Daily Quality Control on the AQUIOS CL System for the Tetra Application

BECKMAN COULTER Life Sciences

Daily Quality Control on the AQUIOS CL System for the Tetra Application

INTRODUCTION

Flow cytometry, especially immunophenotyping by flow cytometry, is used routinely in the diagnosis, prognosis and monitoring of disease. Since cytometry results are used to make clinical decisions, it is essential to maintain the highest quality of all tools, processes, and reagents. Ensuring that all system functions are working accurately and precisely is a process called «Quality Control» (QC) by which scientists review the influence of all factors involved and take corrective actions when appropriate. QC is critical and is therefore an integral part of daily standard operating procedures (SOPs). SOPs are essential to delivering technically sound, reliable, and quality data that clinicians use to make decisions about their patients. The AQUIOS CL flow cytometer has several features that make it a robust clinical instrument which can be used to monitor and help patients. Due to its innovative design, the AQUIOS CL flow cytometer requires no daily manual setup and the QC process is significantly simpler and faster than traditional QC methods.

CURRENT SETUP AND QC METHODS FOR CLINICAL FLOW CYTOMETRY SYSTEMS

For conventional flow cytometers, setup or standardization usually requires checking voltages, gains, and compensation values before Quality Control (QC) is performed. The AQUIOS CL flow cytometer eliminates the need for daily setup. Quality Control is a set of processes that are used to help confirm that the results of a measurement are accurate and consistent on a daily basis and that the system is functioning properly. The primary goals of QC are to minimize variables and to ensure identical analysis conditions with each and every measurement. In flow cytometry, the three elements of QC that require verification for clinical immunophenotyping include [1, 2]:

1. Instrument performance
2. Reagents and standards
3. Sample preparation

Each of these factors needs to be understood and controlled.

RESULTS

1. Instrument Performance

A flow cytometer is a complex instrument comprised of electronics, fluidics, software, and precision optics. Rigorous QC steps monitor instrument performance and perform repeated validation to assure consistent results. It is important to monitor instrument performance daily and between sample runs whenever possible to ensure test results are accurate and reproducible.

The three key areas to understand and monitor in instrument performance include optical stability, fluorescent linearity and separation:

i. Instrument optical stability is a product of optical alignment and robust fluidics, which enables accurate and reproducible optical measurements of cells in the sample fluid stream.

ii. Fluorescent linearity is the behavior of the cytometer in which the reported cellular intensity values are in direct proportion to the actual intensity values, within a pre-defined linear range.

iii. Separation is a function of sensitivity and resolution. Sensitivity is the ability of the instrument to distinguish very low levels of light scatter and fluorescence from background light or electronic noise.[3,4] Resolution is the ability to measure two particles with the same quantity of fluorescence and assign them the same value.

All three performance characteristics are measured and monitored in a conventional cytometer with the help of uniformly sized fluorospheres of known fluorescent intensities.

OPTICAL STABILITY: Optical stability is typically monitored frequently as it is dependent on optimized performance of fluidics and optics. Sometimes, minor, unrelated and uncontrolled environmental factors can affect the alignment of the sample in the optical path leading to inconsistent or irreproducible results. Fortunately, optical stability can be easily measured with defined samples/ fluorospheres and can be normalized. Fluorescent optical stability is checked by running multiple sets of fluorospheres through the cytometer. Mean and Standard Deviation (SD) are calculated using the results from the multiple fluorosphere runs. Once mean and SD are determined, Coefficient of Variation (CV) can be calculated using SD/mean x 100. CV is the percentage ratio of the standard deviation to the mean.

In the AQUIOS CL flow cytometer, optical stability is monitored with AQUIOS IMMUNO-TROL Cells and AQUIOS IMMUNO-TROL Low Cells. These controls reduce multiple fluorosphere and control based steps into two control runs: AQUIOS IMMUNO-TROL Cells/AQUIOS IMMUNO-TROL Low Cells. Additionally, AQUIOS IMMUNO-TROL Cells and AQUIOS IMMUNO-TROL Low Cells are more physiologically representative of a whole blood sample than fluorospheres.

FLUORESCENT LINEARITY: The primary determinant of fluorescent linearity is the photomultiplier tube (PMT) voltage since digital electronics are very linear. Fluorescent linearity is measured by the changing PMT voltages over the entire range of measurement. This measurement determines the optimal range of the fluorescence scale, which is proportional and used to calculate the correct compensation values. It is possible to measure the fluorescent linearity of a flow cytometer by using a mixture of pre calibrated particles with known mean equivalent soluble fluorescence (MESF) units. Fluorescent linearity is not typically monitored daily.

In the AQUIOS flow cytometry system, the user is not required to check linearity, but it should be checked by service after replacement of a PMT, after major changes to the instrument, or during routine service.

