**510(k) Summary for**
**AQUIOS STEM SYSTEM with AQUIOS CL Flow Cytometer**

**510(k) Owner / Submitter Information**

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**Proposed Device Information**

Trade Name: **AQUIOS CL Flow Cytometer**
Common Name: AQUIOS CL
Classification Name: Automated differential cell counter (21 CFR 864.5220)
Classification: Class II
Product Code: OYE
Panel: Hematology

Trade Name: **AQUIOS STEM Software**
Common Name: AQUIOS STEM Software
Classification Name: Automated differential cell counter (21 CFR 864.5220)
Classification: Class II
Product Code: OYE
Panel: Hematology

Trade Name: **AQUIOS STEM Kit**
Common Name: AQUIOS STEM Kit
Classification Name: Automated differential cell counter (21 CFR 864.5220)
Classification: Class II
Product Code: OYE
Panel: Hematology

Trade Name: **AQUIOS STEM CD34 Control Cells**
Common Name: AQUIOS STEM Control Cells Level 1 and Level 2
Classification Name: Hematology quality control mixture (21 CFR 864.8625)
Classification: Class II Exempt, based on regulation 21 CFR 864.8625 which states that Quality Control materials are now exempt from premarket notification subject to the limitations under 21 CFR 862.9
Product Code: JPK
Panel: Hematology

## Predicate Device Information

| Manufacturer | Predicate | 510(k) Number | Product Code | Classification |
|---|---|---|---|---|
| Beckman Coulter Inc | Stem-Kit Reagent | BK210639 BK040032 | GKZ** | Class II (864.5220) Automated differential cell counter |
| | FC500 Flow cytometer | K182886 | GKZ** | |
| | AQUIOS CL Flow Cytometer | K141932 | OYE | |
| | stemCXP software | BK210639 BK040055 | GKZ** | |
| | Stem-Trol control cells | BK040032 | JPK* | Class II Exempt (864.8625) Hematology Quality Control Mixture |

* Based on regulation 21 CFR 864.8625 which states that Quality Control materials are exempt from premarket notification subject to the limitations under 21 CFR 862.9.
** GKZ was the product code for flow cytometer, reagents, controls and software until new product code OYE which is specific to Flow Cytometric products was established.

## Device Description

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

- AQUIOS CL Flow Cytometer
- AQUIOS STEM Software
- AQUIOS STEM Kit Reagent Kit
- AQUIOS STEM CD34 Control Cells

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

AQUIOS STEM Kit contains the following reagents:

- AQUIOS STEM Kit CD45-FITC / CD34-PE – 2 vials
- AQUIOS STEM Kit CD45-FITC / IsoClonic Control-PE – 1 vial
- AQUIOS STEM Kit 7-AAD Viability Dye – 3 vials
- AQUIOS STEM Kit Lysing Solution – 3 vials
- AQUIOS STEM Kit STEM-Count Fluorospheres – 2 vials

The AQUIOS STEM Kit is designed to identify human hematopoietic stem and progenitor cells (HPC) using the following criteria: “True CD34+ cells (a) express CD34 antigen, (b) express CD45 antigen with staining intensity characteristic of blast cells (i.e., readily detectable but at lower levels than lymphocytes and

monocytes), and (c) exhibit low side-angle and low to intermediate forward angle light scatter characteristics of blast cells". Exclusion of dead CD34+ HPC from viable CD34+ HPC enumeration is achieved using the 7-AAD Viability Dye.

The AQUIOS STEM Software is designed for the AQUIOS CL flow cytometer. It includes the algorithms and test definitions that provide automated analysis and results using AQUIOS STEM reagents. AQUIOS STEM Software automatically generates gates and regions based on ISHAGE Guidelines to identify populations of interest. The software enables enumeration of Viable CD34+ and CD45+ cell count based on the identified population of interest and AQUIOS STEM-Count Fluorophore counts.

The AQUIOS Flow Cytometry System also offers an optional standalone offline workstation. This workstation is identical to the workstation that is physically connected to the instrument and can be used for off-line analysis of results generated by the AQUIOS CL Flow Cytometer with AQUIOS STEM Kit reagents and AQUIOS STEM software according to the product labeling.

