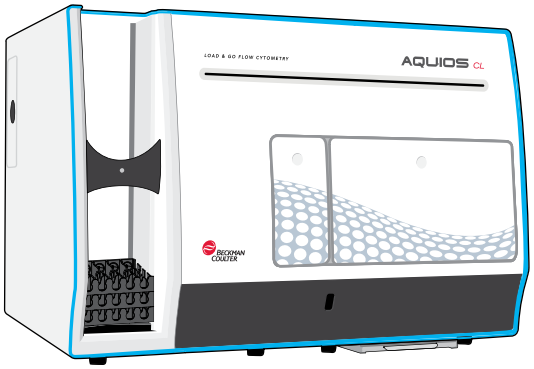
System Guide

AQUIOS Tetra

Rx Only



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BECKMAN COULTER

AQUIOS Tetra Software System Guide
PN B26364AB April 2015

Find us on the World Wide Web at:
www.beckmancoulter.com

Revision History

B26364AA, May 2014
Initial Issue
Software version 1.0

Issue AB, April 2015
Software version 2.0

Updates were made to the following sections:

Introduction

- Before You Begin
- About this System Guide

Chapter 1

- Intended Use
- Consumables and Supplies
- Limitations

Chapter 2

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- Lymphocyte Immunophenotyping Panels
- AQUIOS Tetra-1 Panel
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- Edit Regions for a Review Sample
- Result Screen
- Flags And Notifications

This document applies to the latest software listed and higher versions. When a subsequent software version affects the information in this document, a new issue will be released to the Beckman Coulter Web site. For labeling updates, go to www.beckmancoulter.com and download the latest version of the manual for your instrument.

Chapter 6

- Reference Ranges
- Linearity
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References

This document applies to the latest software listed and higher versions. When a subsequent software version affects the information in this document, a new issue will be released to the Beckman Coulter Web site. For labeling updates, go to www.beckmancoulter.com and download the latest version of the manual for your instrument.

Safety Notice

Read all product manuals and consult with Beckman Coulter-trained personnel before attempting to operate instrument. Do not attempt to perform any procedure before carefully reading all instructions. Always follow product labeling and manufacturer's recommendations. If in doubt as to how to proceed in any situation, contact your Beckman Coulter Representative.

Beckman Coulter, Inc. urges its customers and employees to comply with all national health and safety standards such as the use of barrier protection. This may include, but is not limited to, protective eyewear, gloves, and suitable laboratory attire when operating or maintaining this or any other automated laboratory analyzer.

Alerts for Warning and Caution

⚠ WARNING

WARNING indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury. May be used to indicate the possibility of erroneous data that could result in an incorrect diagnosis.

⚠ CAUTION

CAUTION indicates a potentially hazardous situation, which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices. May be used to indicate the possibility of erroneous data that could result in an incorrect diagnosis.

IMPORTANT IMPORTANT is used for comments that add value to the step or procedure being performed. Following the advice in the IMPORTANT adds benefit to the performance of a piece of equipment or to a process.

NOTE NOTE is used to call attention to notable information that should be followed during use or maintenance of this equipment.

⚠ WARNING

Risk of operator injury if:

- **All doors, covers, and panels are not closed and secured in place prior to and during instrument operation.**
- **The integrity of safety interlocks and sensors is compromised.**
- **Instrument alarms and error messages are not acknowledged and acted upon.**
- **You contact moving parts.**
- **You mishandle broken parts.**
- **Doors, covers, and panels are not opened, closed, removed, and/or replaced with care.**
- **Improper tools are used for troubleshooting.**

To avoid injury:

- **Keep doors, covers, and panels closed and secured in place while the instrument is in use.**
- **Take full advantage of the safety features of the instrument. Do not defeat safety interlocks and sensors.**
- **Acknowledge and act upon instrument alarms and error messages.**
- **Keep away from moving parts.**
- **Report any broken parts to your Beckman Coulter Representative.**
- **Open/remove and close/replace doors, covers, and panels with care.**
- **Use the proper tools when troubleshooting.**

⚠ CAUTION

System integrity could be compromised and operational failures could occur if:

- **This equipment is used in a manner other than specified. Operate the instrument as instructed in the Product Manuals.**
- **You introduce software that is not authorized by Beckman Coulter into your computer. Only operate your system's computer with software authorized by Beckman Coulter.**
- **You install software that is not an original copyrighted version. Only use software that is an original copyrighted version to prevent virus contamination.**

⚠ CAUTION

If you purchased this product from anyone other than Beckman Coulter or an authorized Beckman Coulter distributor, and, it is not presently under a Beckman Coulter service maintenance agreement, Beckman Coulter cannot guarantee that the product is fitted with the most current mandatory engineering revisions or that you will receive the most current information bulletins concerning the

product. If you purchased this product from a third party and would like further information concerning this topic, contact your Beckman Coulter Representative.

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Introduction

Overview

This introduction contains the following information:

- How to Use Your AQUIOS Manuals
- Before You Begin
- About this System Guide
- Conventions
- Safety Symbols
- Graphics

How to Use Your AQUIOS Manuals

Your AQUIOS CL Flow Cytometry system includes the manuals listed below:

- The **AQUIOS CL Flow Cytometer Instructions For Use** manual provides information for the day-to-day running of your AQUIOS CL Flow Cytometer and AQUIOS system software. It also provides procedures for cleaning the instrument and replacement procedures. You can find detailed step-by-step procedures for daily startup for running and reviewing quality control (QC) data, running samples, analyzing data, printing reports, and shutting the instrument down. The Instructions for Use manual also contains information on safety, troubleshooting, error messages, and operation principles. It contains a glossary of terms, a list of abbreviations and acronyms, and the warranty information for the instrument.
- This **AQUIOS Tetra System Guide** provides reference information and instructions for using the AQUIOS System with the Tetra Test. Refer to the glossary of terms in the AQUIOS CL Flow Cytometer Instructions for Use.
- The **AQUIOS Host Transmission** manual provides the information needed to program the transmission interface between the AQUIOS CL Flow Cytometer and the laboratory's host computer.

To quickly determine which manual to read for the information needed, see Related Documents.

Before You Begin

IMPORTANT The AQUIOS Tetra System Guide is not an inclusive manual. It is imperative that an Operator is familiar with the information in the AQUIOS CL Flow Cytometer Instructions for Use manual, and the AQUIOS Tetra Reagent and Consumables Instructions for Use and is capable of performing basic operations.

About Your Cytometer Software

AQUIOS System Software comes pre-installed on your AQUIOS Workstation.

About this System Guide

This AQUIOS Tetra System Guide provides reference information and instructions for using the AQUIOS Tetra System.

Use this guide in conjunction with the AQUIOS CL Flow Cytometer Instructions for Use manual.

Use this guide with the applicable reagent Instructions for Use listed under Consumables and Supplies. These Instructions for Use contain reagent-specific information not contained in this guide.

The information in the AQUIOS Tetra System Guide is organized as follows:

CHAPTER 1, Use and Function
Provides the intended use of the AQUIOS CL Flow Cytometer system as well as information concerning system components (Cytometer, Tetra Test, reagents and consumables) and are referred to as AQUIOS Tetra System application. Limitations are also provided.

CHAPTER 2, Operating Principles
Contains a description of the principles used in AQUIOS Tetra testing, and an overview of the analysis algorithm.

CHAPTER 3, Quality Control
Provides information on running QC samples to verify the performance of the instrument before analyzing non-QC samples.

CHAPTER 4, Sample Analysis
Provides information on the preparation required to successfully run samples using AQUIOS Tetra System reagents, running samples using the autoloader, and running samples using the Single-tube Loader.

CHAPTER 5, Data Review
Provides information on reviewing sample results, including flagged results.

CHAPTER 6, Performance Characteristics
Provides information on clinical performance characteristics such as expected values, reference ranges, linearity, accuracy, precision, and the specificity of AQUIOS Tetra monoclonal antibodies and quality control materials.

CHAPTER 7, Application Specifications
Provides information for storage conditions and stability for whole-blood specimens and reagents as well as sample preparation, throughput, and sample rate.

This manual also includes an appendix, references, and an index.

Conventions

This guide uses the following conventions:

- Throughout this manual, the AQUIOS Tetra System application (Cytometer, Tetra Test, reagents and consumables) may also be referred to as system components.
- Blue text indicates that you can click on the text to access related information.
- **Bold font** indicates a screen icon, menu item, or software option on the Workstation screen.
- *Italics font* indicates screen text displayed on the Workstation.
- The term 'select' is used to indicate either one or both of the following actions:
  - to tap or touch your finger to the touch screen of the computer
  - to click with a mouse
- Tabs in the software are blue when not selected and inactive. Tabs in the software turn green when selected and active.

Safety Symbols

Safety symbols alert you to potentially dangerous conditions. These symbols, together with text, apply to specific procedures and appear as needed throughout this guide.

Symbol	Warning Condition	Action
	**Biohazard**	Use universal precautions when working with pathogenic materials. Means must be available to decontaminate the instrument and to dispose of biohazardous waste.
	Consider all materials (specimens, reagents, controls, and monoclonal antibodies) and areas these materials come into contact with as being potentially infectious.	Wear appropriate barrier protection and follow safe laboratory procedures when handling any material in the laboratory.
	Conditional hazard. Possibility of a hazard based on specific conditions.	Pay close attention to the information provided when you see this symbol.

Graphics

All graphics, including screens and printouts, are for illustration purposes only and must not be used for any other purpose.

CHAPTER 1

Use and Function

Overview

This chapter contains information about:

Intended Use

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). It is used in conjunction with the following reagents and software package.

AQUIOS Tetra-1 Panel and AQUIOS Tetra-2+ Panel monoclonal antibody reagents are for use on the AQUIOS CL Flow Cytometer with peripheral whole blood for immunophenotyping. These reagents are indicated for use in the immunologic assessment of patients having, or suspected of having, immune deficiency. These reagents provide identification and enumeration of:

- AQUIOS Tetra-1 Panel monoclonal antibody reagents.
  - Total CD3+, CD3+CD4+,CD3+CD8+, CD3+CD4+/CD3+CD8+ (ratio only) lymphocyte percentages and absolute counts.
  - CD45+ absolute count
  - CD45+ Low SS (lymphocytes) percentage and absolute count
- AQUIOS Tetra-2+ Panel monoclonal antibody reagents.
  - Total CD3+, CD3-CD19+, CD3-CD56+ and/or CD16+ lymphocyte percentages and absolute counts.
  - CD45+ absolute count
  - CD45+ Low SS (lymphocytes) percentage and absolute count

AQUIOS CL Flow Cytometry system software may be run on an independent computer workstation for off-line analysis of results generated by the AQUIOS CL Flow Cytometer with the monoclonal antibody reagents listed above. The off-line analysis must be performed in accordance with the product labeling.

NOTE An off-line analysis workstation cannot be used for acquisition.

System Components

AQUIOS CL Flow Cytometry System

IMPORTANT Contact your Beckman Coulter Representative if a product is damaged upon receipt.