SEPARATION: Separation is a function of both sensitivity and resolution. Traditional flow cytometers typically focus on resolution as the primary determinant of ideal separation. On these instruments, resolution is measured daily using fluorescent microspheres with uniform size and fluorescent intensity. Sensitivity, however, is not typically monitored daily.

In the AQUIOS CL flow cytometer, separation (both sensitivity and resolution) are monitored daily with the use of AQUIOS IMMUNO-TROL Cells controls and tracked using their Separation Quotient (SQ) values. SQ is the tool the system uses to quantify separation. Refer to Separation Quotient Tracking on page 13, for more information on Separation Quotient and the Separation Quotient statistics.

With the AQUIOS CL flow cytometry system, QC checks associated with instrument performance essentially follow the same principles that are behind the fluorosphere based QC checks performed by general purpose flow cytometers. The key difference is that the AQUIOS CL flow cytometer is designed to perform these QC checks automatically without the need for additional reagents. This helps to simplify the process making QC checks associated with instrument quick and efficient.

The AQUIOS CL flow cytometer, in conjunction with AQUIOS IMMUNO-TROL Cells and AQUIOS IMMUNO-TROL Low Cells, automatically verifies light scatter, electronic volume (EV)[5], fluorescence intensities and color compensation settings for defined assays.

Below are some examples of excellent separation, good separation, moderate separation and poor separation. Refer to Separation Quotient Tracking on page 13, for more information on Separation Quotient and the Separation Quotient statistics used to quantify excellent, good, moderate, and poor separation. Note that each peak represents a different cell population.

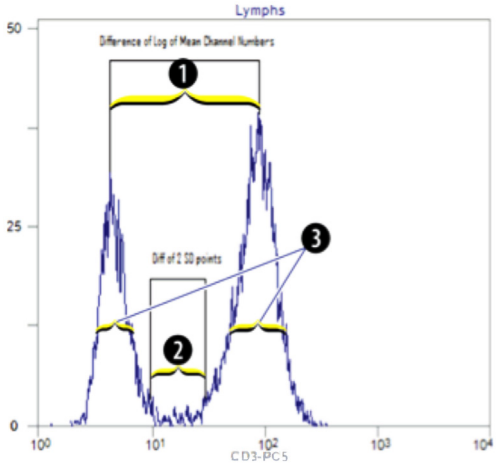
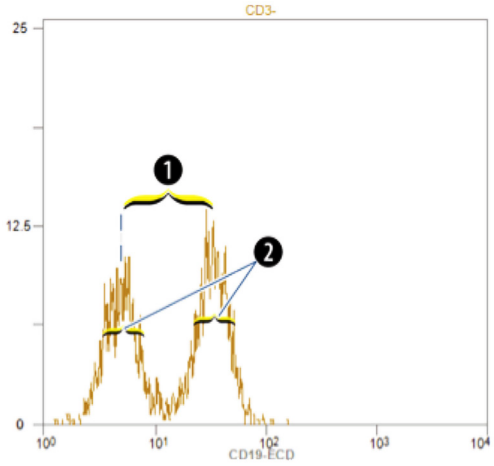


Figure 1 – Separation Quotient = 4.7 (excellent separation)

Note: Figure 1 is representative of excellent separation because it displays both good sensitivity and good resolution. The logarithmic difference of the mean channel numbers (1) is a difference greater than two standard deviation points(2), indicating good sensitivity. In addition, the two cell populations have little variance in the values assigned to each cell population's distinct quantity of fluorescence (3), indicating good resolution.

Figure 2 – Separation Quotient = 2.3 (good separation)

Note: Figure 2 is representative of good separation and displays good sensitivity, but poor resolution. The logarithmic difference of the mean channel numbers (1) is greater than two standard deviation points, indicating good sensitivity. However, the two cell populations have more variance in the values assigned to each cell population's distinct quantity of fluorescence (2), indicating poor resolution.



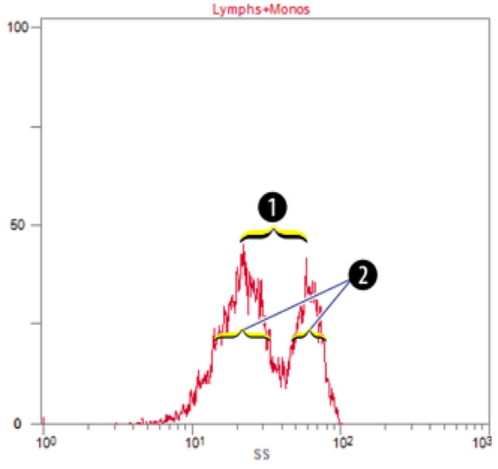


Figure 3 – Separation Quotient = - 0.79 (moderate separation)

Note: Figure 3 is representative of moderate separation and displays moderate sensitivity and moderate resolution. The logarithmic difference of the mean channel numbers (1) is less than two standard deviation points, indicating moderate sensitivity. In addition, the two cell populations have moderate variance in the values assigned to each cell population's distinct quantity of fluorescence (2), indicating moderate resolution.