**Intended Use:**

AQUIOS CL Flow Cytometer

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

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

AQUIOS STEM Kit

The AQUIOS STEM Kit is an in vitro diagnostic medical device intended to be used by laboratory professionals for the simultaneous identification and enumeration of percent (%) CD34+ cell population, CD34+ cells/ µL, and CD45+ cells/ µL in fresh peripheral or mobilized peripheral whole blood, fresh or thawed bone marrow, fresh or thawed apheresis products, and fresh or thawed cord blood on the automated AQUIOS CL Flow Cytometry System.

AQUIOS STEM CD34 Control Cells

AQUIOS STEM CD34 Control Cells Level 1 and Level 2 are in vitro diagnostic medical devices intended to be used by laboratory professionals as process controls for the verification of the parameters CD34 and CD45 as part of the AQUIOS STEM System on the automated AQUIOS CL Flow Cytometry System.

AQUIOS STEM CD34 Control Cells are liquid preparations of stabilized human leukocytes (lymphocytes, monocytes, and granulocytes) and erythrocytes that have lysing, light scatter, antigen expression, and antibody staining properties representative of those found in human whole blood specimens.

The kit contains 2 levels of CD34 positive cell controls with approximately 10 CD34+ cells/µL (level 1) and approximately 30 CD34+ cells/µL (level 2) that are processed in the same manner as whole blood. This allows verification of application performance. AQUIOS STEM CD34 Control Cells Level 1 and Level 2 are quantitative controls used in combination with the AQUIOS STEM Kit as part of the AQUIOS STEM System.

## Substantial Equivalence Comparison between Subject and Predicate Devices

In this submission, AQUIOS STEM Kit System with AQUIOS CL Flow Cytometer (subject device) is compared with Stem Kit Reagents and stemCXP system with the FC500 Flow Cytometer (predicate device). A comparison of the similarities and differences between the subject device and the predicate device is presented in Table 1.

### Table 1 Comparison Between Subject and Predicate Device

| | Predicate | Subject Device | Comments |
|---|---|---|---|
| **Characteristic** | **Stem Kit Reagents and stemCXP system with the FC500 Flow Cytometer** | **AQUIOS STEM Kit System with AQUIOS CL Flow Cytometer** | |
| Intended Use | Stem-Kit Reagents consist of a two-color fluorescent (FITC, PE) murine monoclonal antibody reagent, a two-color murine fluorescent (FITC, PE) isoclonic control, an absolute count reagent, a cell viability reagent, and a lysing reagent. It is intended "For In Vitro Diagnostic Use" for the simultaneous identification and enumeration of CD45+ and dual-positive CD45+ CD34+ cell population percentages and absolute counts in biological specimens by flow cytometry. Biological specimens include fresh normal or mobilized peripheral whole blood, and fresh or thawed apheresis products, cord blood and bone marrow. Cell population measurements may be obtained using either an automated method or a manual method for gating and analysis on the FC500.<br><br>Refer to this Stem-Kit Reagents package insert for complete instructions if using the manual method. Refer to the stemCXP System Guide provided with the stemCXP 2.0 software kit and stemCXP version 2.0, WIN 7 for complete instructions if using the automated method. | The AQUIOS STEM Kit is an in vitro diagnostic medical device intended to be used by laboratory professionals for the simultaneous identification and enumeration of percent (%) CD34+ cell population, CD34+ cells/ µL, and CD45+ cells/ µL in fresh peripheral or mobilized peripheral whole blood, fresh or thawed bone marrow, fresh or thawed apheresis products, and fresh or thawed cord blood on the automated AQUIOS CL Flow Cytometry System. | Subject Device intended use reflects the use of AQUIOS STEM system on AQUIOS CL flow cytometer. Rest of the intended use is updated for clarity. Context of use remains the same. |
| Device Classification | Stem CXP System on FC500 Flow Cytometer: Class II, 864.5220 | AQUIOS CL Flow Cytometer System: Class II, 864.5220 | Same |