The AQUIOS CL Flow Cytometry system applies the principles of flow cytometry to analyze a stained and lysed whole blood sample to identify various cellular populations as determined by the specific monoclonal antibodies and fluorochromes used.

Operation is automatic, initiated as the user loads a cassette containing a specimen tube in the autoloader or a specimen tube in the Single-tube Loader. The blood sample is stained and incubated, the red blood cells are lysed using the AQUIOS Lysing Reagents A and B. The white blood cells are analyzed on the AQUIOS CL Flow Cytometry system with the AQUIOS Tetra Tests. The AQUIOS CL Flow Cytometer, in conjunction with quality control reagents, verify light scatter, electronic volume (EV), and fluorescence intensities and adjustment of color compensation settings.

NOTE Electronic Volume (EV) is a relative measurement which uses the Coulter Principle of impedance to measure relative cell volume.

The AQUIOS Smart Scheduler tracks requests as they proceed from sample preparation to analysis. All AQUIOS reagents are continuously monitored by the system to track reagent consumption and location changes. The acquired data is stored and provides immediate data access for laboratories.

Unique Load & Go Feature

The AQUIOS CL Flow Cytometer is a quantitative automated analyzer that performs the tetra diagnostic applications that can be run in a “no-wash” sample preparation process. Since this system is intended to be an automated analyzer with hands-off processing of samples from specimen introduction to results reports, it is referred to as a Load & Go flow cytometer. The AQUIOS System Software and AQUIOS Tetra Tests and Quality Control Reagents do not require user verification of standardization of light scatter, electronic volume, and fluorescence intensities or verification of color compensation settings.

This “closed” system will only run tests preconfigured by Beckman Coulter and does not allow for user-designed protocols or panels.

AQUIOS Tetra Test

The AQUIOS Tetra Tests are used:

- with quality control reagents on an AQUIOS CL Flow Cytometry system with AQUIOS System Software for system quality control, and
- with AQUIOS Tetra-1 and/or Tetra-2+ four-color monoclonal antibody reagents and AQUIOS System Software for automated analysis.

For more information, see the associated Reagent Instructions for Use.

Consumables and Supplies

The AQUIOS CL Flow Cytometer is optimized to operate with AQUIOS reagents. The stated analytical characteristics and specifications cited in this manual can only be guaranteed by using AQUIOS reagents and the AQUIOS Deep Well Plate.

Additionally, the AQUIOS reagents (with the exception of the AQUIOS Sodium Hypochlorite Solution) and the AQUIOS Deep Well Plate are labeled with bar codes for smart-track monitoring to help minimize potential errors. The system will only run with AQUIOS reagents and the AQUIOS Deep Well Plate.

NOTE AQUIOS reagents are for use on the AQUIOS CL Flow Cytometer only. Do not dilute, aliquot, or freeze the reagents. The product should be used according to labeled instructions.

NOTE Do not use reagents beyond the expiration date printed on the associated labeling and always use good laboratory practices when handling these reagents.

NOTE The AQUIOS CL Flow Cytometer system tracks the AQUIOS Cleaning Agent for the percent remaining and expiration date.

NOTE The AQUIOS CL Flow Cytometer system does not track the AQUIOS Sodium Hypochlorite Solution. You must track the expiration date of the AQUIOS Sodium Hypochlorite Solution upon use.

For more information, see the associated reagent Instructions for Use on the Beckman Coulter Web site at www.beckmancoulter.com or contact your Beckman Coulter Representative.

Preparation

Lysing Reagents

The AQUIOS Lysing Reagent Kit consists of two reagents: Reagent A and Reagent B. Together these two reagents prepare leukocytes from whole blood for flow cytometry.

Reagent A

Reagent A is a cyanide-free lytic reagent that lyses red blood cells in preparation for white blood cell measurement in the flow cell.

Reagent B

Reagent B slows the reaction caused by Reagent A and preserves the white blood cells for measurement in the flow cell.

Reagent A and Reagent B are housed inside the Cytometer behind the reagent door (the larger of the two doors on the Cytometer front cover).

When Lytic Reagent A and Reagent B containers are replaced, the system looks for a specific bar code to ensure that the correct reagent is being loaded and tracked.

Monoclonal Antibodies

The AQUIOS Tetra System utilizes two separate monoclonal antibody panel reagents. Each is a liquid combination of four or five murine monoclonal antibodies. Each antibody is labeled with a different colored fluorochrome.

- AQUIOS Tetra-1 Panel consists of antibody-dye conjugates CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5.
- AQUIOS Tetra-2+ Panel consists of antibody-dye conjugates CD45-FITC/(CD56+CD16)-RD1/CD19-ECD/CD3-PC5.

Sample results may be obtained using the AQUIOS Tetra-1 Panel reagent only, the AQUIOS Tetra-2+ Panel reagent only, or using both the AQUIOS Tetra-1 Panel and AQUIOS Tetra-2+ Panel reagents together, referred to as AQUIOS Tetra Combo.

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 Tetra-1 Panel and AQUIOS Tetra-2+ Panel are each a combination of four or five murine monoclonal antibodies respectively, each conjugated to a specific fluorochrome and specific for a different cell surface antigen.

Analysis

AQUIOS Sheath Fluid

AQUIOS Sheath Fluid is a cyanide-free, isotonic buffered saline solution. It is used to rinse components between sample analysis, and provide a sheath stream to transport the cells in single file through the flow cell.

For more information, see Chapter 1, System Overview of the AQUIOS CL Flow Cytometer Instructions for Use manual.

Quality 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. 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. They also verify the methods used for staining targeted cells, the lysing of erythrocytes, and the analysis of samples by the AQUIOS CL Flow Cytometer.

For more information, see the associated reagent Instructions for Use.

Plate

Tetra sample preparation is optimized to operate using 96-deep well plates with conical-shaped, deep wells. Each well holds up to 600 µL. The plates are made of virgin polypropylene.

Limitations

1. Accurate and reproducible results will be obtained, provided the procedures used are in accordance with the AQUIOS CL Flow Cytometer Instructions for Use manual, the AQUIOS Tetra System Guide, associated reagent Instructions for Use, and good laboratory practices.
2. Histograms must be reviewed before results are reported to ensure analysis was performed correctly.
3. The CD45+ absolute count and CD45+ Low SS percentage and absolute counts should only be used for Immunophenotyping flow cytometric analysis.
4. When modifying the algorithm-generated results through user intervention (regions and quadrants), it is the user's responsibility to follow the instructions appropriately when adjusting the compensation and the region's algorithm generated results.
5. In certain disease states, such as severe renal failure or some hemoglobinopathies, lysis of RBCs may be slow, incomplete, or unattainable. All red blood cells may not lyse under the following conditions: presence of nucleated red blood cells, abnormal protein concentration, or some hemoglobinopathies. This may cause falsely decreased results due to unlysed RBCs being counted as leukocytes.
6. Abnormal states of health are not always represented by abnormal percentages of certain leukocyte populations. An individual in an abnormal state of health may show the same leukocyte percentages as a healthy person. Use test results in conjunction with clinical and other diagnostic data.
7. Very dim or negative CD45 stained lymphocyte cells may affect the results reporting.
8. Results obtained with flow cytometry may be erroneous if the laser is misaligned or the gates and regions are improperly set.
9. Certain patients may present special problems due to altered or very low numbers of certain cellular populations.

10. Erroneous results may be obtained if insufficient white blood cells are used for analysis.
11. Specimens must be maintained at room temperature, between 18°C and 26°C (64.4°F and 78.8°F) prior to placement on the system.
12. Do not refrigerate specimens. Refrigerated specimens may give aberrant results.
13. Do not dilute, aliquot, or freeze reagents. The product should be used according to labeled instructions. In patients treated with anti-human monoclonal antibody therapies, detection of the specific targeted antigens may be diminished or absent due to partial or complete blocking by the treatment antibody. [1, 2, 3, 39, 40, 41, 42].
14. In some specimens, purity of the lymphocyte region may be decreased due to non lymphoid contaminants with low SS and high CD45 fluorescence similar to lymphocyte populations. These samples may meet CD3+ Reliability Check acceptance criteria, as the relative proportion of CD3+CD4+ and CD3+CD8+ cells remains constant, yet the results may not be accurate. A review of all data plots for the presence of the expected staining patterns is recommended for all samples.

CHAPTER 2

Operating Principles

Overview

This chapter contains information about:

- Principles of Tetra Tests
- Light Source Configuration
- Lymphocyte Immunophenotyping Panels
- Analysis Algorithm Overview

Principles of Tetra Tests

Tetra tests are based on the ability of monoclonal antibodies to bind to the surface of cells expressing discrete, or a combination of discrete, antigenic determinants.

The AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and AQUIOS Tetra-2+ Panel CD45-FITC/(CD56+CD16)-RD1/CD19-ECD/CD3-PC5 monoclonal antibody reagents are each a combination of four or five murine monoclonal antibodies respectively, conjugated to the specified fluorochrome and specific for a different cell surface antigen.

Specific staining of leukocytes is accomplished by incubating whole blood with the monoclonal antibody reagent. The red blood cells (RBCs) are then removed by lysis and the leukocytes, which are unaffected by lysis, are analyzed by flow cytometry.

The flow cytometer measures light scatter, electronic volume, and the fluorescence of cells. This allows for the separation of the population of interest within the electronic window defined on various histograms, which correlates with different parameters.

A series of single and dual histograms are used in the gating stage. The fluorescence of the delimited cells is analyzed in order to distinguish the positively-stained events from the unstained ones.

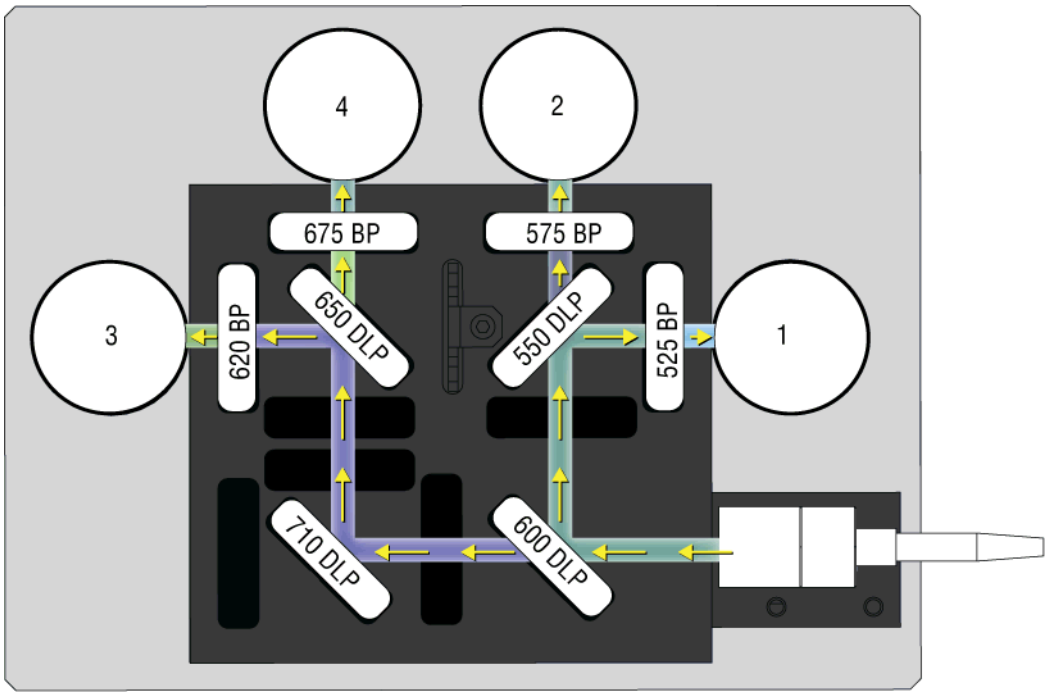
The gating strategy allows detection of circulating leukocytes which are further categorized into various parameters, based on Tetra-1 Panel, Tetra-2+ Panel, or Tetra Combo. See Parameter Results in Table 2.1, Table 2.2, and Table 2.3 for a listing of parameters.