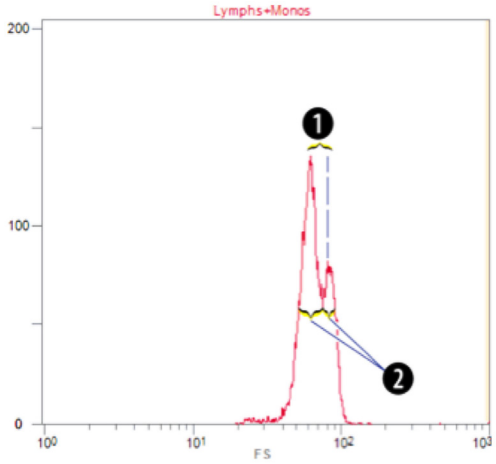


Figure 4 – Separation Quotient = - 4.7 (poor separation)

Note: Figure 4 is representative of poor separation and displays good resolution, but poor sensitivity. The logarithmic difference of the mean channel numbers (1) is less than two standard deviation points, making it difficult to distinguish between the two cell populations, indicating poor sensitivity. However, the two cell populations have little variance in the values assigned to each cell population's distinct quantity of fluorescence (2), indicating good resolution.

2. Reagents and Standards

In a traditional flow cytometer, after an instrument passes instrument performance checks, it is important to verify the system performance with the help of a normal QC material. Positive process controls can also be used to check compensation settings. On current systems, compensation is manually checked on a daily basis. However, the AQUIOS CL flow cytometer uses a simpler and faster method of process control. All control and patient runs are automatically checked for compensation without the need for user initiation. While compensation can be adjusted on the AQUIOS CL flow cytometer, it is typically only set during the manufacturing process, during installation, and after major service.

The AQUIOS CL flow cytometer uses two levels of process control: AQUIOS IMMUNO-TROL Cells and AQUIOS IMMUNO-TROL Low Cells. AQUIOS IMMUNO-TROL Cells and AQUIOS IMMUNO-TROL Low Cells are assayed, lysable whole blood quality control products for immunophenotyping analysis using monoclonal antibody reagents and flow cytometry. AQUIOS IMMUNO-TROL Cells and AQUIOS IMMUNO-TROL Low Cells provide positive cell controls that are processed in the same manner as a whole blood sample. This allows verification of instrument and reagent performance. It also verifies the methods used for staining targeted cells, the lysing of erythrocytes, and the analysis of samples by the AQUIOS CL flow cytometer.

Immunophenotyping analysis using flow cytometry involves the identification and enumeration of targeted cells in whole blood samples. Whole blood samples are stained with monoclonal antibodies and erythrocytes are lysed prior to flow cytometric analysis. A positive cell control is required to verify reagent performance, sample preparation methods, and staining procedures.[6,7] A positive cell control should mimic a representative whole blood sample in terms of monoclonal antibody performance, erythrocyte lysing, and flow cytometric analysis.

AQUIOS IMMUNO-TROL Cells and AQUIOS IMMUNO-TROL Low Cells are a liquid preparation of stabilized human erythrocytes and leukocytes (lymphocytes, monocytes, and granulocytes) that have lysing, light scatter, antigen expression, and antibody staining properties representative of those found in human normal whole blood.

In flow cytometry, Levey Jennings (LJ) charts are used to easily visualize quality control data from run to run or day to day tests. [8]. In LJ plots, the dates of analysis or the number of the control runs are plotted along the x axis with the control values plotted along the y axis. The mean and three standard deviation limits are marked on the y axis. Inspection of the plotted points provides a simple way to detect increased random error and shifts or trends in performance or calibration in the present QC run as well as any day to day variation from previous runs.

The AQUIOS software automatically records and plots the measured values from the various QC runs to three LJ plots for visualization and interpretation: results, instrument and instrument drift. A separate LJ chart is created for each QC value using standard deviation.

AQUIOS Tetra Tests

AQUIOS Tetra tests are indicated for use in immunologic assessment of patients having, or suspected of having, immune deficiency. These tests depend on the ability of a monoclonal antibody to bind to the surface of cells expressing discrete antigenic determinants. Specific cell staining is accomplished by incubating whole blood with the monoclonal antibody reagent. The AQUIOS CL flow cytometer provides the following tests:

AQUIOS Tetra-1 Panel: CD45 FITC/CD4 RD1/CD8 ECD/CD3 PC5. These reagents provide identification and enumeration of total CD3+, CD3+CD4+, CD3+CD8, CD4:CD8 (ratio only), lymphocyte percentages and absolute counts in peripheral whole blood, CD45+ absolute count and CD45+ Low SS (lymphocytes) percentage and absolute count. The CD3+ reliability check is also monitored with this panel.

