer and absorbance spectrometer to be used in conjunction with single use reagent cartridges in performing the direct cell enumeration and /or measurement of absorbance spectrums.<br>•For use with the BD FACSPresto CD4/Hb Cartridge and BD FACSPresto CD4/Hb Cartridge Kit in the direct quantification and enumeration of CD4 absolute count, CD4 of lymphocyte, and determination of hemoglobin concentration in normal and HIV positive patients in conjunction with other laboratory and clinical findings.<br>•For use in children, adolescents, and adults.<br>•For use with human whole blood from fingerstick and/or venous collections in K2EDTA or K3 EDTA blood collection tubes.<br>•Not for point-of-care use.<br>•For in vitro diagnostic use.<br><br>BD FACSPrestoCD4/Hb Cartridge is a single use reagent cartridge to be used with the BD FACSPresto™ System for performing the direct quantification and enumeration of CD4 absolute count, CD4 percentage of lymphocytes, and determination of hemoglobin concentration in normal and HIV positive patients in conjunction with other laboratory and clinical findings.<br>•For use in children, adolescents, and adults.<br>•For use with human whole blood from fingerstick and/or venous collections in K2EDTA or K3 EDTA blood collection tubes.<br>•Not for point-of-care use.<br>•For in vitro diagnostic use. | The intended use of the Sysmex KX-21 is as an automated cell counter for in vitro diagnostic use in clinical laboratories. |
| Sample Volume | 1−2 drops venous or capillary whole blood | 50 µL whole blood |

| Differences | | |
|---|---|---|
| Item | Device<br>BD FACSPresto System for use with BD FACSPresto CD4/Hb Cartridge and BD FACSPresto CD4/Hb Cartridge Kit | Predicate<br>Sysmex Automated Hematology Analyzer KX-21N (K981761) |
| | | 40 μL pre-dilute |
| Sample Preparation | Manual introduction of venous or capillary blood onto BD FACSPresto CD4/Hb Cartridge | Manual placement of blood tube onto sample aspiration arm |
| Sample Analysis | • Broad-spectrum LED light is directed through the blood sample and a diffraction grating to create a spectrum and measure light absorbance at 2 wavelengths: a hemoglobin isosbestic point and a non-hemoglobin-absorbing point.<br>• The light absorbance in the non-hemoglobin-absorbing region measures the amount of light attenuation due to scatter.<br>• Absorbance at the isosbestic point is corrected for scatter and used to calculate hemoglobin concentration. | • Narrow-spectrum LED light is directed through the blood sample to measure light absorbance at a hemoglobin-absorbing wavelength.<br>• Sodium lauryl sulfate lyses the blood cells in the sample, eliminating light attenuation caused by scatter.<br>• Absorbance at the LED wavelength is used to calculate hemoglobin concentration. |
| Assay Principles | Photometric method that detects the presence of predominant forms of Hb, with correction for scatter. | Photometric method with reagent that releases hemoglobin from red cells and forms a stable colored complex. |
| Optics Principles - Hb | Absorbance spectrophotometric method using LED-generated broad spectrum light, diffraction grating, and CCD sensor | Absorbance photometric method using LED-generated monochromatic light and a photosensor |
| Fluidics | Cartridge contains a microfluidic channel through which the sample fills by capillary action. After filling completes, sample is static during data acquisition. | A vacuum pump aspirates sample blood, which passes through a rotor valve and then to volumetric dispensing, mixing, rinsing, and draining. Sample is flowing during data acquisition. |
| Instrument Setup and Quality Control | Setup: Automated instrument setup.<br>Instrument QC: automated verification of instrument performance at power-on-self-test (POST) and during cartridge runs. | Setup: Automated startup check.<br>Instrument QC: Levey -Jennings control that uses data from a single analysis of control sample (Sysmex Eight-Check 3WP X-Tra Controls). |
| Software | Integrated BD FACSPresto System Software | Integrated Sysmex Software |

BD Multi-Check Control

| Similarities and Differences | | |
|---|---|---|
| Item | Device<br>BD Multi-Check Control | Predicate<br>R&D Systems Whole Blood Flow Control (StatusFlow- K961610 & BK990005) |
| Intended Use | The BD Multi-Check control is intended as a complete process control for immunophenotyping by flow cytometry. It is a control for antibody staining, red blood cell (RBC) lysis, instrument setup, instrument performance, and data analysis. The BD™ Multi-Check control is also intended as a CD4 and %CD4 process control for antibody staining, instrument performance, and data analysis on the BD FACSPresto™ system, an imaging cytometer. | R&D Systems' Whole Blood Flow Control (WBFC) is a stabilized preparation of human peripheral leukocytes and erythrocytes to be used as a control in the complete immunophenotyping process which includes: antibody staining, RBC lysis, instrument set-up and instrument performance. |
| Composition | Human leukocytes and erythrocytes in a stabilizing medium | Same |
| Storage Conditions | 2–8°C | Same |
| Open Vial Stability | 9 thermal cycles | Same |
| Closed Vial Stability | 45 days | Same |

BD Multi-Check CD4 Low Control

| Similarities and Differences | | |
|---|---|---|
| Item | Device<br>BD Multi-Check CD4 Low Control | Predicate<br>StatusFlowLo (K982231) |
| Intended Use | The BD Multi-Check CD4 low control is intended as a complete process control for immunophenotyping by flow cytometry. It is a control for antibody staining, red blood cell (RBC) lysis, instrument setup and performance, and data analysis. The BD Multi-Check CD4 low control is also intended as a CD4 and %CD4 process control for antibody staining, instrument performance, and data analysis on the BD FACSPresto™ system, an imaging cytometer. | StatusFlowLo is intended as a complete process control for immunophenotyping by flow cytometry. It is a control for antibody staining, RBC lysis, instrument set-up, instrument performance and data analysis. |
| Composition | Human leukocytes and erythrocytes in a stabilizing medium | Same |
| Storage Conditions | 2−8°C | Same |
| Open Vial Stability | 9 thermal cycles | Same |

| Similarities and Differences | | |
|---|---|---|
| Item | Device<br>BD Multi-Check CD4 Low Control | Predicate<br>StatusFlowLo (K982231) |
| Closed Vial Stability | 45 days | Same |

Eurotrol FACSPresto Hb Control

| Similarities and Differences | | |
|---|---|---|
| Item | Device<br>Eurotrol FACSPresto Hb Control | Predicate<br>Eurotrol Hb 301 Control<br>BK030067 |
| Intended Use | Eurotrol FACSPresto Hb Control is an assayed hemoglobin control intended for in vitro diagnostic use in the verification of the precision and accuracy of the FACSPresto System. | The Eurotrol 301 Hb Control is an assayed hemoglobin control intended for professional use in the verification of the precision and accuracy of the HemoCue Hb 301 System. |
| Product Code | JPK | GGM |
| Composition | Purified bovine hemolysate | Same |
| Open Vial Stability | 30 days at 2–8°C | 30 days at 2–30°C |
| Closed Vial Stability | 1 month at 2–8°C | 25 months at 2–8°C |