| Characteristic | Predicate<br>Stem Kit Reagents and stemCXP system with the FC500 Flow Cytometer | Subject Device<br>AQUIOS STEM Kit System with AQUIOS CL Flow Cytometer | Comments |
|---|---|---|---|
| | Stem-Kit Reagent: Class II, 864.5220<br>Stem-Trol Cells: 21 CFR 864.8625 | AQUIOS STEM Kit: Class II, 864.5220<br>AQUIOS STEM Control Cells: 21 CFR 864.8625 | |
| Product Code | Stem CXP System on FC500 Flow Cytometer: GKZ<br>Stem-Kit Reagent: GKZ<br>Stem-Trol Cells: JPK | AQUIOS CL Flow Cytometer System: OYE<br>AQUIOS STEM Kit: OYE<br>AQUIOS STEM Control Cells: JPK | GKZ was the product code for flow cytometer, reagents, controls and software until new product code OYE which is specific to Flow Cytometric products was established. |
| **Instrument Similarities and Differences** | | | |
| Sample Analysis | • Principle of analysis – Flow cytometry<br>• Detection hardware – Lasers, fluidics, optics, electronics<br>• Sample analysis pathway | Same | Same |
| Optics | Laser light delivered by mirrors, prisms, and lenses | Same | Same |
| Workstation | Software functionality to allow –<br>• Patient data management – storage, review, reporting to LIS<br>• Control data management – storage, review, reporting<br>• System configuration management<br>• System service test and adjustment procedures | Same | Same |
| System Configuration | FC500 System with PC-based workstation running stemCXP application-specific software | AQUIOS System with Reagent Cart and All-in-One Touch Screen Computer loaded with AQUIOS System Software, Database, and AQUIOS STEM Software | Substantially Equivalent |
| Operating System | Microsoft Windows 7 | Microsoft Windows 10 | Substantially Equivalent |
| Controlling software | CXP System Software | AQUIOS System Software | Substantially Equivalent |

| | Predicate | Subject Device | Comments |
|---|---|---|---|
| **Characteristic** | **Stem Kit Reagents and stemCXP system with the FC500 Flow Cytometer** | **AQUIOS STEM Kit System with AQUIOS CL Flow Cytometer** | |
| Lasers | Blue – 488 nm argon ion Laser | Blue – 488 nm Blue Laser | Substantially Equivalent |
| Optics | FC500 flow cell<br>Laser light delivered by mirrors, prisms, and lenses. Emitted light delivered by mirrors | AQUIOS flow cell<br>Laser light delivered by mirrors, prisms, and lenses. Emitted light delivered by mirrors | Substantially Equivalent |
| Sample Preparation | Manual | Automated On-board | Substantially Equivalent |
| Specimen Introduction | Manual | Specimens are loaded in the Autoloader using cassettes / or the single loader as single specimen tubes | Substantially Equivalent |
| Prepared Sample Introduction | Single 12X75 mm test tubes | 96 well plate | Substantially Equivalent |
| Specimen Identification | Manual entry into Worklist | Barcode – positive sample identification or manual entry | Substantially Equivalent |
| Prepared Sample Identification | Worklist and Carousel position | Software tracked by well position. | Substantially Equivalent |
| Standardization | • Automatic voltage/gain adjustments<br>• Compensation matrix calculation | A verification check of standardization is performed with Flow Check and controls | Substantially Equivalent |
| Sample Analysis | • Principle of analysis – Flow cytometric Detection hardware: Lasers, fluidics, optics, electronics.<br>Sample analysis pathway: Manual gating of cellular populations per instrument IFU or **stemCXP** automated gating of cellular populations based on the ISHAGE gating strategy | Same<br>• Principle of analysis - Flow cytometric Detection hardware: Lasers, fluidics, optics, electronics.<br>Sample analysis pathway: Automated gating of cellular population by **AQUIOS STEM System Software**. The software only allows gates/region readjustments by the user. Based on the ISHAGE gating strategy | Same |
| Count Method | Cellular events are compared to Stem Count Fluorospheres events.<br>Automated method: Absolute counts are determined by FC500 stemCXP | Same<br>Absolute counts are determined by AQUIOS STEM Software | Same |