AQUIOS Tetra-2+ Panel: CD45 FITC/(CD56+CD16) RD1/CD19 ECD/CD3 PC5. These reagents provide identification and enumeration of total CD3+, CD3 CD19+, CD3 CD56+ and/or CD16+ lymphocyte percentages and absolute counts in peripheral whole blood, CD45+ absolute count and CD45+ Low SS (lymphocytes) percentage and absolute count.

AQUIOS Tetra Combo: Tetra-1 Panel and Tetra-2+ Panel combination.

3. Sample Preparation

Sample preparation in clinical laboratories requires the operator to follow Good Laboratory Practices (GLP) while keeping detailed laboratory records and preparing samples manually, or through separate sample preparation devices. Separate sample preparation devices, although automated, require a user to physically setup the system prior to use and transfer samples between systems making this process semi-automated. Because manual preparation is subject to technician variability, the optical stability, accuracy, and standardization incorporate that variability. Semi automatic preparation processes also have several shortcomings, including manual worklist generation and sample tracking. Semi automatic systems also require samples to be prepped in batches prior to batch analysis, reducing workflow efficiency in sample preparation. Whether manual preparation or semi automatic preparation are used, both types of sample prep require significant time and attention, increasing the potential for operator error.

In the AQUIOS CL flow cytometer, onboard robotics assure that the sample preparation is consistent and reliable. The system also automatically tracks and maintains records in a centralized location. The automated system is monitored continuously and can notify the operator by email or text in the event of a problem or anomaly.

AQUIOS Tetra-1 or AQUIOS Tetra-2+ Sample Preparation

The method uses 43 µL of whole blood stained with 13 µL of an AQUIOS Tetra 1 Panel or AQUIOS Tetra 2+ Panel monoclonal antibody reagent. After 15 minutes of incubation, the blood is lysed using 335 µL Lysing Reagent A followed by 100 µL Lysing Reagent B. The sample is then aspirated for analysis.

AQUIOS Tetra Combo Sample Preparation

For Tetra Combo, one aspiration from the specimen tube is used to deliver the specimen into two separate wells of the AQUIOS Deep Well Plate. One well is used for AQUIOS Tetra 1 Panel. The second well is used for AQUIOS Tetra 2+ Panel by the method described above.

STARTUP AND QC ON THE AQUIOS CL FLOW CYTOMETER

The AQUIOS CL flow cytometer is an integrated cellular analyzer. It combines automated sample loading, sample preparation and cellular analysis into one compact system. No additional sample preparation, personnel, liquid handling modules or robots are required for tedious and repetitive sample preparation steps. Only a few simple tasks need to be performed before loading specimen tubes and then walking away from the AQUIOS CL flow cytometer. It is not necessary to return to the instrument to transfer samples between preparation and analysis instruments because the AQUIOS CL is capable of performing all of these steps in the precise order and time required by the tests.

The AQUIOS CL flow cytometry system startup consists of a few simple steps as described in detail in the "Instructions for Use" manual (PN B21896). These steps can be summarized as follows:

Table 1 - System Startup and QC Steps: Traditional Flow Cytometer versus AQUIOS CL Flow Cytometer

Steps	Traditional Flow Cytometer	AQUIOS CL Flow Cytometer	Description
Instrument Power Up	Required	Required	Instrument power up is required in both traditional flow cytometers and the AQUIOS CL flow cytometer..
Reagent Management	Mostly Manual	Mostly Automated	**Traditional flow cytometer**: For those cytometers with level sensing capabilities, generally a subset of reagents are tracked. Other cytometers rely on the operator to track this information. **AQUIOS CL flow cytometer**: For most reagents, the system automatically tracks reagent levels and reports reagent level status in real time. [a a] Additionally, the system automatically records and stores information such as lot numbers, expiration dates, date first loaded on the system, etc..
Gains/Voltage Settings Checks and Adjustments	Additional Reagents required	Automated tracking	**Traditional flow cytometer**: Gains and voltage settings are determined by running a fluorosphere based reagent on the system. Gains and voltage settings are adjusted based on these results. **AQUIOS CL flow cytometer**: Gains and voltage settings are factory set. Stability is automatically tracked during QC and patient sample runs.
Optical Alignment Checks	Additional Reagents required	Automated tracking	**Traditional flow cytometer**: Optical alignment is determined by running a fluorosphere based reagent on the system. **AQUIOS CL flow cytometer**: Optical alignment is tracked automatically by the system during QC and patient sample runs.