The parameters, triggers, discriminators, and regions are specific to the test. For immune monitoring panels, the system uses a primary gate on lymphocytes, with double discrimination on CD45-FITC and FS. The absolute counts and cell percentages of interest are collected for every sample.

Light Source Configuration

A 488 nm Solid State Diode Laser provides the light source for scatter and fluorescence measurements. Figure 2.1 shows the optical filter configuration for the 488 nm laser.

Figure 2.1 Optical Filter Configuration



Lymphocyte Immunophenotyping Panels

AQUIOS Tetra-1 Panel

The AQUIOS Tetra-1 Panel reagent contains CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 monoclonal antibodies and allows the simultaneous identification and enumeration of total CD3+, CD3+/CD4+, and CD3+/CD8+, T lymphocyte population percentages, and absolute counts as well as CD45+ absolute count and CD45+ Low SS absolute count and percentage. The system also provides the CD4:CD8 ratio.

The AQUIOS Tetra-1 Panel reagent also functions as an analytic reliability check for a specimen by monitoring the Total CD3+ absolute count. The sum of the percentages of CD3+CD4+ and CD3+CD8+ cells should equal the total percentage of CD3+ cells ±5%[4]

Table 2.1 contains the parameter results generated when a Tetra-1 Panel is used for analysis.

Table 2.1 Parameter Results Provided by Tetra-1 Panel Analysis

Parameter Result
CD3+ (T-cells) Percent
CD3+ (T-cells) Count/µL
CD3+/CD4+ (Helper T-cells) Percent
CD3+/CD4+ (Helper T-cells) Count/µL
CD3+/CD8+ (Suppressor T-cells) Percent
CD3+/CD8+ (Suppressor T-cells) Count/µL
CD4:CD8 Ratio
*CD3+ Reliability Check
CD45+ Count/µL
CD45+Low SS Percent
CD45+Low SS Count/µL

NOTE QC Results are notated with a "*".

AQUIOS Tetra-2+ Panel

The AQUIOS Tetra-2+ Panel reagent contains CD45-FITC/(CD56+CD16)-RD1/CD19-ECD/CD3-PC5 monoclonal antibodies and allows simultaneous identification and enumeration of total CD3+, total CD19+, and CD3-/CD56+ and/or CD3-/CD16+ lymphocyte population percentages, and absolute counts as well as CD45 absolute count and CD45+ Low SS absolute count and percentage.

This reagent reflects the distribution of the three major subsets comprising the lymphocyte populations upon which other lymphocyte enumeration studies are based and provides the total lymphocyte percentage.

The combination of AQUIOS Tetra-2+ Panel monoclonal antibody reagents can function as a quality control check for a specimen in terms of total lymphocyte percentage determined using the following formula:[4]

$$\text{Total lymphocyte percentage (\%)} = \begin{array}{l}\text{\% CD3+ (T) lymphocytes + \% CD19+ (B) lymphocytes +} \\ \text{\% CD3-/CD56+ and/or CD16+ (NK) lymphocytes}\end{array}$$

Table 2.2 contains the parameter results generated when an AQUIOS Tetra-2+ Panel is used for analysis.

Table 2.2 Parameter Results Provided by Tetra-2+ Panel Analysis

Parameter Result
CD3+ (T-cells) Percent
CD3+ (T-cells) Count/µL
CD3-/CD19+ (B-cells) Percent
CD3-/CD19+ (B-cells) Count/µL
CD3-/CD56+ CD16+ (NK Cells) Percent
CD3-/CD56+ CD16+ (NK Cells) Count/µL
*%Total Lymphocytes (T+B+NK) Percent
CD45+ Count/µL
CD45+Low SS Percent
CD45+Low SS Count/µL

NOTE QC Results are notated with a "*".

AQUIOS Tetra Combo Panel

The AQUIOS Tetra Combo Panel (both AQUIOS Tetra-1 Panel and AQUIOS Tetra-2+ Panel monoclonal antibodies) provides the same ability to enumerate an individual's major lymphocyte subsets: T, B, and NK, as well as provide information for the T-Cell subsets, CD3+/CD4+ and CD3+/CD8+, as its constituent Panels. The combination of monoclonal antibody reagents can function as a quality control check for a specimen in terms of total lymphocyte percentage determined using the following formula:[4]

$$\text{Total lymphocyte percentage (\%)} = \begin{array}{l}\text{\% CD3+ (T) lymphocytes + \% CD19+ (B) lymphocytes +} \\ \text{\% CD3-/CD56+ and/or CD16+ (NK) lymphocytes}\end{array}$$

Table 2.3 contains the parameter results generated when a Tetra Combo Panel is used for analysis.

CD3+ Reliability Check is the sum of the percentages of CD3+CD4+ and CD3+CD8+ cells and should be within ±5% of the total percentage of CD3+.[4]

CD3+ Intrapanel Check is the variability between Tetra-1 and Tetra-2+ for CD3+ and serves as an internal control. Differences between replicate CD3 percent positive results should be ±3.5%.

Table 2.3 Parameter Results Provided by Tetra Combo Analysis

Parameter Result
Average CD3+ (T-cells) Percent
Average CD3+ (T-cells) Count/µL
CD3+/CD4+ (Helper T-cells) Percent
CD3+/CD4+ (Helper T-cells) Count/µL
CD3+/CD8+ (Suppressor T-cells) Percent
CD3+/CD8+ (Suppressor T-cells) Count/µL
CD3-/CD19+ (B-cells) Percent
CD3-/CD19+ (B-cells) Count/µL
CD3-/CD56+CD16+ (NK Cells) Percent
CD3-/CD56+CD16+ (NK Cells) Count/µL
CD4:CD8 Ratio
*Total Lymphocytes (T+B+NK) Percent
*CD3 Reliability Check
*CD3 Intrapanel Check
CD45+ Count/µL
CD45+Low SS Percent
CD45+Low SS Count/µL
CD3+ (T-cells) Percent Tetra 1
CD3+ (T-cells) Count/µL Tetra 1
CD3+ (T-cells) Percent Tetra 2
CD3+ (T-cells) Count/µL Tetra 2

NOTE QC Results are notated with a "*".

Analysis Algorithm Overview

The AQUIOS Tetra algorithm is the software analysis component that works in conjunction with AQUIOS Tetra-1 Panel and AQUIOS Tetra-2+ Panel monoclonal antibody reagents to automatically identify and enumerate lymphocyte populations. The algorithm combines information from seven parameters (FS, SS, Fluorescence FL1-FL4 and Electronic Volume) to automatically generate gates and regions to identify lymphocytes and separate them into their components.

Introduction to Gating Strategy

Software algorithms automatically detect the subset populations of interest to calculate and report the results for the predefined tests. The software generates flags and notifications after analyzing samples.

Monocytes and granulocytes in a sample can be excluded by proper gating on lymphocytes on the flow cytometer.[5] To ensure method specificity, the AQUIOS Tetra System software is designed to automatically identify and optimize the lymphocyte gate based on CD45 bright positive, electronic volume, and light scatter characteristics. To further ensure method specificity, the AQUIOS Tetra software monitors nonspecific antibody binding to lymphocytes by automatically placing cursors based on the separation of positive and negative peaks. This eliminates the need for an isotypic control.

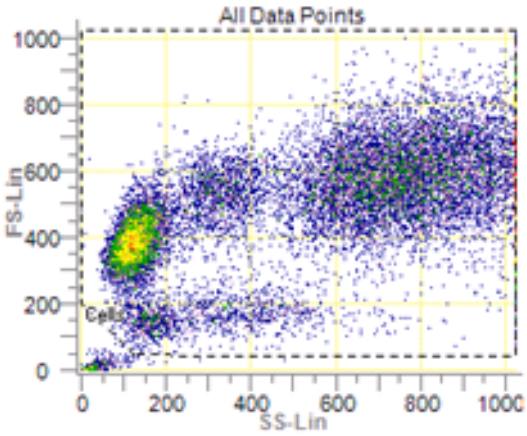
Gating Strategy: AQUIOS Tetra-1

The Lymphs region is defined as Lymphs(45) AND Lymphs EV. The CD3- region is defined on a single-parameter histogram of CD3 gated on Lymphs. Lymphs Total is defined as CD3+All or CD3- OR CD3-Capture.

Plots 1 through 5 determine the CD3+ cell (T-cells) enumeration.

Plot 1 Forward Scatter (FS) versus Side Scatter (SS) Plot
The **All Data Points** gate is used to identify all cells of interest, and eliminate debris. This FS/SS DotPlot identifies the Cells region consisting of leucocytes, and removes debris (lower left). See Figure 2.2.

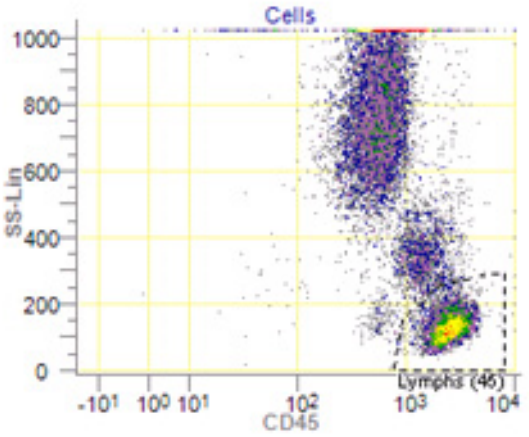
Figure 2.2 FS versus SS



Plot 2 SS-Lin versus CD45 Plot

From the **Cells** region a CD45-FITC/SS dot plot is created to define the first lymphocyte gate, **Lymphs(45)**. See Figure 2.3.