| Characteristic | Predicate<br>Stem Kit Reagents and stemCXP system with the FC500 Flow Cytometer | Subject Device<br>AQUIOS STEM Kit System with AQUIOS CL Flow Cytometer | Comments |
|---|---|---|---|
| Photomultipler Tubes (PMTs) / Colors | • Forward Scatter – Solid state detector<br>• Side Scatter Detector – photodiode<br>• 4 Fluorescent Detectors –Photomultipliers<br>  ○ 525nm BP Filter<br>  ○ 575nm BP Filter<br>  ○ 620nm BP Filter<br>  ○ 675nm BP Filter | • FS Solid State Detector<br>• SS Solid State Detector – (488LP Dichroic)<br>• 4 Fluorescent Detectors<br>  ○ 525nm BP Filter<br>  ○ 575nm BP Filter<br>  ○ 620nm BP Filter<br>  ○ 675nm BP Filter | Substantially Equivalent |
| Detectors/Parameters | Six (FS, SS, FL1-FL4) | Seven (EV, FS, SS, FL1 – FL4) | Substantially Equivalent |
| Off-line analysis software | Analysis of Listmode files with stemCXP | Stand-alone workstations with AQUIOS STEM Software to review data offline | Substantially Equivalent |
| **Reagent and Controls Similarities and differences** | | | |
| Reagent Form | Liquid form | Same<br>Liquid form | Same |
| Storage Conditions | 2 – 8 °C | Same except for lysing solution storage<br>2 – 8 °C<br>Lysing solution to be stored at 18-25°C upon receipt | Substantially Equivalent |
| Set-up Reagents<br><br>Standardization | Flow Set | Set at installation by Service. Requires only standardization verification via the AQUIOS STEM Controls. | Substantially Equivalent |
| Verification of Optical alignment and fluidics | Flow Check Fluorospheres | Same<br>Flow Check Fluorospheres | Same |
| Control cell assay parameter | One Control Level: Positive | Two Control Levels | Substantially Equivalent |
| Reagent Assay | Stem Reagent Kit:<br>• CD45-FITC / CD34-PE<br>• CD45-FITC / IsoClonic Control-PE<br>• Stem-Count Fluorospheres.<br>• 7-AAD Viability Dye<br>• 10X NH4Cl Lysing Solution | Same except for lysing solution<br>AQUIOS STEM Reagent Kit:<br>• AQUIOS STEM Kit CD45-FITC / CD34-PE<br>• AQUIOS STEM Kit CD45-FITC / IsoClonic Control-PE | Substantially Equivalent |

| Characteristic | Predicate<br>Stem Kit Reagents and stemCXP system with the FC500 Flow Cytometer | Subject Device<br>AQUIOS STEM Kit System with AQUIOS CL Flow Cytometer | Comments |
|---|---|---|---|
| | | • AQUIOS STEM Kit STEM-Count Fluorospheres<br>• AQUIOS STEM Kit 7-AAD Viability Dye<br>• AQUIOS STEM Kit Lysing Solution | |
| Clones | Specificity CD45 CD34<br>Clone J33 581 | Same<br>Specificity CD45 CD34 Clone J33 581 | Same |
| Sample Preparation with Monoclonal Antibodies | Manually prepared by pipetting and adding specimen to reagents, incubating, lysing, adding Fluorospheres | On-Board Automated Preparation of the same steps as the predicate. | Substantially Equivalent |
| Lyse Reagents | Ammonium Chloride NH4CL | AQUIOS STEM Kit Lysing Solution | |
| Reagents for Color Compensation | Quick Comp 2 | Set at installation by Service and verified with the AQUIOS STEM control cells | Substantially Equivalent |
| Closed Vial Reagent Stability | 365 Days | 325 Days | Substantially Equivalent |
| Open Vial Reagent Stability Claim | 30 Days | 75 Days | Substantially Equivalent |
| On Board Stability | N/A | 70 Hours | N/A |
| Specimen Stability | • Whole Blood, and Mobilized Whole Blood - 20 hours at room temperature (18 - 25°C)<br>• Cord Blood - 24 hours at room temperature (18 - 25°C)<br>• Apheresis products and Bone Marrow - 24 hours at 2- 8°C | • Whole Blood, Mobilized Whole Blood, and Cord Blood - 24 hours at room temperature (18 - 25°C)<br>• Bone Marrow and Apheresis 24 hours at 2- 8°C | Substantially Equivalent |
| Prepared Specimen Stability | • 60 minutes on ice after the addition of Stem Count Fluorospheres for Apheresis, Cord Blood, Bone Marrow Samples<br>• 45 minutes on ice after the addition of Stem Count Fluorospheres for Whole Blood and Mobilized Whole Blood | Prepared Specimen Stability not applicable. After Lyse incubation of 15 - 20 minutes, the AQUIOS STEM Fluorospheres are added, and the sample is immediately analyzed | N/A |
| **Software Similarities and differences** | | | |
| System Configuration | FC500 System with PC-based workstation running stemCXP application-specific software | AQUIOS System with Reagent Cart and All-in-One Touch Screen Computer workstation running AQUIOS STEM application-specific software | Substantially Equivalent |