Steps	Traditional Flow Cytometer	AQUIOS CL Flow Cytometer	Description
Compensation Checks and Adjustment	Additional reagents required	Automated tracking	**Traditional flow cytometer**: Compensation is checked daily and determined by running a fluorosphere based reagent or stained control material on the system. Adjustments are made as necessary. **AQUIOS CL flow cytometer**: Compensation is tracked automatically by the system during QC and patient sample runs. Adjustments are rarely, if ever required.
Sensitivity Testing	Additional reagents required	Automated tracking	**Traditional flow cytometer**: Sensitivity is checked running multi peak fluorospheres on the system. **AQUIOS CL flow cytometer**: Both sensitivity and resolution are tracked automatically as a function of separation with the Separation Quotient during QC and patient sample runs.
Preparation of QC Samples	Manual/ Semi-Automated	Automated (Load & Go System)	**Traditional flow cytometer**: QC samples are either prepared manually or using a separate semi automated prep system. Both methods require operator interaction, increasing the potential for user error. **AQUIOS CL flow cytometer**: The Load & Go system automatically prepares the QC samples eliminating the potential for user error..
Running QC Samples	Required	Required	**Traditional flow cytometer**: Requires the operator to manually load QC samples onto the system and manually enter worklists, introducing the potential for user error. **AQUIOS CL flow cytometer**: Once a QC specimen's bar code is scanned, the system seamlessly moves from preparation to running QC samples because of its unique integrated design and Load & Go functionality..
Analysis of QC Pass/Fail	Manual	Automated	**Traditional flow cytometer**: Many traditional flow cytometers require the operator to manually enter QC results to generate LJ charts in order to review. This introduces the potential for user error. **AQUIOS CL flow cytometer**: The system automatically generates LJ charts and flags failed results and will send a text or email notification to the operator.

[a] The AQUIOS CL Flow Cytometer system tracks the remaining tests and expiration dates for all reagents with the exception of AQUIOS Sodium Hypochlorite Solution, which isn't tracked.

Once system startup is complete, the AQUIOS CL flow cytometer is ready to run patient samples. The first test result after QC is passed will be available in about 20 minutes using AQUIOS Tetra-1 Panel or AQUIOS Tetra-2+ Panel. Subsequent results will be available approximately every two minutes thereafter for the first 96 well plate when running AQUIOS Tetra Combo.

The only QC samples needed are two levels of whole blood process controls, AQUIOS IMMUNO-TROL Cells and AQUIOS IMMUNO-TROL Low Cells. The AQUIOS flow cytometry system automatically measures attributes such as: instrument drift through abrupt changes or trends in mean channel number, fluorescent compensation values and Separation Quotient, a novel measurement defined later in this document.

Load & Go Process

Running samples on the AQUIOS CL flow cytometer with the AQUIOS Tetra panel is a true **Load & Go** process.

First, AQUIOS system startup is simple. There is no need for complex checks and adjustment of instrument settings. QC is performed using just two controls: AQUIOS IMMUNO-TROL Cells and AQUIOS IMMUNO-TROL Low Cells. These two whole blood process control cells are all it takes to ensure that the AQUIOS CL flow cytometer is working accurately and precisely.

Second, once the AQUIOS system QC is completed, the operator can load the samples and walk away to perform other functions in the lab.

Third, the system automatically stains and prepares the cells for analysis. The system does so by adding precise amounts of blood and reagents then mixes and incubates the sample for preset times. Since sample preparation is integrated, there is no need to transfer prepared samples from one module to another as with conventional flow cytometers.

All that is required of the operator is to start the instrument and the software, **load** the controls, reagents and samples, and **go**. The process is automatic and the operator is free to perform other laboratory functions.

WHY DOES THIS WORK ON THE AQUIOS CL FLOW CYTOMETER?

In traditional clinical flow cytometry, optical stability, alignment and sensitivity are measured and monitored periodically using several reagents containing uniform size fluorospheres of known characteristics and known fluorescent intensities. In the AQUIOS CL flow cytometer, the QC process is automated and simplified, but is still as thorough as traditional methods. There are several reasons why this QC process works efficiently and continuously on the AQUIOS CL flow cytometer.

i. **Defined Reagents**: The AQUIOS CL flow cytometer is designed to run pre defined and well characterized tests. Lymphocyte subset analysis tests like Tetra 1, Tetra 2+, and Tetra Combo panel applications are thoroughly tested and pre-configured with instrument settings of PMT voltages, gains, discriminators, and compensation values. The AQUIOS system is designed to offer hands off freedom which is accomplished by providing the user with preconfigured, **Load & Go** protocols. System settings may not be changed by the user with the exception of gating and region adjustments and in rare instances, compensation. When well assayed and characterized fluorescent monoclonal antibody reagents are combined with pre configured instrument settings, the result is a well defined assay that is stable and consistent.