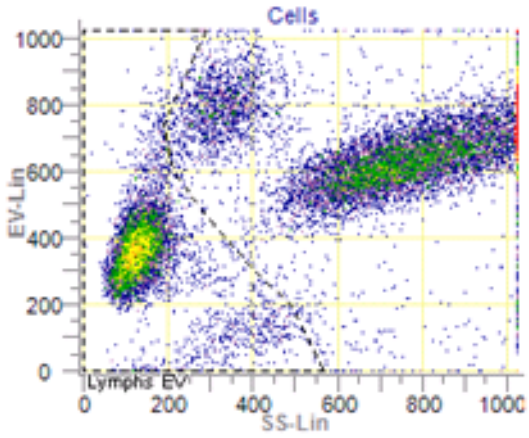
Figure 2.3 SS-Lin versus CD45



Plot 3 Electronic Volume (EV) versus Side Scatter (SS) Plot

From the **Cells** region, an EV/SS dot plot is created to optimize the population of interest and define an additional lymphocyte gate. See Figure 2.4.

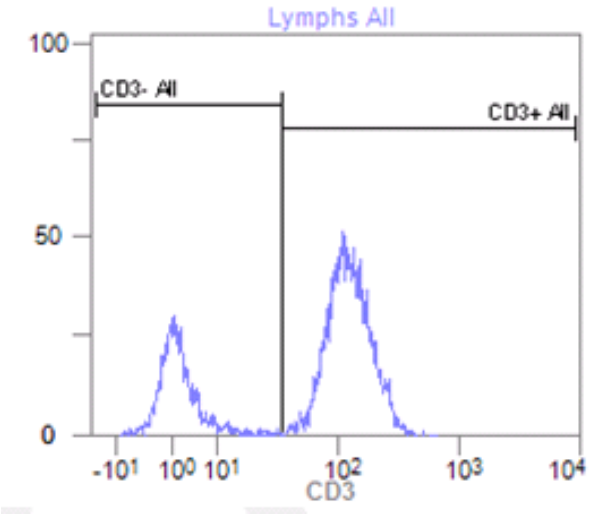
Figure 2.4 EV vs SS



Plot 4 CD3 Histogram

The Lymph All region is defined as Lymphs(45) OR (Lymphs EV AND CD45Pos). This histogram is used to distinguish CD3+ events (CD3+ALL) from CD3- events (CD3-ALL). See Figure 2.5.

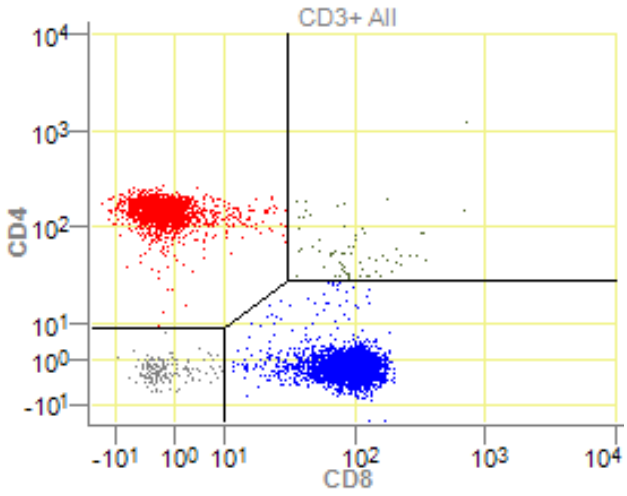
Figure 2.5 CD3 Histogram



Plot 5 CD4-RD1 versus CD8-ECD Plot

From **CD3+All** cells, quad-stat regions are defined to identify the positive populations CD3+CD4+ and CD3+CD8+. See Figure 2.6.

Figure 2.6 CD4-RD1 versus CD8-ECD

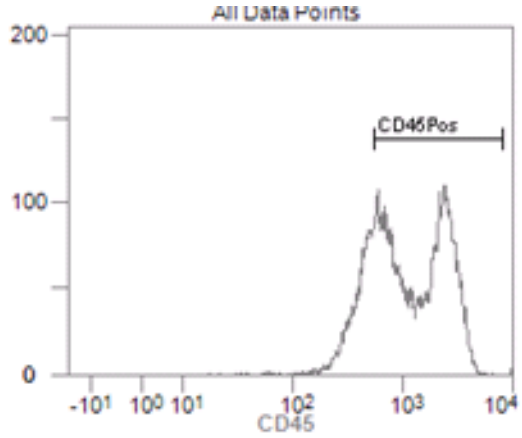


Plots 6 and 7 describe the approach in determining the CD3- (non-Tcell) population. Both of these values determine the total lymphocyte population and can then be applied to determine percentage.

Plot 6 Single Parameter CD45 Histogram

A single parameter histogram of CD45 is created to define the CD45 positive cells, **CD45Pos**. This region defines the left edge of lymph to ensure that CD3- are high CD45 bright cells. See Figure 2.6.

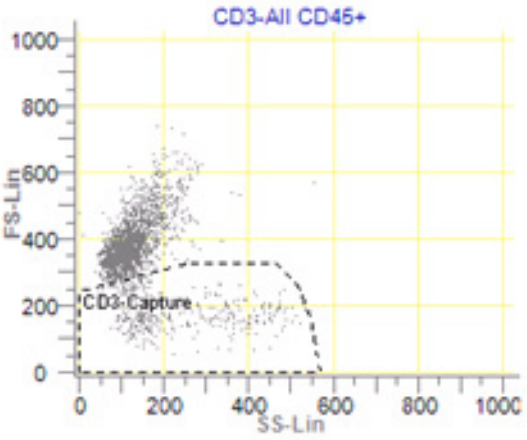
Figure 2.7 CD45 Histogram



Plot 7 FS-Lin versus SS-Lin Plot (CD3-All + CD45+)

This plot is gated on the **CD3-All CD45+** events and is used to capture CD3- cells with low forward scatter and high side scatter events. See Figure 2.8.

Figure 2.8 FS-Lin versus SS-Lin (CD3-All + CD45+)

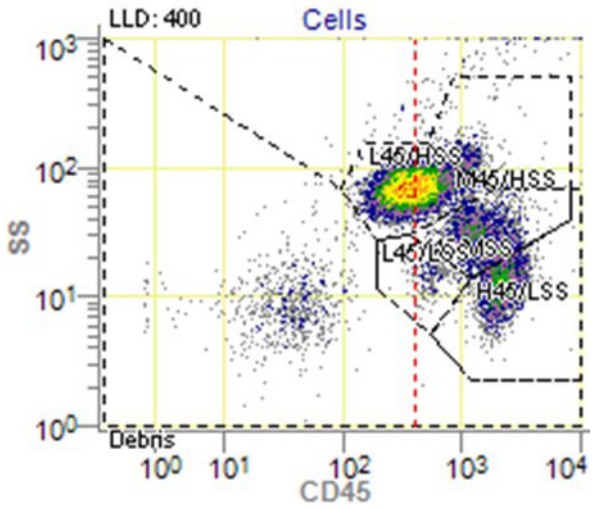


NOTE **CD3-All and CD45+:** This logic region is created from **CD3-All AND CD45Pos** to ensure that cells that are included in **CD3-capture** region are CD45+ bright.

Plot 8 SS vs. CD45 (CD45+ Total)

"CD45+ Total" is defined as a logical region on the five parts of the CD45+'s. These five regions are defined on the CD45/SS graph filtered on Cells ("H45/LSS", "M45/MSS", "M45/HSS", "L45/HSS", "L45/LSS"). The region OR's all five regions to create the total CD45+. This plot is not intended to represent a 5-part differential. This plot is for illustration purposes only to situate the populations relative to each other. See Figure 2.9.

Figure 2.9 Plot 8 SS vs. CD45 (CD45+ Total)



Gating Strategy: AQUIOS Tetra-2+

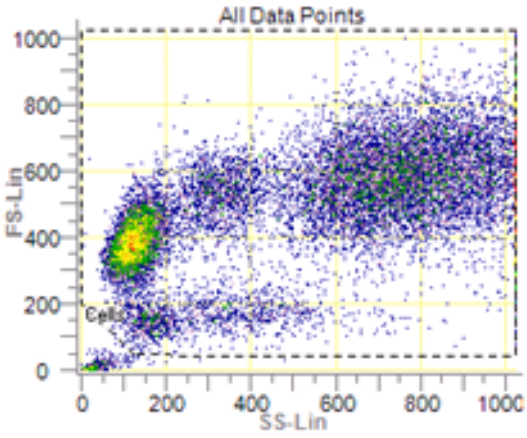
The Lymphs region is defined as Lymphs(45) AND Lymphs EV. The CD3+ and CD3- regions are defined on a single-parameter histogram of CD3 gated on Lymphs. Lymphs Total is defined as CD3+ ALL or CD56-CD16-/CD19+ or NK Total OR CD56-CD16-/CD19- or CD56+CD16+/CD19+.

Plots 1 through 4 determine the CD3+ cell (T-cells) enumeration.

Plot 1 FS versus Side Scatter (SS) Plot

All Data Points is used to identify all cells of interest, and eliminate debris. This FS/SS dot plot identifies the Cells region consisting of leucocytes, and removes debris (lower left). See Figure 2.10.

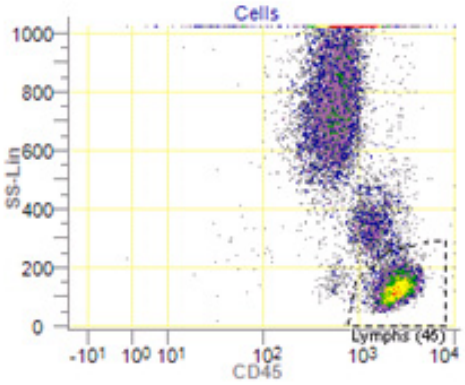
Figure 2.10 FS versus SS



Plot 2 Side Scatter (SS) and CD45-FITC Plot

From the **Cells** region a CD45/SS dot plot is created to define the first lymphocyte gate, **Lymphs(45)**. See Figure 2.11.

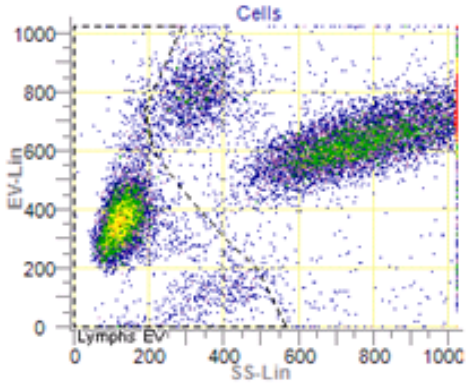
Figure 2.11 SS versus CD45



Plot 3 Electronic Volume (EV) versus Side Scatter (SS) Plot

From the **Cells** region, an EV/SS dot plot is created to optimize the population of interest and define an additional lymphocyte gate. See Figure 2.12.