| | Predicate | Subject Device | Comments |
|---|---|---|---|
| **Characteristic** | **Stem Kit Reagents and stemCXP system with the FC500 Flow Cytometer** | **AQUIOS STEM Kit System with AQUIOS CL Flow Cytometer** | |
| Operating System | Microsoft Windows WIN7 | Microsoft Windows WIN10 | Substantially Equivalent |
| Programming Language | C++ | C#<br>VB.Net | Substantially Equivalent |
| Workstation Interface | USB to fiber optic | USB to Serial | Substantially Equivalent |
| Data Acquisition | Digital Pulse Processing | SAME | Same |
| General | Software functionality to allow:<br>• Patient data management – storage, review, reporting<br>• Control data management – storage, review, reporting<br>• System configuration management<br>System service test and adjustment procedures. | SAME | Same |
| Data Processing | • Region/gates evaluation<br>• Statistics generation<br>Export of results to MS Excel/PDFfile | SAME | Same |
| Standardization | • Automatic voltage/gain adjustments | Automatic gain calibration using target value | Substantially Equivalent |
| User interface | • Plots and reports | SAME | Same |
| Control Software | • The FC500 acquisition software enables the user to acquire data from the cytometer and to analyze, display, print and export acquired listmode data. The embedded software resides in the FC500 instrument. It controls the instrument functionality including the multi-carousel loader (MCL) for sample introduction.<br>• The embedded software controls the instruments' lasers, acquisition system and fluidics. The instrument fluidics aspirates the sample and performs instrument maintenance functions such as startup/shutdown. The embedded software also captures and provides the data to the workstation for processing. | • The Aquios software enables the user to acquire data from the Aquios flow cytometer instrument and to analyze, display, print and export acquired fcs file.<br>• The Aquios software controls the instrument functionality including the cassettes multi-loader for sample introduction.<br>• The Aquios software controls the instruments' lasers, acquisition system and fluidics. The instrument fluidics aspirates the sample and performs instrument maintenance functions such as startup/shutdown. | Substantially Equivalent |

| Characteristic | Predicate<br>Stem Kit Reagents and stemCXP system with the FC500 Flow Cytometer | Subject Device<br>AQUIOS STEM Kit System with AQUIOS CL Flow Cytometer | Comments |
|---|---|---|---|
| | | The Aquios Software also captures and provides the data to the workstation for processing. | |
| Data Processing | • Region and Gate processing<br>• Statistics Generation<br>• Export to Excel<br>○ Incoming data stream formatted into per-parameter vectors for improved performance of lin/log data transformations and compensation calculations | SAME | Same |
| User Account Control | • Multiple role assignments<br>• User account information stored in password protected database. | SAME | Same |
| Report generator | • Panel reports & QC | SAME | Same |