ii. **Stable instrumentation**: The AQUIOS CL flow cytometer is precisely engineered with closed, fixed optics, which are designed for long term stability.

iii. **Daily QC**: The AQUIOS CL flow cytometer daily routine uses two levels of AQUIOS IMMUNO-TROL AQUIOS IMMUNO-TROL Cells and AQUIOS IMMUNO-TROL Low Cells rather than both controls and fluorospheres to verify cytometer performance. Seven parameters including, forward scatter, EV, side scatter and four independent fluorescent parameters make monitoring the controls an exact and accurate process. A compensation check is also performed simultaneously.

iv. **Separation Quotient tracking**: *Separation Quotient* (SQ) is a calculated parameter that is specific to the AQUIOS CL flow cytometer and is used as an indicator of relative sensitivity and resolution. SQ is tracked in real time and checks the quality of the sample.

The detailed definition of Separation Quotient can be found in the AQUIOS Tetra System Guide but can be understood as the ratio of channels between the inner edges (2 SD) of two populations AND the channels between the mean of those two populations. The values are then expressed on a scale of <0 to 10, with 10 being the best separation. See Table 2.

Separation Quotient is an indicator for the separation between two different populations; some examples include: positive versus negative cell populations in fluorescence parameters, sample versus debris, or lymphocytes versus monocytes. The larger the separation quotient, the better the separation.

The *Separation Quotient* index is measured continuously for both samples and QC controls to determine whether the cytometer and Tetra panel of reagents are performing optimally for each and every patient sample and control. Table 2 lists the different ranges of *Separation Quotient* and their meaning.

Table 2 - Separation Quotient Statistics

Numeric Range	Implication for Results
3 to 10	Excellent separation such that >99% of the data points in the populations are separated from each other. See Figure 1.
0 to 3	Good separation >2 SD or approximately 95% of the data points in the populations are separated from each other. See Figure 2.
0	Point at which populations of 2 SDs touch approximately 5% of the data points in the populations overlap.
-3 to 0	Moderate separation at which >5% of the data points in the populations overlap. See Figure 3.
< 3	Poor separation which worsens as the numbers decrease. See Figure 4.

v. **Real time tracking and continuous QC**: The AQUIOS CL flow cytometer tracks the changes over time using Separation Quotient, the mean channel of each parameter, absolute counts and cell percentages for the controls. Additional checks are performed on every patient sample as described in Special QC Features. When QC samples are run, the system automatically measures assay values and checks for instrument drift by tracking abrupt changes in mean channel numbers. It also checks Separation Quotients, fluorescence compensation, and event rate /sec in real time.

   The results from running QC are recorded and plotted graphically in LJ plots. Measured values are then compared to AQUIOS IMMUNO-TROL assay values. If they fall outside of the assay ranges, then the system generates appropriate flags and alerts the operator of the QC failure by triggering a pop up error message on screen followed by an optional email and/or text alert.

vi. **Precision syringe for volumetric absolute counting**: AAbsolute count measurement in a conventional flow cytometer is derived by adding reference fluorospheres of known concentration and calculating the ratio of cells to the reference fluorospheres. In the AQUIOS Tetra application, a precise and calibrated syringe dispenses an accurate and fixed volume of liquid in a defined time. This syringe pump delivers a metered amount of specimen over a preset acquisition time eliminating the need for added fluorospheres in the samples.

One of the design goals for the AQUIOS CL flow cytometer was to develop a system that is simple, smart and efficient. Hematology analyzers serve as good models for these characteristics. Hematology analyzers are rugged, consistent, require minimal setup, are precisely calibrated and work robotically to analyze samples continuously without elaborate setup or constant physical monitoring. They also use well defined and tested reagents that enable the system to be run with little variability. Simplification of the QC process by eliminating the use of fluorospheres and utilizing all the novel technologies mentioned above in one platform makes the AQUIOS CL flow cytometer function more like a hematology analyzer in simplicity. This results in less hands on time for the user and the ability to use operators to perform other testing and to use less experienced flow cytometry operators.

SPECIAL QC FEATURES

QC is important when running clinical flow cytometry applications. However, performing QC testing is tedious for the operator and can consume a significant amount of time and a significant amount of reagents. The AQUIOS CL flow cytometer alleviates the problems of repetitive tests, long setup times and reagent waste by requiring QC after instrument startup followed by real time QC during the sample analysis. The AQUIOS CL flow cytometer offers a set of quality control tools with special features like internal QC for patient samples, **Pause for QC**, text and email alerts, and a Smart Track reagent monitoring system.

i. **Internal QC for patient samples**

The QC check of every patient sample happens in real time as the sample is run on the system. For every QC and patient sample, several QC checks are tracked; some of them are listed in Table 3.