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

1. CLSI EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition
2. CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline;
3. CLSI EP07-A2: Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition
4. CLSI EP09-A2-IR, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition (Interim Revision).
5. CLSI EP17-A ,Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline
6. CLSI EP25-A; Evaluation of Stability of In Vitro Diagnostic Reagents. Approved Guideline.
7. CLSI EP28-A3c Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition
8. CLSI GP42-A6 (formerly H04-A6): Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard – Sixth Edition
9. CLSI H15-A3: Reference and Selected Procedures for the Quantitative Determination of Hemoglobin in Blood; Approved Standard – Third Edition

10. ANSI/AAMI/ISO 10993-5:2009, Biological Evaluation of Medical Devices-Part 5: Test for in vitro cytotoxicity
11. ASTM F2148:2007 – Standard Practice for Evaluation of Delayed Contact hypersensitivity using the Murine Local Lymph Node Assay (LLNA)
12. ANSI/AAMI/ISO 10993-10:2010, Biological Evaluation of Medical Devices-Part 10: Tests for irritation and skin sensitization

**L. Test Principle:**

The BD FACSPresto instrument is an automated multicolor fluorescent imaging cytometer and absorbance spectrometer with camera, light-emitting diode (LED) illumination, touchscreen user-interface, battery backup power, and onboard software algorithms that analyze images and report results. The instrument acquires images of the cartridge at multiple fields of view and images are analyzed using on-board BD FACSPresto Software to determine CD4, %CD4, and Hb. Additionally, the instrument has on-board, built in QC features and algorithms to verify its performance at power-on-self-test (POST) and during cartridge runs.

The BD FACSPrestoCD4/Hb Cartridge is designed to take whole blood from a venipuncture or fingerstick, incubate sample for 18 minutes to 2 hours in the BD FACSPresto CD4/Hb Cartridge, and then run on the BD FACSPresto instrument to obtain accurate results. When using an aliquot of blood from an EDTA tube, a pipette will be required to transfer the blood to the cartridge. The BD FACSPresto CD4/Hb Cartridge product provides a disposable transfer pipette for each cartridge to be used for this purpose.

The dried reagent in the BD FACSPresto CD4/Hb Cartridge features controlled release of the reagent upon rehydration and contains EDTA to prevent coagulation of fingerstick specimens. CD4 PE-Cy5 stains CD4-positive cells, while CD3-APC and CD45RA-APC stain total lymphocytes for use in the %CD4 calculation. CD14-PE is used for staining monocytes to exclude CD4 and/or CD45RA expressing monocytes from analysis.

Image processing and gating is performed automatically in the FACSPresto software. Briefly, the gating logic is first to identify CD14 expressing cells and exclude them from further analysis, second to identify CD3-APC and CD45RA-APC expressing cells to generate the total lymphocyte population, and third to identify CD4-PECy5 expressing cells within the total lymphocyte population. Percent CD4 is calculated directly from the ratio of CD4-expressing to total lymphocytes. CD4 absolute count is the CD4-expressing lymphocyte count divided by the volume of the imaged field of views.

**M. Performance Characteristics (if/when applicable):**

1. <u>Analytical performance:</u>

   *a. Precision/Reproducibility:*

   <u>Assay Repeatability and Reproducibility Using Control Material</u>

Bench-Total precision for the BD FACSPresto system was measured for 21 operational days using duplicate Streck CD-Chex Plus BC Normal and CD4-Low controls for CD4 and %CD4 and duplicate Eurotrol Hb 301 Controls (Levels 1−3) for Hemoglobin. Daily precision measurements were performed on alternating BD FACSPresto systems (three instruments in total) in two separate runs per day (morning/afternoon) by three operators. The study was performed with one instrument and operator using one lot for seven days, then the next instrument and operator with a second lot was run for the next seven days, and then the final instrument and operator with a third lot was run for the final seven days. All acceptance criteria were met.

Sponsor's Precision Acceptance Criteria:

| | Range | %CV or %SD |
|---|---|---|
| CD4 | Mean: 50–199 cells/µL | CV ≤ 20% |
| | Mean: ≥ 200 cells/µL | CV ≤ 10% |
| %CD4 | Mean: < 25% | SD ≤ 2.5% absolute |
| | Mean: ≥ 25% | CV ≤ 10% |
| Hemoglobin | 2–20 g/dL | CV ≤ 7% |

| Sample Type | N | Mean | Between Run (%CV) | Within Run (%CV) | Total |
|---|---|---|---|---|---|
| CD4 Control Low | 84 | 155.96 | 0.00 | 6.69 | 6.81 |
| CD4 Control Normal | 84 | 940.93 | 0.00 | 2.99 | 3.34 |
| CD4% Control Low | 84 | 12.55 | 0.00 | 5.98 | 5.98 |
| CD4% Control Normal | 84 | 43.90 | 0.00 | 1.73 | 1.73 |
| Hb Control Low | 84 | 6.90 | 0.60 | 2.34 | 2.44 |
| Hb Control Medium | 84 | 12.74 | 0.00 | 1.47 | 1.52 |
| Hb Control High | 84 | 16.87 | 0.11 | 1.13 | 1.14 |

Multi-Site Reproducibility Study using Control Material

The study was designed using CLSI EP05-A3. Each sample was measured in triplicate, two times a day by one operator only at each of the three sites with the same lot of cartridges. All acceptance criteria were met.

| Sample Type | N | Mean | Between Site (%CV) | Between Run (%CV) | Within Run (%CV) | Total |
|---|---|---|---|---|---|---|
| CD4 Control Low | 90 | 138.93 | 0.00 | 0.52 | 5.29 | 5.32 |
| CD4 Control Normal | 90 | 848.32 | 0.66 | 0.00 | 2.44 | 2.56 |
| CD4% Control Low | 90 | 12.75 | 0.00 | 0.00 | 4.63 | 4.63 |
| CD4% Control Normal | 90 | 44.16 | 0.25 | 0.48 | 1.68 | 1.77 |
| Hb Control Low | 90 | 7.23 | 0.00 | 0.09 | 2.66 | 2.83 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Hb Control Medium | 90 | 12.93 | 0.00 | 0.22 | 1.52 | 1.53 |
| Hb Control High | 90 | 17.12 | 0.29 | 0.00 | 1.13 | 1.26 |