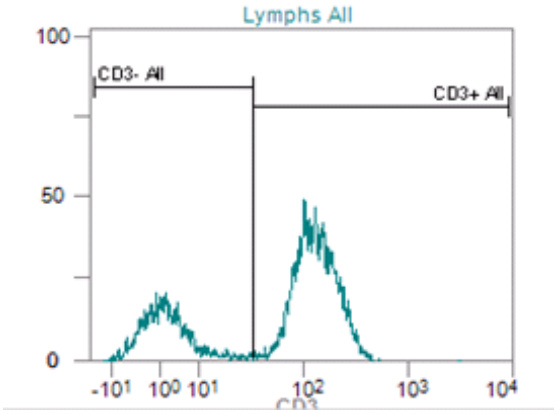
Figure 2.12 EV versus SS



Plot 4 CD3 Histogram

The CD3 histogram (not shown) is gated on the Lymph gate to distinguish CD3+ events from CD3- events. The Lymph gate is defined as Lymphs(45) and Lymphs(EV). The CD3 histogram is gated on the Lymph All region. The Lymph All region is defined as Lymphs(45) OR (Lymphs EV AND CD45Pos). This histogram is used to distinguish CD3+ events (CD3+All) from CD3- events (CD3-All). See Figure 2.13.

Figure 2.13 CD3 Histogram

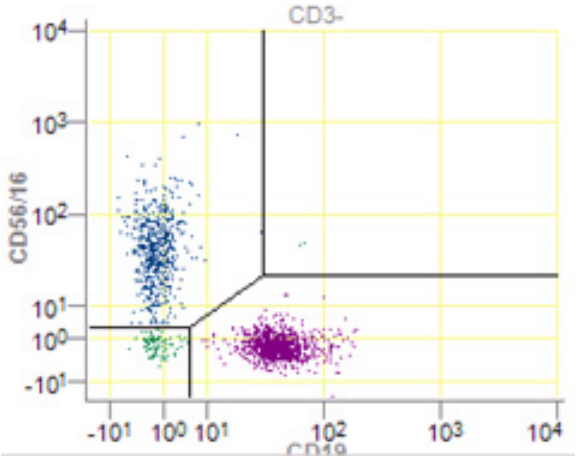


Plots 5, 6 and 7 describe the approach in determining the CD3- subsets. These populations are used for both enumeration and total lymphocyte population and can then be applied to determine percentage.

Plot 5 CD56+CD16+ versus CD19+ Plot

The following graph is gated two ways. One gate is **CD3-** and the other is **CD3-ALL.** Cursor placement is kept in sync by the software automatically. See Figure 2.14.

Figure 2.14 CD56+CD16+ versus CD19+



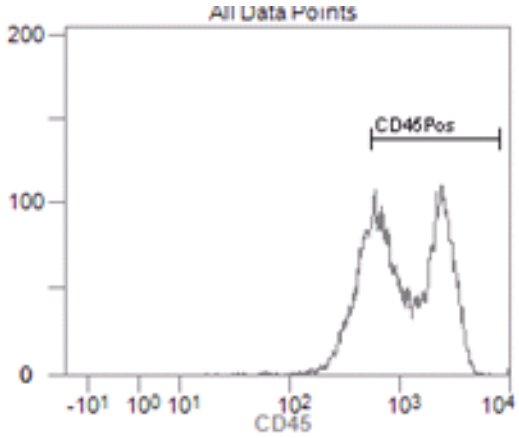
Population	Gate
CD56+CD16+/CD19-	CD3- (*)
CD56+CD16+/CD19+	CD3-
CD56-CD16-/CD19+	CD3-All
CD56-CD16-/CD19-	CD3-

*CD56+CD16+/CD19- subset is added to **NKCapture** to create **NKTotal**.

Plot 6 Single Parameter CD45 Histogram

A single parameter histogram of CD45 is created to define the CD45 positive cells, **CD45Pos**. This region defines the left edge of the lymphocytes to ensure that CD3- are high CD45 bright cells. See Figure 2.15.

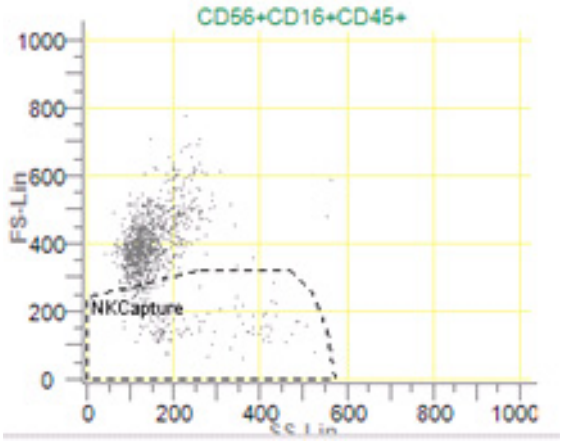
Figure 2.15 CD45 Histogram



Plot 7 Forward Scatter (FS) versus Side Scatter (SS) Plot (CD56+CD16+CD45+)

The *AND* region **CD56+CD16+CD45+** is created from CD56+CD16+ *AND* CD45Pos. It is increased to include the low forward scatter and high side scatter events in the equation that will define the total of CD56+CD16+ positive cells. See Figure 2.16.

Figure 2.16 FS versus SS (CD56+CD16+CD45+)

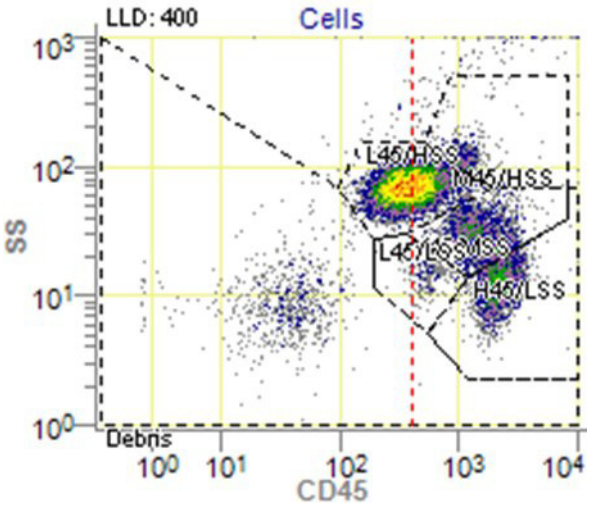


NOTE CD45Pos *AND* those cells in the CD56+CD16+ region is gated on CD3-All.

Plot 8 SS vs. CD45 (CD45+ Total)

"CD45+ Total" is defined as a logical region on the five parts of the CD45+'s. These five regions are defined on the CD45/SS graph filtered on Cells ("H45/LSS", "M45/MSS", "M45/HSS", "L45/HSS", "L45/LSS"). The region OR's all five regions to create the total CD45+. This plot is not intended to represent a 5-part differential. This plot is for illustration purposes only to situate the populations relative to each other.See Figure 2.17.

Figure 2.17 Plot 8 SS vs. CD45 (CD45+ Total)



CHAPTER 3

Quality Control

Overview

Run QC samples to verify the performance of the instrument before analyzing non-QC samples.

This chapter contains information about:

QC Materials

The following Beckman Coulter reagents are required to perform the daily QC for the AQUIOS Tetra-1 and AQUIOS Tetra-2+ panels:

- AQUIOS Tetra-1 Monoclonal Antibody Panel
- AQUIOS Tetra-2+ Monoclonal Antibody Panel
- AQUIOS Controls:
  - AQUIOS IMMUNO-TROL Cells
  - AQUIOS IMMUNO-TROL Low Cells
- AQUIOS Lysing Reagent Kit
  - AQUIOS Lysing Reagent A
  - AQUIOS Lysing Reagent B

Quality Control Checks

The system automatically verifies the optical, fluidic and electronic stability of the instrument. The control reagent data is automatically populated into the Levey-Jennings charts of the QC screens: Results, Instrument and Instrument Drift.

A compensation check is automatically performed while running the control reagents. The message **Compensation Passed** or **Compensation Failed** is then displayed in the Status Field of the control run results.

The AQUIOS system results are displayed and flagged based on the generated values. In general, the system displays fewer digits than that which is generated.

Results are rounded using standard rounding procedures. As a reminder, to round off decimals:

1. Find the place value you want (the 'rounding digit') and look at the digit to the immediate right of it.
2. If the digit to the right of the place value is less than 5, keep the original place value, and drop all digits to the right of the place value.
3. If the digit to the right of the place value is 5 or greater, add one to the place value, and drop all digits to the right of the place value.

For instance, 4.94 would display as 4.9, but 4.95 would display as 5.0.

For control results, if the assay range is, for example, 20-25, a result of 20 could be flagged as being out of range low, if the generated result is actually 19.9.

The same applies when comparing sample results to the normal and action ranges.

Daily QC

Daily Quality Control is a critical component of ensuring the system's performance for the AQUIOS Tetra application. Refer to Daily QC Procedure in the AQUIOS CL Flow Cytometer Instructions for Use manual.

IMPORTANT When running QC for Tetra Combo, if either Tetra-1 or Tetra-2+ fail, then Tetra Combo will issue a flag stating QC for Tetra Combo failed. Check the individual runs for Tetra-1 or Tetra-2+ to determine why QC failed.

Run QC Samples

⚠ CAUTION

Risk of erroneous results. When using Tetra-1 Panel or Tetra-2+ Panel tests, there are 15 tests per IMMUNO-TROL tube. Each cap should have no more than 15 piercings. When using the Tetra Combo, there are 10 tests per IMMUNO-TROL tube. Cap-piercing beyond the allotted number may clog the instrument.

Before running the AQUIOS IMMUNO-TROL controls, verify that:

1. The Daily Startup was performed. See Startup in Chapter 3, Daily Startup in AQUIOS CL Flow Cytometer Instructions for Use manual for details.
2. The Beckman Coulter reagents and the reagent levels are suitable and sufficient for the QC procedure. To verify reagent availability, select 

on the Status Bar. To replace a reagent, see Replacing Reagents and Consumables in Chapter 11, Replacement Procedures in the AQUIOS CL Flow Cytometer Instructions for Use manual.
3. The control assay values have been previously registered into the system. See Reagent Info Screen in Chapter 8, Setup in the AQUIOS CL Flow Cytometer Instructions for Use manual for more information. If the control lot number information has not been registered in the system, scan the control assay sheet before adding a QC request. See Starting a New Lot of Controls in Chapter 11, Replacement Procedures in the AQUIOS CL Flow Cytometer Instructions for Use manual.

For instructions on running Daily Quality Control, see the Daily QC Procedure in Chapter 4, Quality Control of the AQUIOS CL Flow Cytometer Instructions for Use manual.

NOTE If QC Results are not acceptable, refer to Chapter 9, Troubleshooting of the AQUIOS CL Flow Cytometer Instructions for Use manual.

QC Screen

The Results tab on the QC screen (Figure 3.1) tracks the changes over time in absolute counts (cells/µL) and cell percentages for AQUIOS IMMUNO-TROL and AQUIOS IMMUNO-TROL Low Cells. This data reflects performance and stability of the instrument over the specified period.