Table 3 - Real time QC check points

Tracking	What is tracked	What is accomplished
Mean channel of key cell populations	Lymphocytes and monocytes (Scatter and Fluorescence)	Detects any drift and ensures instrument stability
Event rate/sec	All Events	Monitors flow rate for bubbles or flow irregularity
Compensation	Compensation matrix	Ensures that compensation values are appropriate for the application being performed
Cell debris	Percent debris	Monitors the percent of unlysed cells or unusual contamination or debris in the sample
Separation Quotient	The distance between relevant populations as a means of measuring both system sensitivity and resolution as a function of separation	Ensures that sample preparation, fluidics, and alignment are performing sufficiently

The real time QC check points listed in Table 3 continuously monitor the quality of the analysis during the sample run to ensure that the system is working acceptably even after the initial QC check. These methods of operation are similar to the methodology of a hematology system in terms of ensuring consistent performance and continuous monitoring.

ii. **Laboratory SOP: Pause for QC**

Pause for QC is an important user-selectable feature when creating a test request on the AQUIOS CL flow cytometer's **Load & Go** workflow. If **Pause for QC** is selected, the operator can then load controls and all patient samples at the same time and walk away. The AQUIOS CL flow cytometer will process the controls and NOT process any of the patient samples until the controls are analyzed and the system determines whether QC passed or failed. If it passes QC, the system automatically begins to process the patient samples that are loaded on the instrument. If it fails QC, the system remains paused so that the problem can be corrected before running subsequent samples. Upon failure of QC, the system stops and alerts the operator (via an optional pop up message on screen, email and/or text message) that QC has failed. This allows the operator to return to the instrument and correct the problem. The most common action is to repeat QC runs.

If the user turns off the **Pause for QC** feature, the patient samples will be analyzed irrespective of whether QC passed or failed. Although the operator may choose to select this option to save time, there may be a loss of reagents and patient samples if QC fails.

Ultimately, this feature enables the user to select the benefits of this important **Load & Go** capability based on the laboratory's SOPs.

iii. **Text and email if QC or run fails**

In current flow cytometry laboratories [7, 8], an instrument operator sets up the cytometer and monitors it continuously as the sample is run. This also implies that each operator usually runs only one instrument and must be available to monitor the run from start to finish.

The AQUIOS CL flow cytometer monitors the sample in real time and, if 3 consecutive runs are flagged, it can notify the operator by a text message or an email. Once notified, the operator can address the issue right away. This ability allows an operator to run multiple instruments, perform other tasks in the laboratory as samples are being processed and walk away knowing that the flow cytometer can notify the operator of any significant issues. This is unique to the AQUIOS CL flow cytometer and not available on any other flow cytometer.

SMART-TRACK REAGENT MONITORING

Clinical laboratory SOP's require monitoring of reagents[8,9], keeping records, determining the availability/usage of reagents, writing down procedures followed in preparing the sample and tracking continuously. The AQUIOS CL flow cytometer is engineered with several systems to aid the user and minimize errors in this process.

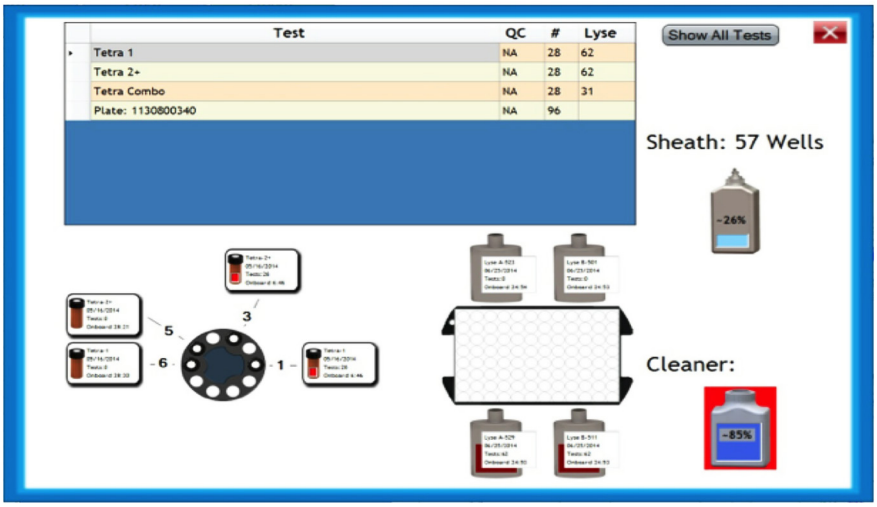
The AQUIOS reagents use a unique barcode identity for tracking the expiration date, on board expiration, lot and container numbers. The reagent consumption and plate usage are monitored by the system as the samples are processed. Updates of new reagents, plate usage, reagent availability and reagent location are displayed after opening and closing the reagent door. This is possible using a barcode on the label, which houses most of this information. The bar code reader scans a reagent or plate upon first use, ensuring that the consumable information is error free. The system considers reagent containers or plates to be at full capacity the first time they are seen by the system.