## Summary of AQUIOS CL Flow Cytometer Performance Testing

| Study | Testing Approach | FDA Guidance Documents | Standards/ References | Testing Results |
|---|---|---|---|---|
| Instrument Linearity | Verify fluorescence detection is linear using standard AQUIOS CL settings. | None | None | Linearity of fluorescence measurements was demonstrated. |
| Laser Performance Characteristics | Verify stability of the laser performance of the AQUIOS CL flow cytometer over time. | None | None | Analysis of the data collected demonstrates that the AQUIOS CL laser performance is stable over time. |
| Instrument Carryover | To verify instrument carryover using SPHERO AccuCount Ultra-Rainbow Fluorescent Particles meets performance specifications. | Special Controls Guidance Document: Premarket Notifications for Automated Differential Cell Counters for Immature or Abnormal Blood Cells - Carryover (Section 12) | CLSI H26-A2; FDA Standards Recognition #7-210 | Analysis of the data collected demonstrates that the AQUIOS CL meets the whole blood carryover performance requirements. |

## Summary of AQUIOS STEM Kit Reagents Characterization and Analytical Testing:

| Study | Objective | FDA Guidance Documents | Standards/ References | Testing Results |
|---|---|---|---|---|
| Antibody Specificity | Identify specific antibody clones used for the manufacturing of the AQUIOS STEM Kit Reagents. | None | None | Specific antibody clones that are used in the AQUIOS STEM Kit Reagents are identified. |
| Dose Titration | Determine the optimal dose (in µg/test) of each antibody conjugates and nucleic acid DNA dye 7AAD used in AQUIOS STEM Reagent Kit | None | None | Optimal dosage for antibody conjugates and 7-AAD determined. |

| Study | Objective | FDA Guidance Documents | Standards/ References | Testing Results |
|---|---|---|---|---|
| Antibody Staining and Lyse incubation Range | Verify the acceptable performance of varying antibody reagent and lyse incubation times on the AQUIOS CL Flow Cytometry System when processing multiple samples in parallel. | None | None | Acceptable staining and lyse incubation times verified. |
| Frozen specimen handling | Define the maximum time between the thawing of a frozen sample and the sample processing with the AQUIOS STEM SYSTEM on the AQUIOS CL Flow Cytometer | None | None | The study established the maximum time between the thawing of a frozen sample and the sample processing with the AQUIOS STEM system. |
| Lot to Lot Reproducibility - Reagent | Verify acceptable variability of multiple lots of AQUIOS STEM Kit | None | CLSI EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Third Edition. FDA Standards Recognition #7-251 | Analysis of the data collected demonstrates that the AQUIOS STEM Kit reagents have acceptable lot variability performance. |
| Lot to Lot Reproducibility – Control Cells | Verify acceptable variability of multiple lots of AQUIOS STEM CD34 Control Cells | None | CLSI EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Third Edition. FDA Standards Recognition #7-251 | Analysis of the data collected demonstrates that the AQUIOS STEM CD34 Control Cells have acceptable lot variability performance. |
| Reagent Stability | Demonstrate the shelf life of the AQUIOS STEM Kit reagents. | None | CLSI EP25-A – Evaluation of Stability of In Vitro Diagnostic Reagents. Approved Guideline. FDA Standards Recognition #7-235 | Analysis of the data collected demonstrates that the AQUIOS STEM Kit reagents meet performance requirements in support of the product's stability claims. |

| Study | Objective | FDA Guidance Documents | Standards/ References | Testing Results |
|---|---|---|---|---|
| Interference | Determine the impact of interfering substances on the results from AQUIOS STEM SYSTEM. | None | CLSI EP07 Edition A3: Interference testing in clinical chemistry. Third edition. April 2018. FDA Recognition Number 7-275<br><br>CLSI EP37: Supplemental Tables for interference testing in clinical chemistry, 1st edition- April 2018.<br>FDA Recognition Number 7-284 | Impact of interfering substances on AQUIOS STEM SYSTEM results determined. |
| Analytical Measuring Interval | Establish the Analytical Measuring Interval for the AQUIOS STEM Kit reagents when analyzed on the AQUIOS CL Flow Cytometry system. | None | • CLSI EP17-A2. Evaluation of the detection capability for clinical laboratory measurement procedures. Approved guideline. FDA Recognition Number 7-233<br>• CLSI EP-06 Ed2 Nov 2020 Evaluation of Linearity of Quantitative Procedures: Approved Guideline. FDA Recognition Number 7-306 | Analytical Measuring Intervals for each of the markers in AQUIOS STEM SYSTEM have been established for use with the AQUIOS CL flow cytometer. |