Figure 3.1 Results Tab Screen

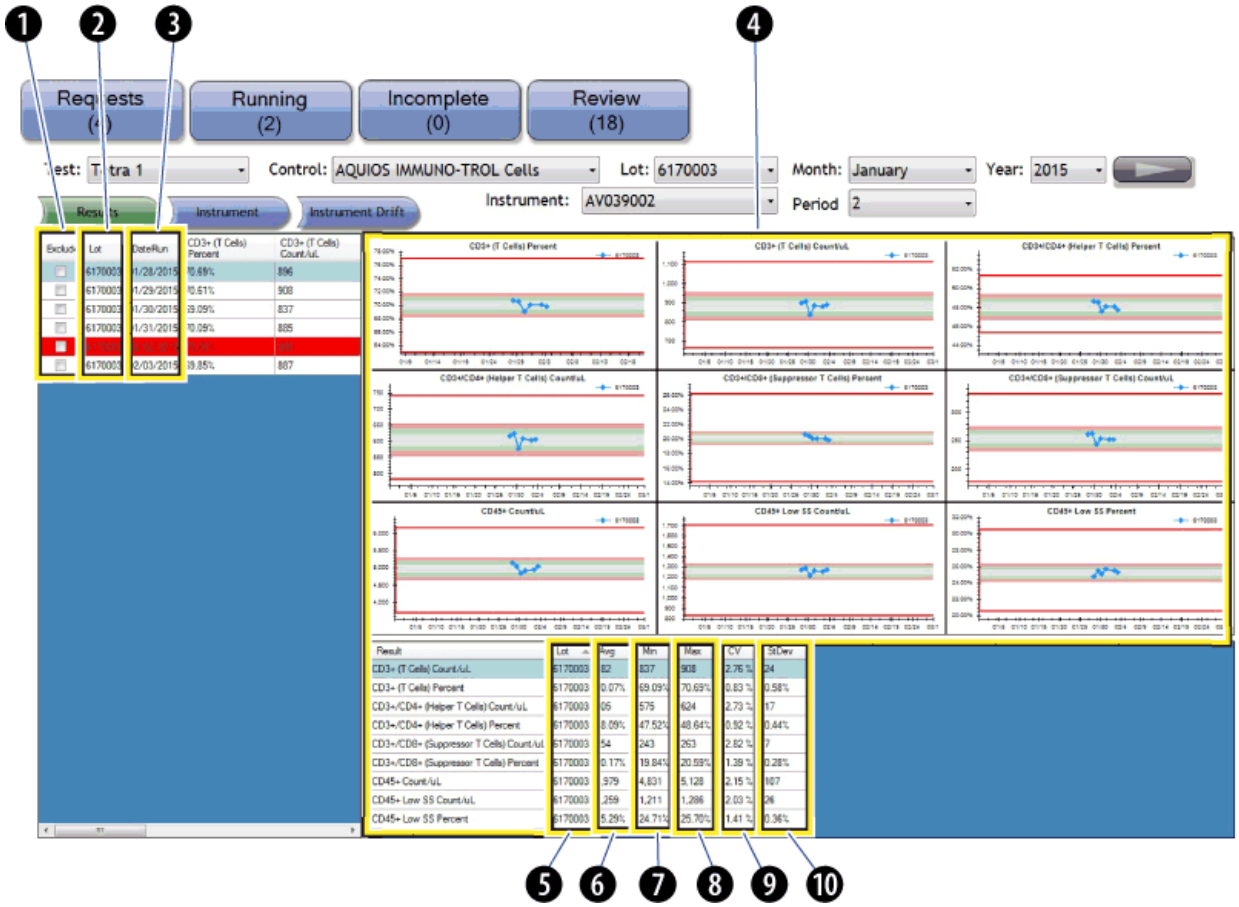
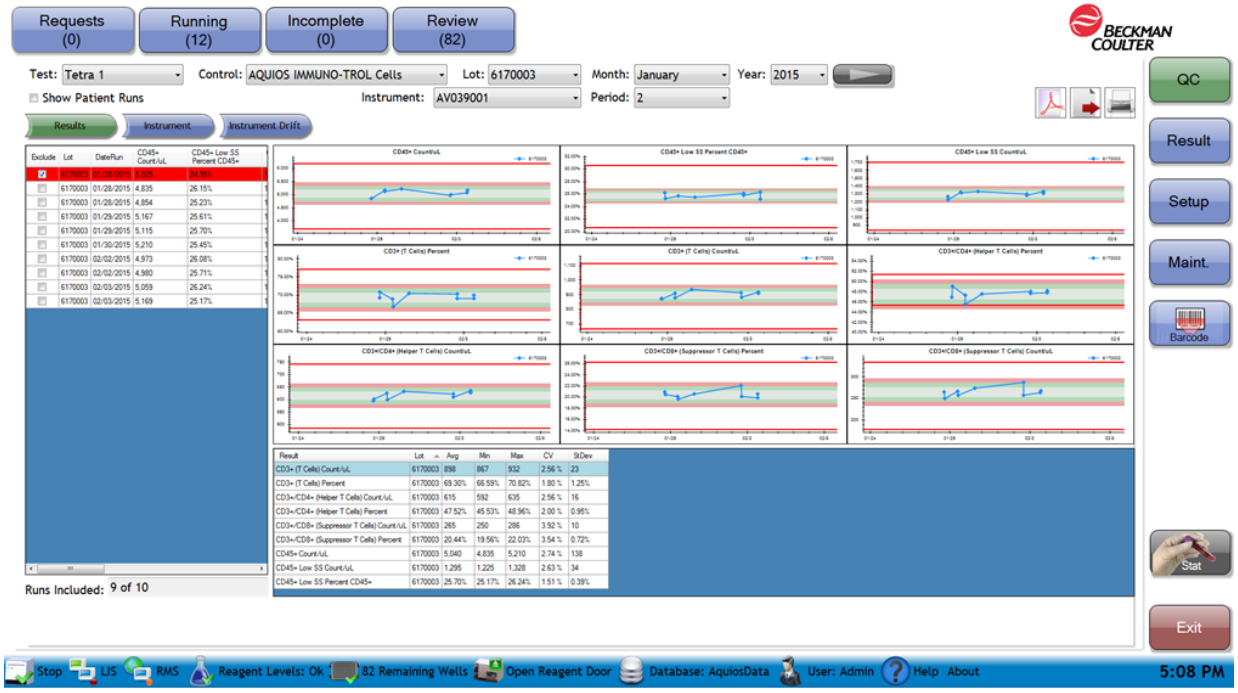


Table 3.1 General Table on QC Screens: Defined

1	**Exclude Checkbox**: Use the checkbox to eliminate this run from the QC range calculation and select .	6	**Average**: The average of all the non-excluded runs listed.
2	**Lot**: Control reagent lot number used for the specified QC run.	7	**Min**: The lowest value of all the non-excluded runs listed.
3	**Date run**: The date on which the selected QC run was performed.	8	**Max**: The highest value of all the non-excluded runs listed.
4	**Results**: The QC results quantitatively for the selected QC run. The results displayed depend on the selected test.	9	**CV**: The coefficient of variation of all the non-excluded runs listed (Standard Deviation/Average) x 100.
5	**Lot**: The lot numbers of the control reagent for all of the runs listed.	10	**StDev**: The standard deviation of all the non-excluded runs listed.

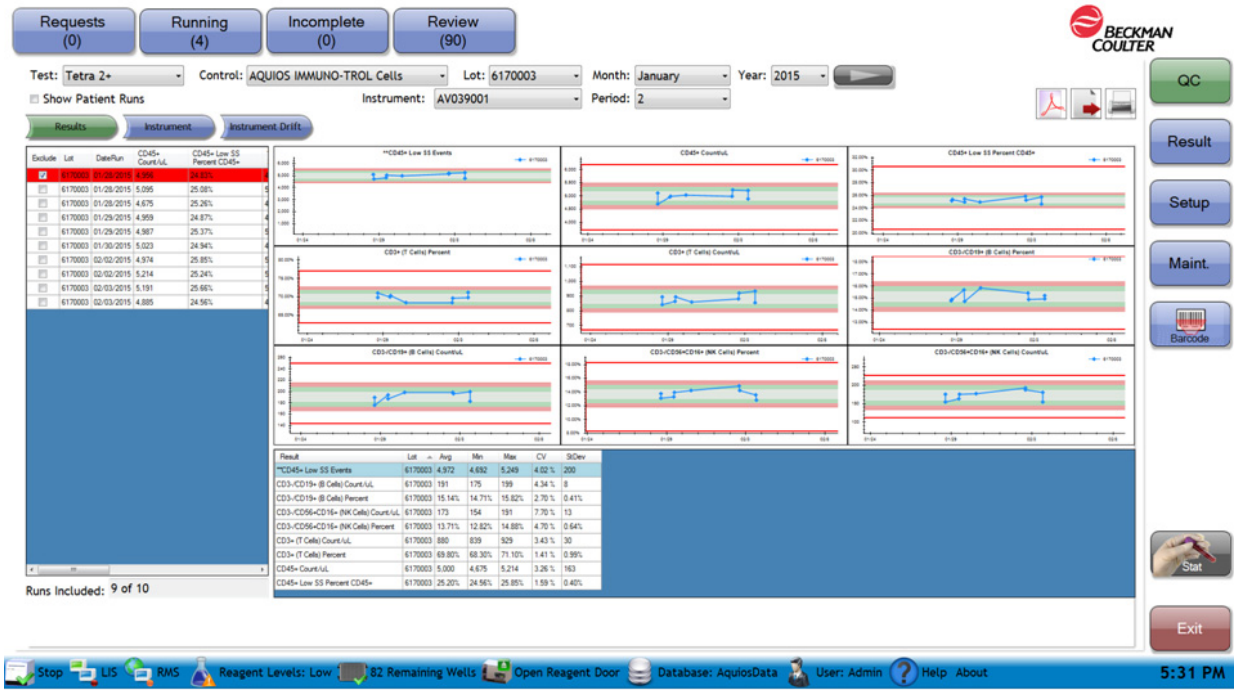
NOTE Summary statistics are listed at the bottom of the graphs. Use the vertical scroll bar to access all statistics.