Note: The AQUIOS CL Flow Cytometer system does not track the remaining tests and expiration dates of the AQUIOS Sodium Hypochlorite Solution. You must track the expiration date of the AQUIOS Sodium Hypochlorite Solution upon use.

The Smart Track reagent monitoring system is only available on the AQUIOS system and is a first in flow cytometry. Onboard reagents, when placed in their designated location, are automatically detected and read by the system. Reagents that are not onboard also carry barcodes that can be presented to the external barcode reader to enter them into the system. The system records all the demographics per reagent (i.e., Identity, lot number, number of tests, number of days until open and close vial expiration, onboard stability and container number.) The Smart Track reagent tracking system provides real time consumable tracking to ensure that reagents with proper dating are used, and that there is sufficient reagent for each sample. All consumables are bar coded and are automatically read into the system when placed onboard or presented to the external barcode reader.

The Smart Track system updates the Reagent Levels screen (see Figure 5) of the AQUIOS system software and gives information about how much volume of reagent is remaining and/or the number of tests remaining, making it easy for the operator to monitor.

Figure 5 - Reagent Levels Screen



CONCLUSIONS

The AQUIOS CL flow cytometer is a true **Load & Go** system. It does not require adjustments or any setup process on a daily basis. The AQUIOS CL flow cytometer's design removes the bulk of tedious sample preparation and instrument setup work allowing the operator to focus on the results.

The AQUIOS CL flow cytometer provides simplicity, speed, and comprehensive QC solutions for running patient samples in routine immunophenotyping analysis with **accuracy, reliability, and consistency**.

The system offers simplification of the QC process by elimination of various types of quality control materials and procedures in clinical laboratories and combining sample preparation, reagent monitoring, tracking, run and analysis into **one platform**.

REFERENCES

1. Owens MA, Vall HG, Hurley AA, Wormsley SB (2000) Validation and quality control of immunophenotyping in clinical flow cytometry. J Immunol Methods. 243(1-2):33-50.
2. Tanqri S, Vall H, Kaplan D, Hoffman B, Purvis N, Porwit A, Hunsberger B, Shankey TV; (2013) ICSH/ICCS Working Group. Validation of cell-based fluorescence assays: practice guidelines from the ICSH and ICCS - part III - analytical issues. Cytometry B Clin. Cytom. 84(5):291-308.
3. Hoffman RA, Wood JCS. (2007). Current protocols in cytometry. New York: John Wiley & Sons, Inc. Section 1.8.
4. Shapiro HM. (2003) Practical flow cytometry fourth edition. New York: Wiley-Liss.
5. Coulter, Wallace H., (1953). Means for counting particles suspended in a fluid, Patent number: 2656508.
6. National Committee for Clinical Laboratory Standards. Clinical Applications for Flow Cytometry: Quality Assurance and Immunophenotyping of Lymphocytes; Approved Guideline. 1998. NCCLS document H42-A.
7. 1997 Revised Guidelines for the Performance of CD4+ T-Cell Determination in Persons with Human Immunodeficiency Virus (HIV) Infection. MMWR, January 10, 1997, p. 11.
8. Levey S., Jennings, E.R., (1950) The use of control charts in the clinical laboratory. Am. J. Clin. Pathol. 20: 1059.
9. Calvelli, T, Denny, TN, Paxton, H, Gelman R, and Kagan, J. (1993) Guideline for Flow Cytometric Immunophenotyping: A Report From The National Institute of Allergy and Infectious Diseases, Division of AIDS, Cytometry 14:702-714.

ACRONYMS

CV:	Coefficient of Variation
EV:	Electronic Volume
GLP:	Good Laboratory Practices
Tetra:	AQUIOS Tetra-1 panel / AQUIOS Tetra-2+ panel / AQUIOS Tetra combo panel
LJ:	Levey-Jennings
MESF:	Mean equivalent soluble fluorescence
PMT:	Photomultiplier tube
QC:	Quality Control
SD:	Standard Deviation
SOP:	Standard Operating Procedures
SQ:	Separation Quotient

TRADEMARKS

CLASS 1 LASER PRODUCT

FLOW-172TCH06.15-C