Venipuncture Whole Blood Repeatability Study

Three lots of cartridges and three instruments were used by three operators during the study. Each operator was assigned to one instrument for the duration of the study at one clinical site. Each donor was tested using 18 replicates. All acceptance criteria were met.

| Sample Type | N | Mean | Within Run | | Between Operator/ Instrument | | Between Lot | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | %CV |
| CD4 | 67 | 623.60 | 21.74 | 3.49 | 6.38 | 1.02 | 4.25 | 0.68 | 3.70 |
| CD4% | 67 | 26.92 | 0.72 | 2.69 | 0.19 | 0.69 | 0.10 | 0.37 | 2.80 |
| Hb | 68 | 13.49 | 0.39 | 2.92 | 0.55 | 4.11 | 0.11 | 0.85 | 5.11 |

Repeatability study results broken down by CD4 level:

| Sample CD4 Level (cells/µL) | N | Mean | Within Run (%CV) | | Between Operator/ Instrument | | Between Lot | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | %CV |
| ≤ 200 | 12 | 97.26 | 6.94 | 7.13 | 2.43 | 2.49 | 0.79 | 0.81 | 7.60 |
| 201–500 | 17 | 327.02 | 17.28 | 5.28 | 4.45 | 1.36 | 4.76 | 1.46 | 5.65 |
| 501–1000 | 25 | 691.35 | 22.25 | 3.22 | 5.53 | 0.80 | 0.57 | 0.08 | 3.32 |
| 1001–5000 | 13 | 1367.00 | 32.29 | 2.37 | 10.95 | 0.80 | 7.9 | 0.58 | 2.57 |

*b. Linearity/assay reportable range:*

Five samples with evenly spaced CD4 concentrations were evaluated in the lower CD4 range of 40−200 cells/µL. Twelve samples with evenly spaced CD4 concentrations were evaluated in the CD4 range of 40–2500 cells/µL and the total lymphocyte range of 200–5000 cells/µL. Eleven evenly spaced samples were evaluated for hemoglobin (Hb) in the range of 2–26 g/dL. These samples were prepared by manipulating whole blood samples, which were collected in EDTA tubes. The concentration pools for CD4, lymphocytes, and Hb were prepared by proportionally mixing pools of samples with high and low analyte concentrations. Samples were tested in triplicate on BD FACSPresto Cartridges for each study. Three BD FACSPresto instruments and three lots of BD FACSPresto Cartridges were used for this evaluation.

Because %CD4 is not an analyte by itself but is determined by CD4 and lymphocyte absolute counts (numerator and denominator, respectively), BD evaluated CD4 and lymphocyte linearity in this study. Linearity for lymphocyte absolute counts was

evaluated for the range of 200−5,000 cells/µL on the BD FACSPresto system.

The reportable ranges of the BD FACSPresto system include: CD4 (40−2500 cells/µL), %CD4 (5–60%), and Hb (5−18 g/dL).

Sponsor's Acceptance Criteria

CD4 Absolute Counts: Across the dynamic ranges of the assay for CD4 (40–200 cells/µL and 40−2,500 cells/µL), the system shall be linear if 2nd and 3rd order coefficients of the regression lines are not significant. If the coefficients from the higher order polynomial fit (2nd and 3rd) tested are statistically significant, then the difference between the first order linear fit and the higher order linear fit must be within 10% of the first order linear fit for concentration levels that are >200 cells/µL, or within ± 20 cells/µL of the first order linear fit for concentration levels that are ≤ 200 cells/µL. The coefficient of determination (R2) shall be >95% for linear fit.

Total Lymphocyte Counts: Across the dynamic range of the assay for total lymphocyte count (200 to 5,000 cells/µL), the system shall be linear if 2nd and 3rd order coefficients of the regression lines are not significant. If the coefficients from the higher order polynomial fit (2nd and 3rd) tested are statistically significant, then the difference between the first order linear fit and the higher order linear fit must be within 10% of the first order linear fit for concentration levels that are > 200 cells/µL, or within ± 20 cells/µL for concentration levels that are ≤ 200 cells/µL. The coefficient of determination (R2) shall be >95% for linear fit.

| Sample | Lot | Test Range | R2 | Recovery Range |
|---|---|---|---|---|
| CD4 | 1 | 40-2500 cells/µL | 0.999 | 36-3176 |
| | 2 | 40-2500 cells/µL | 0.998 | 30-3160 |
| | 3 | 40-2500 cells/µL | 0.998 | 34-3270 |
| CD4 low | 1 | 40-200 cells/µL | 0.994 | 30-262 |
| | 2 | 40-200 cells/µL | 0.995 | 31-265 |
| | 3 | 40-200 cells/µL | 0.995 | 30-262 |
| Total Lymphocytes | 1 | 200-5000 cells/µL | 0.999 | 157-6183 |
| | 2 | 200-5000 cells/µL | 0.998 | 156-6171 |
| | 3 | 200-5000 cells/µL | 0.998 | 159-6280 |

The linearity results for the two CD4 ranges (40−200 cells/µL and 40−2,500 cell/µL) using EDTA anticoagulated blood met the acceptance criteria. Similarly, the total lymphocyte linearity results (200−5,000 cells/µL) met the acceptance criteria. Hemoglobin linearity evaluation was passed.

| | Lot | Order coefficients | Criteria | P value Test for Linearity | Criteria | %CV | Pass/Fail |
|---|---|---|---|---|---|---|---|
| Hemoglobin | 1 | 3 | ±0.5 | 0.09 | <10% | 4.6 | Pass |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2 | 2 | ±0.5 | 0.04 | <10% | 3.1 | Pass |
| | 3 | 3 | ±0.5 | 0.15 | <10% | 4.1 | Pass |

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

Traceability:

There are currently no international reference standards for CD4 marker antibodies. The BD Multi Check Controls were manufactured by diluting human whole blood containing leukocytes and erythrocytes in a stabilizing medium. The human whole blood was obtained from commercial sources and was tested for markers of infectious substances.

The FACSPresto system was evaluated for its accuracy performance against the HiCN method for hemoglobin determination. Performance was evaluated for the range of 2−22 g/dL on the BD FACSPresto system and the HiCN reference method system using manipulated and donor samples. Manipulated levels were spaced equidistant from each other. These equidistant levels were prepared by manipulating whole blood samples collected in EDTA tubes. A minimum of one FACSPresto instrument and one lot of BD FACSPresto Cartridges were used for this evaluation. The BD FACSPresto System demonstrated agreement with the HiCN method. The Hb assay is linear and accurate across the reported linear range (2−20 g/dL) as compared to the HiCN method.