Figure 3.2 Results Tab: AQUOS IMMUNO-TROL, Tetra-1



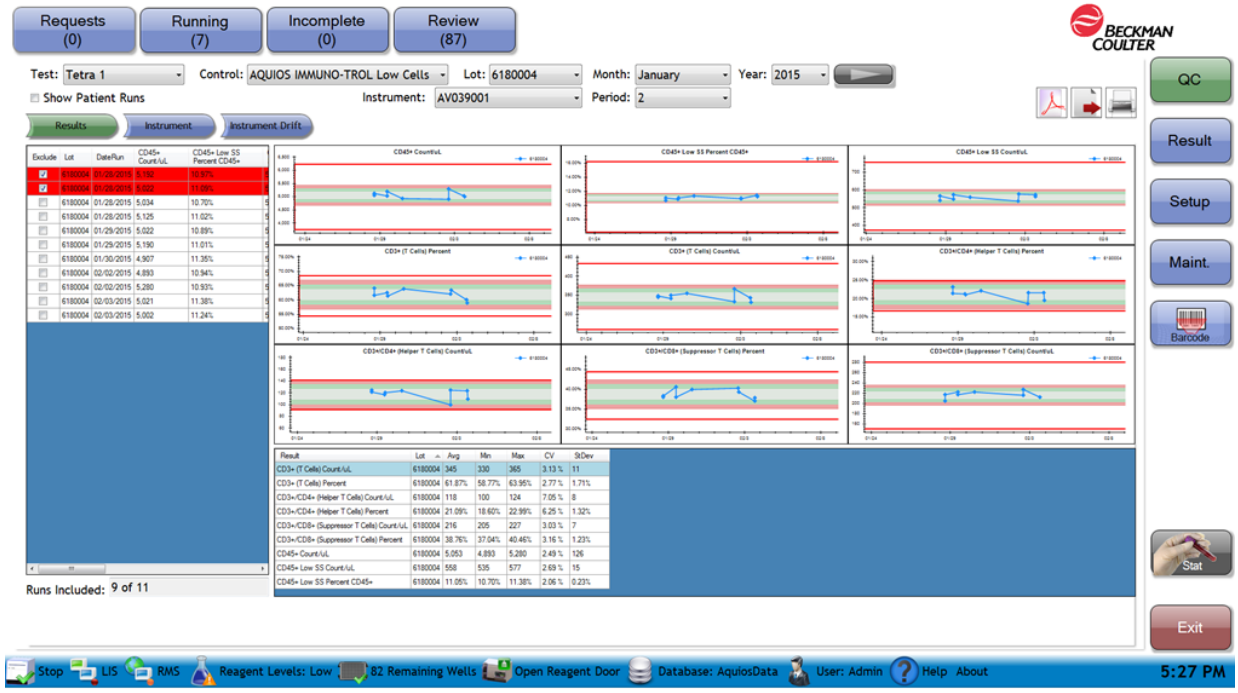
NOTE There is a data table listed on the left of the QC screen with the same information as the data plots. Use the horizontal and vertical scroll bar to access all statistics.

Figure 3.3 Results Tab: AQUIOS IMMUNO-TROL, Tetra-2+



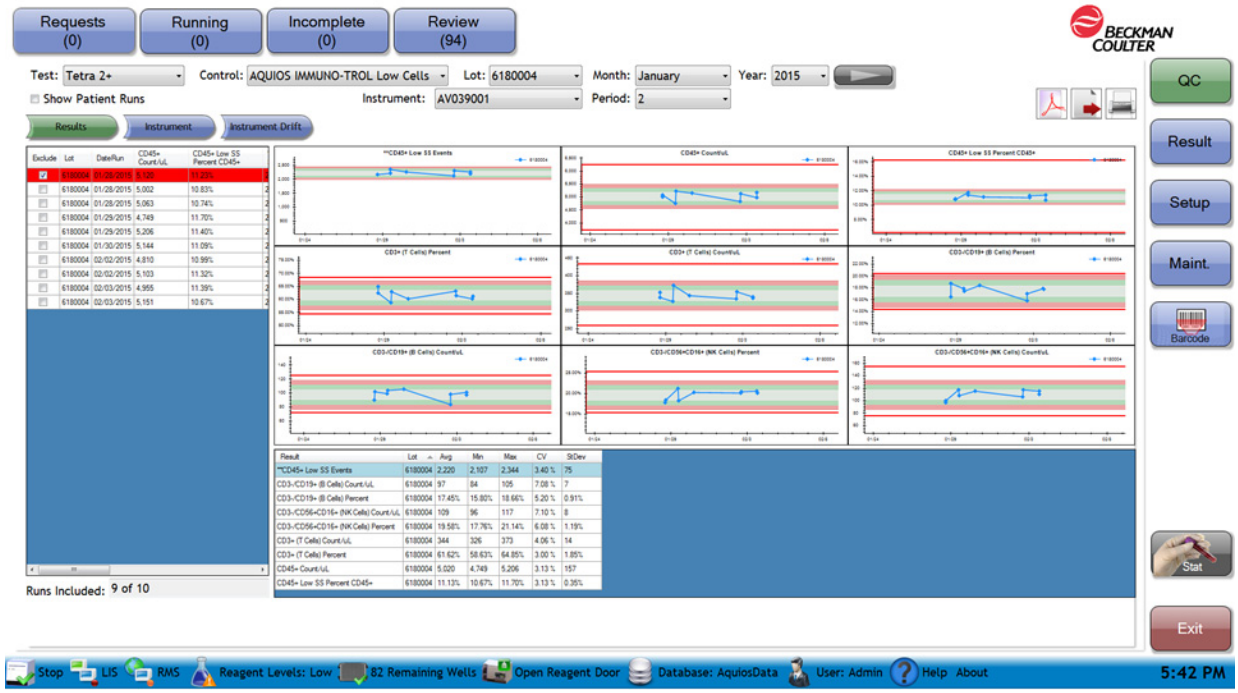
NOTE There is a data table listed on the left of the QC screen with the same information as the data plots. Use the horizontal and vertical scroll bar to access all statistics.

Figure 3.4 Results Tab: AQUIOS IMMUNO-TROL Low Cells, Tetra-1



NOTE There is a data table listed on the left of the QC screen with the same information as the data plots. Use the horizontal and vertical scroll bar to access all statistics.

Figure 3.5 Results Tab: AQUIOS IMMUNO-TROL Low Cells, Tetra-2+



NOTE There is a data table listed on the left of the QC screen with the same information as the data plots. Use the horizontal and vertical scroll bar to access all statistics.

Instrument Tab

The Instrument Screen Levey-Jennings charts keep track of the changes over time in the Separation Quotient for AQUIOS IMMUNO-TROL and AQUIOS IMMUNO-TROL Low Cells. See Figure 3.6.