## Summary of AQUIOS STEM Kit System Performance Testing:

| Study | Testing Approach | FDA Guidance Documents | Standards/ References | Testing Results |
|---|---|---|---|---|
| Assay Linearity | Verify the linear range of absolute count of CD34+ cells/µL and the absolute count of CD45+/µL using the AQUIOS STEM Kit reagents on | Special Controls Guidance Document: Premarket Notifications for Automated Differential Cell Counters for | CLSI EP-06 Ed2 Nov 2020 Evaluation of Linearity of Quantitative Procedures: Approved Guideline. FDA Recognition Number 7-306 | Analysis of the data collected demonstrates that the AQUIOS STEM SYSTEM on AQUIOS CL Flow Cytometer meets the linearity performance requirements. |

| Study | Testing Approach | FDA Guidance Documents | Standards/ References | Testing Results |
|---|---|---|---|---|
| | the AQUIOS CL flow cytometer. | Immature or Abnormal Blood Cells - Linearity (Section 11) | | |
| Assay Carryover – Specimen and Reagent | Verify carryover of specimens and reagents on the AQUIOS CL meets performance specifications. | Special Controls Guidance Document: Premarket Notifications for Automated Differential Cell Counters for Immature or Abnormal Blood Cells - Carryover (Section 12) | CLSI H26-A2 Validation, Verification, and Quality Assurance of Automated Hematology Analyzers, Approved Standard - 2nd Edition; June 2010; FDA Standards Recognition # 7-210<br><br>ICSH Guideline Clin Lab Haematol, 16(2):157-174, 1994 | Analysis of the data collected demonstrates that the AQUIOS STEM SYSTEM meets carryover performance requirements. |
| Detection Capability | To establish and verify Limit of Blank (LoB), Lower Limit of Detection (LLoD), Lower Limit of Quantitation (LLoQ) for the AQUIOS STEM Kit reagents when analyzed on the AQUIOS CL instrument. | None | CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition; FDA Standards Recognition #7-233 | Analysis of the data collected established the Limit of Blank, Limit of Detection, and Limit of Quantitation values for each AQUIOS STEM Kit reagent when evaluated on the AQUIOS CL Flow Cytometry System for Detection Capability. |
| Specimen Age Stability and Acceptable Lyse Incubation Duration | Stability of various specimens was evaluated to verify specimen and acceptable lyse incubation duration. | Special Controls Guidance Document: Premarket Notifications for Automated Differential Cell Counters for Immature or Abnormal Blood Cells - Specimens (Section 13) | ICSH Guideline: Am J Clin Path, 100(4)371-372, 1993. | Analysis of the data collected demonstrates that the AQUIOS STEM SYSTEM when analyzed on the AQUIOS CL meet the requirements for specimen and lyse incubation duration. |
| Anticoagulant Equivalency | Demonstrate the equivalent performance of whole blood, mobilized peripheral whole blood, bone marrow, apheresis product, and cord blood specimens when collected in different anticoagulants K2EDTA, K3EDTA, Heparin, | Special Controls Guidance Document: Premarket Notifications for Automated Differential Cell Counters for Immature or Abnormal Blood Cells - Accuracy (Section 8) | CLSI EP09c: Measurement Procedure Comparison and Bias Estimation Using Patient Samples - Third Edition. FDA Standards Recognition 7-296 | Analysis of the data collected demonstrated equivalence of performance of various clinical specimens across anti coagulants when processed with AQUIOS STEM SYSTEM on AQUIOS CL Flow Cytometer. |