Control Value Assignment:

The value assignment for BD Multi-Check Control and BD Multi-Check CD4 Low Control included controls were tested on three instruments, with three lots of reagent cartridge, at two incubation time points: 18 minutes and two hours, for a total of 18 values to determine the final value assignment. The BD Multi-Check results acquired on the BD FACSPresto system shall fall within the FACSPresto-specific ranges at least 95% of the time and meet the FACSPresto system error rate of < 5%. All acceptance criteria were met. Precision testing was performed on the controls using three lots of BD Multi-Check Control and BD Multi-Check CD4 Low Controls performed at three sites on three instruments spanning a minimum of 20 days. In addition to verifying the precision of the process controls on the BD FACSPresto system, this study further verifies the acceptability of the FACSPresto-specific assay ranges.

Eurotrol FACSPresto Hb Control value assignment was established using vials from each level (1, 2, and 3) of Eurotrol FACSPresto Hb Control. Three lots of BD FACSPresto Cartridges are tested on three FACSPresto Instruments at three incubation time-points: 1−5 min, 18−30 min and 2 hours (±15min), for a total of 27 values to determine the final value assignment. The hemoglobin results acquired on the BD FACSPresto system shall fall within the FACSPresto specific ranges at least 95% of the time and meet the FACSPresto system < 5% error rate at each site. All

acceptance criteria were met. Three lots of Eurotrol FACSPresto Hb Control were run on three instruments to test precision over 20 days verifying the FACSPresto-assigned ranges were acceptable for use.

Stability:

Stability studies have been performed and the results support the following claims:

*Sample Stability*:
For specimens acquired via the venipuncture method, staining was performed after age of blood reached 22−24 hours. These specimens were stained then analyzed at 18−22 minutes later and then again at 2 hours and 15 min after initiating staining. The acceptance criteria was that a 24 hours post-drawn venipuncture blood in EDTA shall remain readable and the bias of CD4 count, %CD4, and Hb results shall be within ±15% with 95% confidence interval of fresh (< 6 hours post-drawn) venipuncture blood in EDTA. Samples were shown to be stable for up to 22 hours at room temperature.

*Age of Stain*:
The acceptable age of staining after reconstitution was determined through the time course evaluation of the BD FACSPresto Cartridge using fresh (within six hours of draw) patient blood (both capillary and venous). These specimens were stained and analyzed for 18−22 minutes after incubation (i.e., standard operating procedures) and then analyzed again at 2 hours and 15 min after initiating staining. For specimens acquired via the venipuncture method, age of stain was also performed after age of blood reached 22−24 hours. These specimens were stained then analyzed at 18−22 minutes later and then again at 2 hours and 15 min after initiating staining. The staining of the cells was shown to be stable for one hour after the sample has been applied to the cartridge.

*Reagent Cartridge Stability*:
Shelf Life: Cartridge for closed pouch expiration is assigned a shelf life of one year in a temperature and humidity range of 4°C to 31°C and 10% to 95% relative humidity. The acceptance criteria of CD4, %CD4, or Hb results within ± 10% of reference reagent (pouched cartridges at -20°C) were met. Real time stability is ongoing and currently supports a claim of one year.

*Open/In use Stability*: Open pouch expiration for the cartridges is assigned 30 minute stability in a temperature and humidity range of 10°C to 40°C and 10% to 95% relative humidity. The acceptance criteria of CD4, %CD4, or Hb results within ±10% of reference reagent (pouched cartridges at -20°C) were met.

*Control Stability*:
Shelf Life: The acceptance criteria for the BD Multi-Check Control and BD Multi-Check CD4 Low Control results acquired on the BD FACSPresto system shall fall within the FACSPresto-specific ranges at least 95% of the time and meet the

FACSPresto system error rate of < 5%. Three lots of controls were tested. All lots of the BD Multi-Check Control and BD Multi-Check CD4 Low Control were within the established acceptance criteria. Expiration dating reflects the validated time period that assures adequate device performance throughout the shelf life of 45 days.

The acceptance criteria for shelf life stability of the Eurotrol FACSPresto Hb Control was the measurement results of the controls must demonstrate no more than 7% allowable drift with 95% confidence interval from the mean value at T=0 throughout the claimed shelf life. All three lots of the Eurtotrol FACSPresto Hb Control were within the established acceptance criteria. Expiration dating reflects the validated time period that assures adequate device performance throughout the shelf life of one month.

*Open Vial/In-use Stability*:
Real time stability testing of the BD Multi-Check Control and BD Multi-Check CD4 Low Control passed the acceptance criteria for all stability tests through the stated open vial shelf life of nine thermal cycles, stored at 2-8°C and sampled at room temperature.

Real time stability testing of two lots of the Eurotrol FACSPresto Hb Control (Levels 1–3) passed the acceptance criteria for all stability tests through the stated open vial shelf life of 30 days, stored at 2–8°C and sampled at room temperature.

*d. Detection limit:*

Limit of Blank (LoB):
For the FACSPresto CD4 assay, the LoB was determined by producing a blank sample for the CD4 assay by blocking all CD4 binding sites with CD4-FITC. The CD4-PECy5 will be prevented from binding. There will be no CD4 signal and the algorithm will not count and gate any CD4 cells. The instrument average of blank samples, after running the blank 60 times, was 5.02 counts, with a SD of 3.16, and the $95^{th}$ percentile of blank sample of 9.92.

The sponsor has stated that it is not feasible to use the BD FACSPresto system to perform the Hb assay at zero g/dL for LoB determination due to an internal algorithm quality control process.

Limit of Detection (LoD):
For the FACSPresto CD4 assay, the LoD was determined by testing 20 replicates from a sample with the lowest detectible level of analyte and 20 replicates from the blank sample were tested for CD4 per lot of cartridges per instrument. The LoD of the CD4 assay was determined to be 22 cells/µL.

For the FACSPresto Hemoglobin assay the LoD was determined by following the Probit approach in accordance with CSLI EP-17-A2. The LoD of the Hemoglobin assay was determined to be 0.91 g/dL.

Limit of Quantitation (LoQ):
For the FACSPresto CD4 assay the LoQ was determined by following the variant approach described in Section 6.6 of the CSLI EP17-A2 guideline. Five samples were tested in six replicates for each of two lots of cartridges. The LoQ of the CD4 assay was determined to be 35 cells/µL.

The LoQ for Hemoglobin (Hb) was determined to be 2.0 g/dL. To determine the LoQ of Hb, 20 replicates from a sample with the lowest detectible level of analyte were tested for Hb per lot of cartridges per instrument (60 replicates total). Whole blood was concentrated by removing the plasma to obtain a sample with high Hb level and then diluted with plasma to target an Hb level of 2 g/dL.

*e. Analytical specificity:*

The endogenous and exogenous interference studies were performed in accordance with CLSI EP07-A2 guidelines. Clinically significant means a 15% or lower difference from a negative control. The acceptance criteria for CD4, %CD4, and Hb results were: for each of the sample results, intra-sample precision shall have a CV under 7% and for each of the analytes, with 95% confidence interval applied, the mean difference (or bias) between the sample with target concentration and the negative control sample shall be less than 15%. The following interfering substances were tested and passed the acceptance criteria for the concentrations stated below.

| **Analyte** | **Max Concentration** |
|---|---|
| Acetaminophen | 11.5 mg/dL |
| Albumin | 5 g/dL |
| Ascorbic Acid | 6 mg/dL |
| Conjugated Bilirubin | 5 mg/dL |
| Creatinine | 5 mg/dL |
| Ethambutol | 12µg/mL |
| Glucose | 120 mg/dL |
| Hemolysis | 20% |
| Iron | 150 µg/dL |
| Isoniazid | 40 µg/mL |
| Lipemia (intralipid) | 2400 mg/dL |
| Magnesium | 6.3µg/dL |
| Methemoglobin | 14% |
| Nevirapine | 7 µg/mL |
| Quinine | 16 µg/mL |
| Rifampicin | 32 µg/mL |
| Tenofovir | 1000 ng/mL |
| Urea | 40 mg/dL |
| Zidoyudine | 1000 ng/mL |
| Amodiaquine | 60 ng/mL |
| Artesunate | 600 ng/mL |
| Efavirenz | 16 µg/mL |

| Analyte | Max Concentration |
|---|---|
| Gamma Globulin | 40 mg/mL |
| Ibuprofen | 500 µg/mL |
| Quinine | 48 µg/mL |
| Rifampicin | 64 µg/mL |
| Salicylic Acid | 200 µg/mL |
| Tetracycline | 150 µg/mL |
| Uric Acid | 90 µg/mL |
| Thrombocytes (Platelets) | $1.541 \times 10^6$ cells/µL |
| White Blood Cells* | $2.5 \times 10^3$ cells/µL |
| Disease Condition | |
| Rouleaux Formation | No interference |
| Cold Agglutinin | No interference |

*Applicable to Hb results only

*f. Assay cut-off:*

Not applicable

2. Comparison studies:

*a. Method comparison with predicate device:*

A study population of HIV+ paired fingerstick (FS) and venipuncture (VP) samples measured in duplicate on the FACSPresto system and the predicate devices. Remnant venous blood samples without corresponding capillary specimens were also included. The tests were performed using three lots of the BD FACSPresto cartridge, three instruments, and two operators.

Venipuncture samples were prospectively obtained from 796 de-identified and de-linked donors, of which 692 provided matched capillary blood specimens. Male (322) and female (410) subjects from apparently healthy, HIV negative subjects with other diseases, and HIV positive patients were included. Seven hundred seventeen (717) subjects from CHIL, DIT, KEM, NAR, PEK SIR and SFG were enrolled in the study, and 75 venous blood specimens were procured from an external vendor for sample manipulation at MED and added to the venous portion of the study. The samples were manipulated by combining cell fractions and plasma in appropriate ratios to reach target concentrations of analyte(s) to fill the low bins (CD4 and Hb) or high bins (CD4 and Hb) in the accuracy evaluation. Of the non-manipulated samples, 57 samples were from children (2 to 11 years), 68 samples were from adolescents (12 to 21 years), and 592 samples were from adults (≥ 22 years). None of the capillary blood specimens was manipulated.

Acceptance criteria were as follows:

| Specimen Type | Parameter | Criteria | N | Results | Pass or Fail |
|---|---|---|---|---|---|
| Venous Blood (all samples) | CD4 | Slope: 0.9 to 1.1 with 95%CI | 785 | Slope:0.96 (0.96,0.97) | Pass |
| | | $R^2$: ≥ 0.90 | | $R^2$: 0.97 | |
| | | Intercept: ± 20 counts | | Intercept: -0.36 counts | |
| | %CD4 | Slope: 0.9 to 1.1 with 95%CI | 785 | Slope:1.01 (1.00,1.02) | Pass |
| | | $R^2$: ≥ 0.90 | | $R^2$: 0.97 | |
| | | Intercept: ±3% | | Intercept: 0.40% | |
| | Hb | Slope: 0.9 to 1.1 with 95%CI | 796 | Slope: 1.01 (0.99,1.03) | Pass |
| | | $R^2$: ≥ 0.90 | | $R^2$: 0.94 | |
| | | Intercept :±0.5 g/dL | | Intercept: -0.35 g/dL | |
| Capillary Blood (Non-Manipulated samples only) | CD4 | Slope: 0.9 to 1.1 with 95%CI | 683 | Slope:1.03 (1.02,1.05) | Pass |
| | | $R^2$: ≥ 0.90 | | $R^2$: 0.97 | |
| | | Intercept : ± 20 counts | | Intercept: 0.72 counts | |
| | %CD4 | Slope: 0.9 to 1.1 with 95%CI | 683 | Slope: 1.01 (1.00,1.03) | Pass |
| | | $R^2$:≥ 0.90 | | $R^2$:0.96 | |
| | | Intercept : ±3% | | Intercept: -0.31% | |
| | Hb (data from original 3 sites only) | Bias at 10.5 ±1 g/dL (clinical decision point) <7% with 95%CI | 87 | 1.83% (0.78%, 2.8%) | Pass |
| | Hb (data from new 7 sites only) | Slope: 0.9 to 1.1 with 95%CI | 692 | Slope: 1.02 (1.00,1.05) | |
| | | $R^2$: ≥0.90 | | $R^2$: 0.89 | |
| | | Intercept : ±0.5 g/dL | | Intercept: -0.37 g/dL | |

A performance evaluation per age group:

| Parameter | N | Children (2 to 11 years) | N | Adolescent (12 to 21 years) | N | Adults (≥ 22 years) |
|---|---|---|---|---|---|---|
| Venous Blood | | | | | | |
| CD4 | 57 | Range: 329−4020 cells/µL | 68 | Range: 5−5204 cells/µL | 592 | Range: 14-1624 cells/µL |
| | | Slope: 0.97 (0.94,1.00) | | Slope:0.98 (0.95, 1.01) | | Slope: 0.96 (0.95,0.98) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | Intercept: 3.84 counts | | Intercept: -0.78 counts | | Intercept: 5.36 counts |
| %CD4 | 57 | Slope: 0.97 (0.93,1.01)<br>Intercept : 1.74% | 68 | Slope:1.00 (0.96,1.04)<br>Intercept: 0.84% | 592 | Slope: 1.01 (1.00,1.02)<br>Intercept : 0.43% |
| Hb | 57 | Slope: 1.05 (0.94,1.17)<br>Intercept : -1.06 g/dL | 68 | Slope: 0.97 (0.90,1.04)<br>Intercept: -0.19 g/dL | 596 | Slope: 1.02 (0.99,1.05)<br>Intercept : -0.50 g/dL |
| Capillary Blood | | | | | | |
| CD4 | 54 | Range: 306−3969 cells/µL<br>Slope: 1.02 (0.95,1.09)<br>Intercept : 17.11 counts | 65 | Range: 8−5216 cells/µL<br>Slope:1.05 (1.01,1.08)<br>Intercept: 1.76 counts | 563 | Range: 8−1509 cells/µL<br>Slope: 1.03 (1.01,1.05)<br>Intercept : -0.35 counts |
| %CD4 | 54 | Slope: 0.99 (0.90,1.08)<br>Intercept : 0.19% | 65 | Slope: 1.00 (0.95,1.05)<br>Intercept: -0.30% | 563 | Slope: 1.02 (1.00,1.04)<br>Intercept : -0.39% |
| Hb | 54 | Slope: 1.08 (0.81,2.07)<br>Intercept : 1.08 g/dL | 65 | Slope: 1.05 (0.95,1.15)<br>Intercept : -0.58 g/dL | 572 | Slope: 1.02 (0.99,1.05)<br>Intercept : -0.37 g/dL |

Eight individual sites covered different parts of the analytical measuring ranges as shown below.

VEN=venous (venipuncture); CAP= capillary (fingerstick)

| Parameter | Sample type | Site ID | N | Mean counts | Median | Min | Max | Total |
|---|---|---|---|---|---|---|---|---|
| CD4 | VEN | CHL | 19 | 850.74 | 828 | 227 | 1190 | 785 |
| | | DIT | 132 | 372.38 | 373 | 5 | 982 | |
| | | KEM | 177 | 651.10 | 522 | 18 | 5204 | |
| | | MED | 58 | 424.52 | 292 | 8 | 1301 | |
| | | NAR | 139 | 470.48 | 408 | 41 | 1274 | |
| | | PEK | 93 | 739.56 | 719 | 85 | 1624 | |
| | | SFG | 103 | 525.04 | 517 | 14 | 1240 | |
| | | SIR | 64 | 884.02 | 730.5 | 176 | 4020 | |
| | CAP | CHL | 16 | 925.56 | 871.5 | 282 | 1399 | 682 |
| | | DIT | 127 | 389.57 | 409 | 8 | 1002 | |
| | | KEM | 163 | 699.10 | 588 | 15 | 5216 | |
| | | NAR | 132 | 501.28 | 452 | 84 | 1534 | |

| Parameter | Sample type | Site ID | N | Mean counts | Median | Min | Max | Total |
|---|---|---|---|---|---|---|---|---|
| | | PEK | 90 | 773.7 | 765.5 | 73 | 1532 | |
| | | SFG | 92 | 555.76 | 535 | 8 | 1291 | |
| | | SIR | 62 | 946.18 | 773 | 234 | 3969 | |

| Parameter | Sample type | Site ID | N | Mean % | Median | Min | Max | Total |
|---|---|---|---|---|---|---|---|---|
| CD4% | VEN | CHL | 19 | 35.41 | 35.06 | 15.31 | 50.02 | 785 |
| | | DIT | 132 | 22.35 | 23.435 | 0.63 | 43.6 | |
| | | KEM | 179 | 25.90 | 25.94 | 0.96 | 53.77 | |
| | | MED | 58 | 27.53 | 28.76 | 0.3 | 47.17 | |
| | | NAR | 139 | 24.64 | 23.84 | 5.55 | 44.23 | |
| | | PEK | 93 | 38.78 | 38.71 | 13.03 | 59.82 | |
| | | SFG | 103 | 26.51 | 25.9 | 1.69 | 54.26 | |
| | | SIR | 64 | 28.45 | 29.22 | 11.15 | 45.72 | |
| | CAP | CHL | 16 | 35.53 | 34.25 | 15.13 | 47.34 | 682 |
| | | DIT | 127 | 21.63 | 22.45 | 0.73 | 42.33 | |
| | | KEM | 166 | 25.26 | 24.92 | 0.57 | 56.2 | |
| | | NAR | 132 | 23.37 | 22.925 | 6.43 | 44.49 | |
| | | PEK | 90 | 39.11 | 38.15 | 13.44 | 58.5 | |
| | | SFG | 92 | 25.87 | 26.21 | 1.44 | 55.2 | |
| | | SIR | 62 | 27.28 | 27.72 | 10.73 | 48.38 | |

| Parameter | Sample type | Site ID | N | Mean g/dL | Median | Min | Max | Total |
|---|---|---|---|---|---|---|---|---|
| Hb | VEN | CHL | 19 | 13.28 | 12.9 | 11 | 16.7 | 796 |
| | | DIT | 134 | 14.31 | 14.7 | 4.9 | 19.2 | |
| | | KEM | 179 | 11.97 | 11.5 | 4.7 | 21.2 | |
| | | MED | 64 | 12.34 | 17.5 | 3 | 20.7 | |
| | | NAR | 139 | 12 | 11.9 | 8.5 | 16.7 | |
| | | PEK | 94 | 13.27 | 13.5 | 7.1 | 17 | |
| | | SFG | 103 | 13.39 | 13.7 | 5.2 | 16.9 | |
| | | SIR | 64 | 12.64 | 12.6 | 6.8 | 16.6 | |
| | CAP | CHL | 17 | 13.88 | 13.6 | 11.1 | 17 | 692 |
| | | DIT | 128 | 14.34 | 14.8 | 5.2 | 18.7 | |
| | | KEM | 166 | 11.79 | 11.7 | 4.7 | 17.1 | |
| | | NAR | 132 | 12.21 | 12.1 | 8.8 | 16 | |
| | | PEK | 92 | 13.45 | 13.85 | 7 | 16.7 | |
| | | SFG | 95 | 13.55 | 13.9 | 5.4 | 17.3 | |
| | | SIR | 62 | 12.99 | 12.85 | 7.1 | 16.9 | |