| Study | Testing Approach | FDA Guidance Documents | Standards/ References | Testing Results |
|---|---|---|---|---|
| | Heparin/ACD-A mix, ACD-A, and CPD when processed with the AQUIOS STEM Kit on the AQUIOS CL instrument. | | | |
| AQUIOS STEM Test Option equivalency study | Demonstrate the equivalent performance of AQUIOS STEM system in enumerating viable CD34+ cells/µL, percent (%) CD34+ and the count of viable CD45+ cells/µL when the samples are processed across these AQUIOS STEM system analysis options. | None | CLSI EP09c: Measurement Procedure Comparison and Bias Estimation Using Patient Samples - Third Edition | Equivalent performance of the three AQUIOS STEM system analysis options demonstrated. |
| Precision Study using Control Material | Demonstrate system precision for total CD45+ cellsµL), total CD34+ cells/µL and total percent (%) CD34+ using control material as a surrogate for a stabilized sample | Special Controls Guidance Document: Premarket Notifications for Automated Differential Cell Counters for Immature or Abnormal Blood Cells - Precision (Section 9) | CLSI EP5-A3, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Third Edition.<br>FDA Standard Recognition # 7-251 | Analysis of the data collected demonstrates that the AQUIOS STEM SYSTEM on AQUIOS CL meets performance requirements for repeatability and reproducibility using control material. |
| Precision Study using Clinical Specimens (Specimen Repeatability) | Demonstrate assay precision for viable CD45+ cells/µL, viable CD34+ cells/µL and viable percent (%) CD34+ using clinical specimens | Special Controls Guidance Document: Premarket Notifications for Automated Differential Cell Counters for Immature or Abnormal Blood Cells - Precision (Section 9) | CLSI H26-A2 Validation, Verification, and Quality Assurance of Automated Hematology Analyzers, Approved Standard – 2nd Edition; June 2010; FDA Standards Recognition # 7-210 | Analysis of the data collected demonstrates that the AQUIOS STEM SYSTEM on AQUIOS CL meets repeatability performance requirements for clinical specimens. |

| Study | Testing Approach | FDA Guidance Documents | Standards/ References | Testing Results |
|---|---|---|---|---|
| Precision Study using Clinical Specimens (frozen apheresis) | Demonstrate site-to-site system precision for viable CD34+ cells/µL using frozen apheresis specimens | Special Controls Guidance Document: Premarket Notifications for Automated Differential Cell Counters for Immature or Abnormal Blood Cells - Precision (Section 9) | CLSI EP5-A3, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Third Edition.<br>FDA Standard Recognition # 7-251 | Analysis of the data collected demonstrates that the AQUIOS STEM Kit on AQUIOS CL meets performance requirements for repeatability and reproducibility for frozen apheresis samples. |
| Measurement Procedure Comparison (Method comparison to predicate) | Demonstrate the substantial equivalency of the AQUIOS STEM System for the simultaneous identification and enumeration of viable CD45+ cells/µL, viable CD34+ cells/µL and viable percent (%) CD34+ | Special Controls Guidance Document: Premarket Notifications for Automated Differential Cell Counters for Immature or Abnormal Blood Cells - Accuracy (Section 8) | CLSI EP09c: Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guidelines – Third Edition #7-296 | Analysis of the data collected demonstrates that the AQUIOS STEM Kit reagent shows acceptable substantial equivalence as compared to the predicate stemCXP SYSTEM. |
| Reference Interval | Establish adult reference interval for viable CD34+ cells/µL, viable percent (%) CD34+, and viable CD45+ cells/µL on the AQUIOS STEM Kit system. | Special Controls Guidance Document: Premarket Notifications for Automated Differential Cell Counters for Immature or Abnormal Blood Cells – Reference Values (Section 14) | CLSI EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory. Third Edition FDA Standard Recognition # 7-224 | Adult reference intervals were established. |

**Substantial Equivalence Conclusion to Demonstrate Safety, Effectiveness & Equivalent Performance to Predicate:**

In conclusion, the AQUIOS STEM System as described in this submission is substantially equivalent in terms of safety and effectiveness to its predicate devices.

This summary of safety and effectiveness is being submitted in accordance with the requirements of the Safe Medical Device Act of 1990 and the implementing regulation 21 CFR 807.92.