Site-to-Site Comparison of Venous Non-Manipulated Samples

| Specimen Type | Site ID | Parameter | N | Slope | Intercept | % Bias |
|---|---|---|---|---|---|---|
| VEN | CHL | CD4 | 19 | 0.93 | -6.06 | -6.1% |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | %CD4 | 19 | 1.01 | 0.69% | 2.5% |
| | | Hb g/dL | 19 | 0.92 | 0.99 | 0.9% |
| | DIT | CD4 | 132 | 0.94 | -0.96 | -6.3% |
| | | %CD4 | 132 | 1.05 | -0.26% | 2.7% |
| | | Hb g/dL | 134 | 1.03 | 0.18 | 4.5% |
| | KEM | CD4 | 167 | 0.98 | 5.05 | -0.1% |
| | | %CD4 | 167 | 1.02 | 0.45% | 4.6% |
| | | Hb g/dL | 168 | 0.94 | 0.25 | -3.9% |
| | NAR | CD4 | 139 | 0.96 | -8.59 | -6.3% |
| | | %CD4 | 139 | 1.03 | 0.04% | 3.3% |
| | | Hb g/dL | 139 | 1.01 | -0.49 | -3.3% |
| | PEK | CD4 | 93 | 1.00 | 9.38 | 1.6% |
| | | %CD4 | 93 | 0.98 | 1.29% | 1.7% |
| | | Hb g/dL | 94 | 0.98 | 0.13 | -0.8% |
| | SFG | CD4 | 103 | 0.95 | 3.94 | -3.6% |
| | | %CD4 | 103 | 1.00 | 0.71% | 3.2% |
| | | Hb g/dL | 103 | 0.99 | -0.61 | -5.6% |
| | SIR | CD4 | 64 | 0.98 | -10.01 | -3.9% |
| | | %CD4 | 64 | 0.98 | 0.58% | 0.1% |
| | | Hb g/dL | 64 | 0.93 | 0.2 | -6.0% |

Site-to-Site Comparison of Capillary Non-Manipulated Samples

| Specimen Type | Site ID | Parameter | N | Slope | Intercept | % Bias |
|---|---|---|---|---|---|---|
| CAP | CHL | CD4 | 16 | 1.03 | 7.49 | 4.1% |
| | | %CD4 | 16 | 0.94 | 2.34% | 1.1% |
| | | Hb g/dL | 17 | 1.01 | 0.47 | 4.6% |
| | DIT | CD4 | 127 | 1.00 | 2.1 | 1.5% |
| | | %CD4 | 127 | 1.02 | -0.23% | 0.8% |
| | | Hb g/dL | 128 | 1.03 | 0.24 | 4.5% |
| | KEM | CD4 | 163 | 1.06 | 1.94 | 6.1% |
| | | %CD4 | 163 | 1.02 | 0.29% | 0.1% |
| | | Hb g/dL | 166 | 0.98 | 0.52 | 1.2% |
| | NAR | CD4 | 132 | 0.99 | 4.55 | 0.6% |
| | | %CD4 | 132 | 0.99 | 0.09% | -1.0% |
| | | Hb g/dL | 132 | 1.06 | -0.99 | -1.8% |
| | PEK | CD4 | 90 | 1.05 | 13.79 | 7.6% |
| | | %CD4 | 90 | 0.97 | 2.21% | 3.1% |
| | | Hb g/dL | 92 | 0.97 | 0.52 | 0.8% |
| | SFG | CD4 | 90 | 1.03 | -1.76 | 2.3% |
| | | %CD4 | 92 | 0.98 | 0.38% | 0.3% |
| | | Hb g/dL | 95 | 0.99 | -0.45 | -4.5% |
| | SIR | CD4 | 62 | 1.05 | -23.69* | 1.6% |
| | | %CD4 | 62 | 1.00 | -1.25% | -4.6% |
| | | Hb g/dL | 62 | 0.94 | 0.43 | -3.1% |

The clinical method comparison data supports the following analytical measuring ranges (AMR):

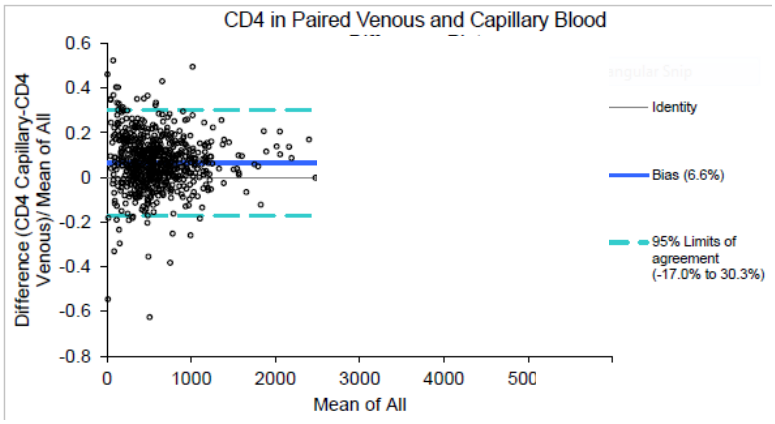
| Assay output | AMR |
|---|---|
| CD4 (cells/µL) | 40 to 2,500 |
| %CD4 | 5 to 60 |
| Hemoglobin (Hb) (g/dL) | 5 to 18 |

*b. Matrix comparison:*

The sponsor provided matrix comparison studies that included 678 matched pairs of fingerstick blood samples and venous blood samples for measurement of CD4 absolute counts, %CD4, and hemoglobin measurements in whole blood.

Venous Peripheral Blood vs. Fingerstick linear regression

| | N | Slope | 95% CI | y-Intercept | 95% CI | $R^2$ |
|---|---|---|---|---|---|---|
| CD4 | 678 | 1.07 | 1.05 to 1.09 | 0.58 | -6.44 to 7.59 | 0.96 |
| %CD4 | 678 | 1.00 | 0.99 to 1.02 | -0.80 | -1.17 to -0.44 | 0.96 |
| Hb | 685 | 1.00 | 0.97 to 1.02 | 0.23 | -0.08 to 0.53 | 0.89 |




Note that for 14 of the 692 paired capillary vs venous blood samples, the difference in results between matrices would result in different result interpretation based on the cutoff of 200 cells/µL. However, as a clinician would not use 200 cells/µL alone as an absolute cutoff value for decision making, and all clinical factors are considered important to evaluate the patient diagnosis, having a discordant reading between capillary and venous blood samples in these cases would not result delayed or missed treatment.

Multiple sources of CD4 count variability are recognized, including intra-laboratory measurements and individual patient physiologic factors. CD4 results must be interpreted in conjunction with other laboratory and clinical findings

3. Clinical studies:

*a. Clinical Sensitivity:*

Not applicable

*b. Clinical specificity**:***

Not applicable

*c. Other clinical supportive data (when a. and b. are not applicable):*

Additional data was collected from seven of the clinical sites clinical sites to evaluate agreement around the primary clinical decision points of values less than 200 cells/µL to confirm performance of the device in the clinically relevant ranges for HIV-infected US patients.

The sponsor included 717 non-manipulated venous samples and 682 non-manipulated capillary samples in the comparison study between the new and predicate device.

Agreement with cutoff of 200 CD4 Cells/µL in Non-Manipulated Venous Blood

| **Method** | | **Predicate (FACSCalibur)** | | |
|---|---|---|---|---|
| | | Positive (< 200) | Negative (> 200) | Total |
| **Test (FACSPresto)** | Positive (< 200) | 75 | 10 | 85 |
| | Negative (> 200) | 1 | 631 | 632 |
| | Total | 76 | 641 | 717 |

Positive Percent Agreement (75/76): 98.7% (95% CI: 92.89–99.97%)
Negative Percent Agreement (631/641): 98.4% (95% CI: 97.48–99.40%)
Overall Percent Agreement (706/717): 98.5%

Agreement at 200 CD4 Cells/µL in Non-Manipulated Capillary Blood

| **Method** | | **Predicate (FACSCalibur)** | | |
|---|---|---|---|---|
| | | Positive (< 200) | Negative (> 200) | Total |
| **Test (FACSPresto**) | Positive (< 200) | 62 | 5 | 67 |
| | Negative (> 200) | 11 | 604 | 615 |
| | Total | 73 | 609 | 682 |

Positive Percent Agreement (62/73): 84.9% (95% CI: 76.73–93.34%)
Negative Percent Agreement (604/609): 99.2% (95% CI: 98.46–99.90%)
Overall Percent Agreement (666/682): 97.7%

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

Two BD FACSPresto instruments and three lots of BD FACSPresto Cartridges were used for testing venous and capillary blood specimens collected from 133 male and 142 female apparently healthy subjects, between 18 to 70 years of age, at one clinical site (BCW). On each day of testing, BD FACSPresto instruments were set up and process controls were run obtaining passing results. The first replicate was used to calculate CD4, %CD4, and Hb values. Data were analyzed using parametric (ANOVA) and non-parametric methods described in Section 7.3 of CLSI Guideline EP28-A3c *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition.* We recommend that laboratories and other users establish their own reference intervals for their patient populations using the BD FACSPresto system to reflect potential sources of variability, such as patient gender, race, age, and preparation techniques.

| Sample Type | Parameter | Gender | N | Mean | Reference Interval |
|---|---|---|---|---|---|
| Venous | CD4 | Male | 129 | 836 cells/µL | 256−1652 cells/µL |
| | | Female | 142 | 1071 cells/µL | 522−1902 cells/µL |
| | %CD4 | Male | 129 | 46.35% | 31.51–61.02% |
| | | Female | 142 | 49.91% | 34.67–63.28% |
| | Hb | Male | 129 | 14.8 g/dL | 12.3–16.6 g/dL |
| | | Female | 142 | 13.1 g/dL | 11.3–14.9 g/dL |
| Capillary | CD4 | Male | 133 | 856 cells/µL | 276−1515 cells/µL |
| | | Female | 140 | 1131 cells/µL | 536−2031 cells/µL |
| | %CD4 | Male | 133 | 46.14% | 31.33−62.11% |
| | | Female | 140 | 50.50% | 33.06−66.48% |
| | Hb | Male | 133 | 15.0 g/dL | 12−17.3 g/dL |
| | | Female | 140 | 13.4 g/dL | 11−15.4 g/dL |

**N. Instrument Name:**

BD FACSPresto™ System

**O. System Descriptions:**

1. Modes of Operation:

Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

Yes _x__ or No _____

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes _____ or No ___x___

2. Software:

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

   Yes ____x___ or No _____

3. Specimen Identification:

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

4. Specimen Sampling and Handling:

   Collection is by aliquot from an EDTA-coated tube containing whole blood from venipuncture or from fingerstick via lancet. To prepare the cartridge, the user transfers the whole blood specimen directly from an EDTA tube or fingerstick to the inlet port of the cartridge. Once blood flows in the cartridge, the cap is closed and the cartridge automatically performs all sample preparation. During this process the sample flows through the reagent disk and into the cartridge channel where staining of white cells by fluorescent-antibody conjugates occurs. The tear-strip on the cartridge label protects the stained sample from light exposure. After 18 minutes (and up to two hours) stain time, the tear-strip is removed and the cartridge can be read on the instrument.

5. Calibration:
   Performed by manufacturer

6. Quality Control:

   BD Multi-Check Control and BD Multi-Check CD4 Low Control are assayed whole blood quality control products for analysis using monoclonal antibody reagents and image cytometry. They provide positive cell controls that are processed in the same manner as a whole blood sample. This allows verification of instrument and reagent performance.

   Eurotrol FACSPresto Hb Control is an assayed hemoglobin control product for analysis using spectrophotometry. The control is processed in the same manner as a whole blood sample. This allows verification of instrument and reagent performance.

**P. Other Supportive Instrument Performance Characteristics Data Not Covered In the "Performance Characteristics" Section above:**

Comparison of Traditional gating and CD3+CD45RA single color gating strategy for determination of total lymphocytes:

#Events acquired in total, in the CD45 gate, CD3+CD45RA gate and in both gates

| Sample | All events | $CD45^+$ | $CD3^+CD45RA^+$ | $CD3^+CD45RA^+CD45^+$ |
|---|---|---|---|---|
| 1 | 32705 | 7711 | 7741 | 7662 |
| 2 | 16305 | 6356 | 6319 | 6286 |
| 3 | 25577 | 11449 | 11433 | 11262 |
| 4 | 37034 | 13131 | 13054 | 12999 |
| 5 | 33982 | 15866 | 15817 | 15724 |

Note: events are not per µL

Fifty seven (57) samples were analyzed, comprised of 18 HIV- samples and 39 HIV+ samples on two different BD FACSPresto systems: a BD FACSCalibur instrument with BD Tritest CD3/4/45 (the predicate assay), and a BD FACSCalibur instrument with the BD Multitest Immune Monitoring Kit (IMK) consisting of CD3/4/8/45 and CD3/16+56/19/45 (2 tube T/B/NK assay), to compare total lymphocyte counts between methods. The Tritest assay uses SSC and CD45 signals to identify lymphocytes as SSC low and CD45 high. The IMK assay similarly uses SSC and CD45 to identify lymphocytes and then uses CD3, CD19 and CD16+CD56 to identify these lymphocytes as T-cells, B-cells and NK-cells respectively. Both FACSCalibur methods used BD Trucount beads for calculating absolute lymphocyte counts.

Lymphocytes/µL

| | Presto vs. Tritest | Presto vs. IMK | Presto vs. Tritest (MC) |
|---|---|---|---|
| N | 57 | 57 | 717 |
| Sample Range | 656−4530 | 701−4486 | 225−11883 |
| Slope | 0.96 | 1.01 | 0.93 |
| Slope 95% CI | 0.91 to 1.01 | 0.97 to 1.05 | 0.92 to 0.95 |
| Intercept | 113.3 | 24.3 | 13.15 |
| $R^2$ | 0.97 | 0.98 | 0.99 |

**Q. Proposed Labeling:**

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

**R. Conclusion:**

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