Figure 3.6 Instrument Tab: AQUOS IMMUNOTROL, Tetra-1

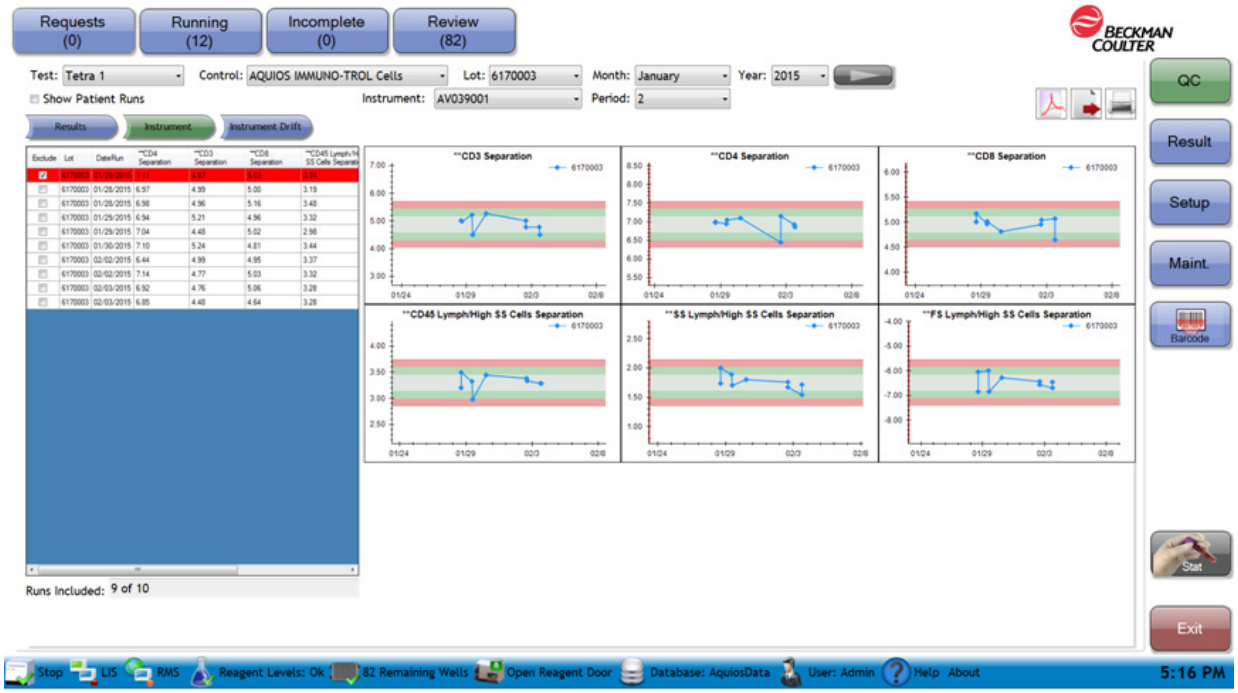


Figure 3.7 Instrument Tab: AQUIOS IMMUNO-TROL, Tetra-2+

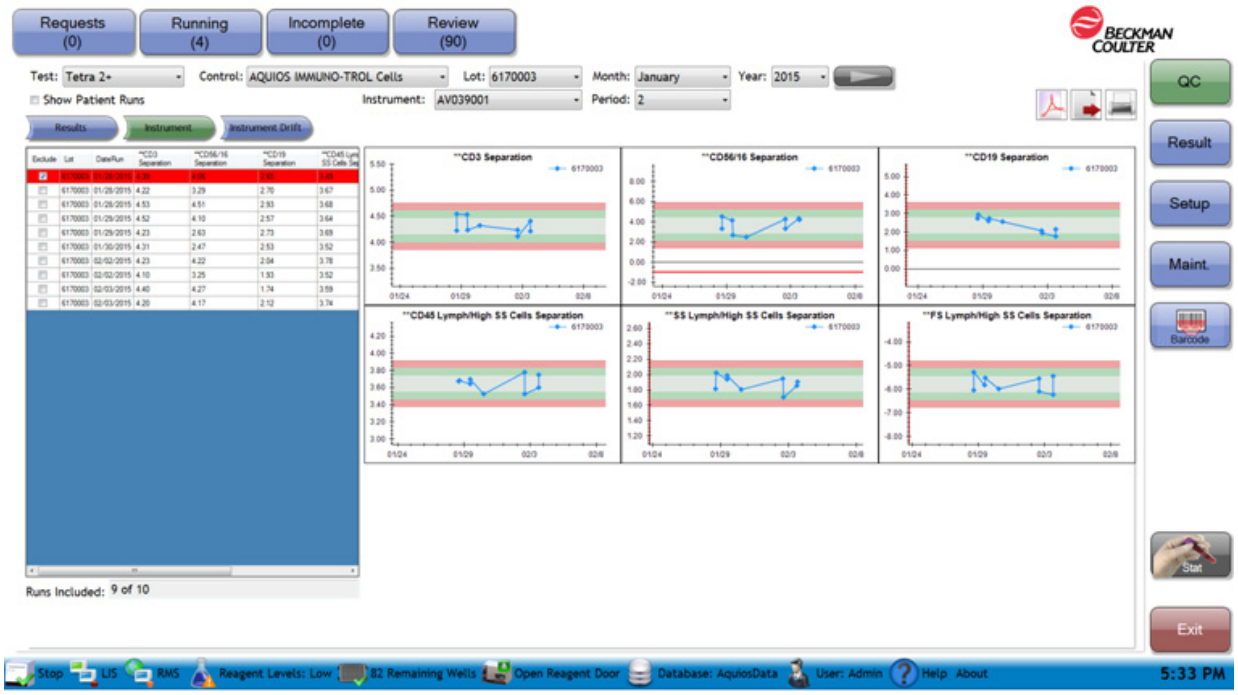


Figure 3.8 Instrument Tab: AQUIOS IMMUNO-TROL Low Cells, Tetra-1

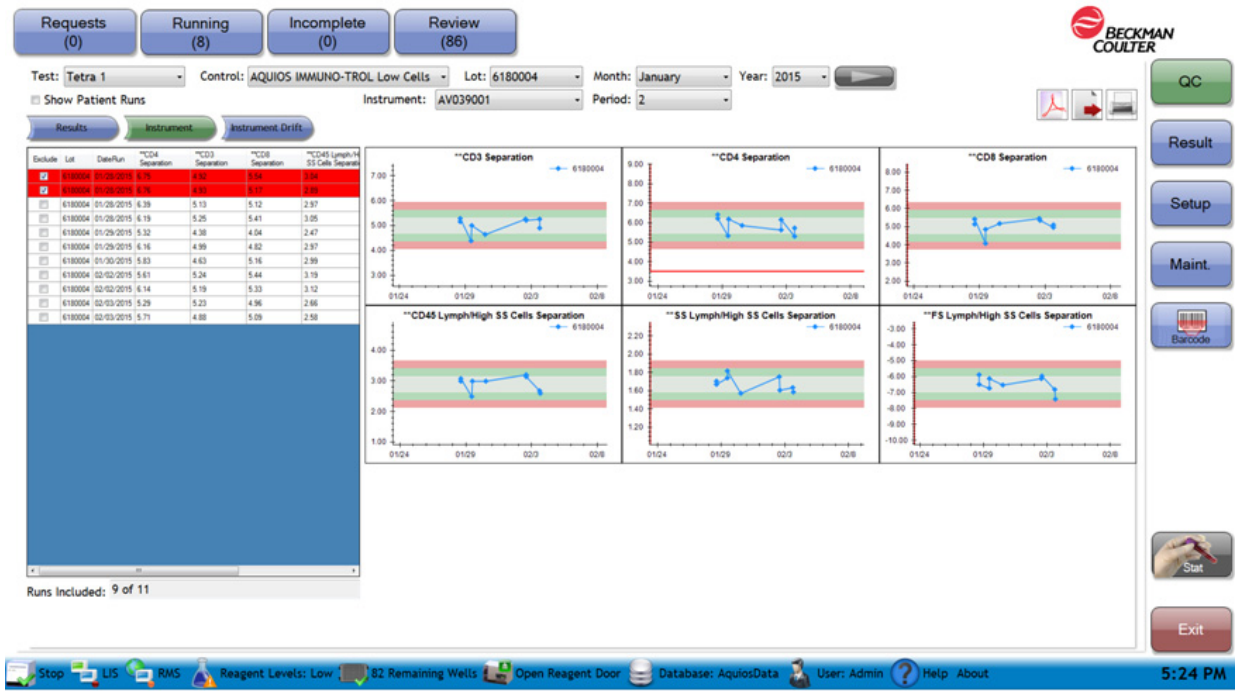
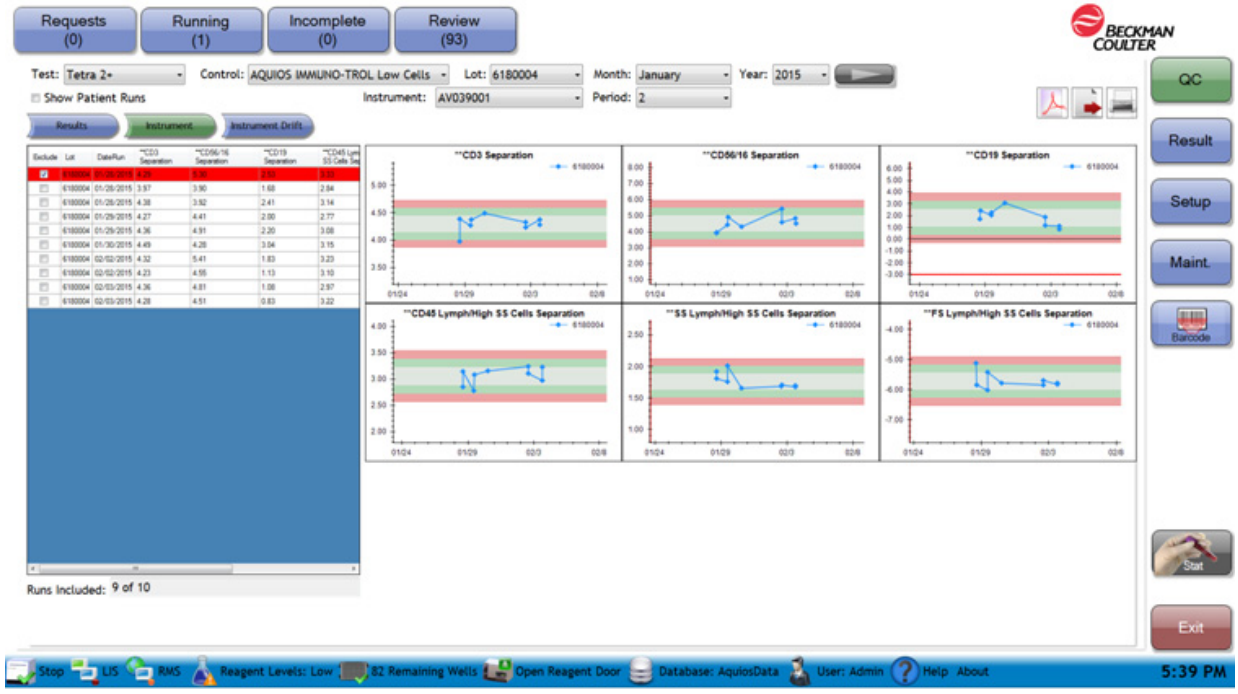


Figure 3.9 Instrument Tab: AQUIOS IMMUNO-TROL Low Cells, Tetra-2+



There is a data table listed on the left of the QC screen with the same information as the data plots. Use the horizontal scroll and vertical bar to access all statistics.

NOTE This varies depending on the test selected and the control being viewed.

The definition of the Separation Quotient Statistic is as follows:

Separation Quotient (SQ) is an indicator of relative sensitivity and resolution and can be used as an index that indicates how well separated two different populations are, such as sample versus debris, or lymphocytes versus monocytes: The larger the separation quotient, the better the separation.

The separation quotient is used for quality control.

For two populations the separation between them for a given parameter is defined as:

- The difference between the 2 SD points is divided by the difference of the mean channel numbers. All calculations are done using the log of the data to take the variation between channel number and standard deviation out of the function.
- The above function returns a number less than or equal to one.
- For readability the number is multiplied by 10.

The end result is a numeric means of qualifying separation of populations in the data plots for a test.

Table 3.2 Separation Quotient Statistics

Numeric Range	Meaning
3 to 10	Excellent separation such that >99% of the data points in the populations are separated from each other
0 to 3	Good separation >2 SD or approximately 95% of the data points in the populations are separated from each other.
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.
<-3	Poor separation which worsens as the numbers decrease.

Examples:
Below are examples of excellent, good, moderate, and poor separation.

Figure 3.10 Separation Quotient = 4.7 (Excellent Separation)

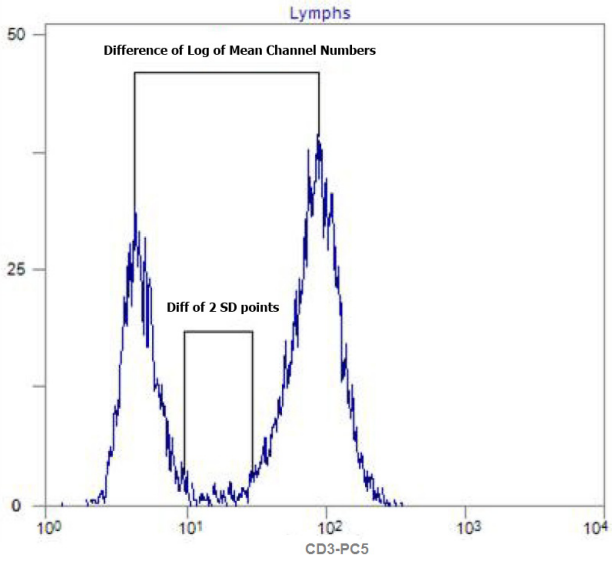


Figure 3.11 Separation Quotient = 2.3 (Good Separation)

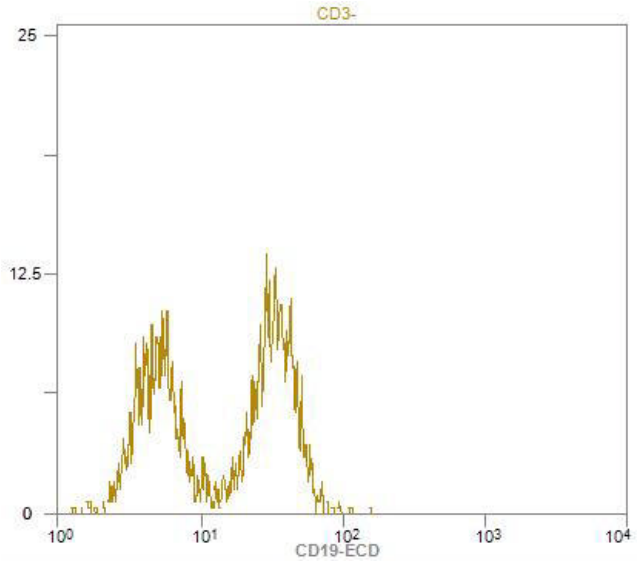


Figure 3.12 Separation Quotient = -0.79 (Moderate Separation)

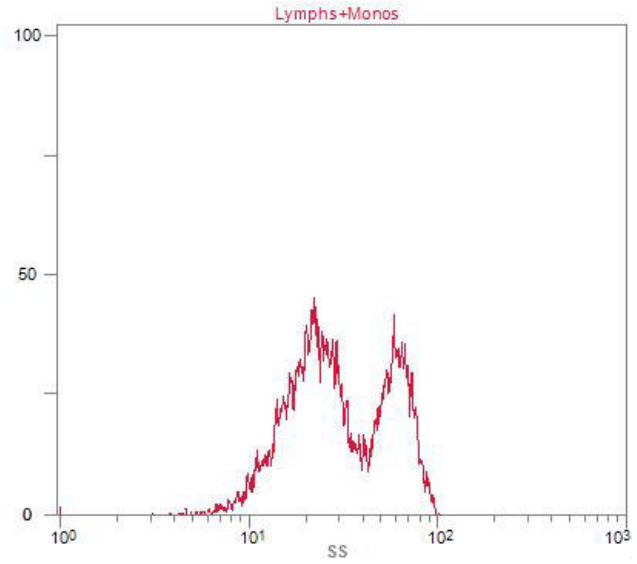
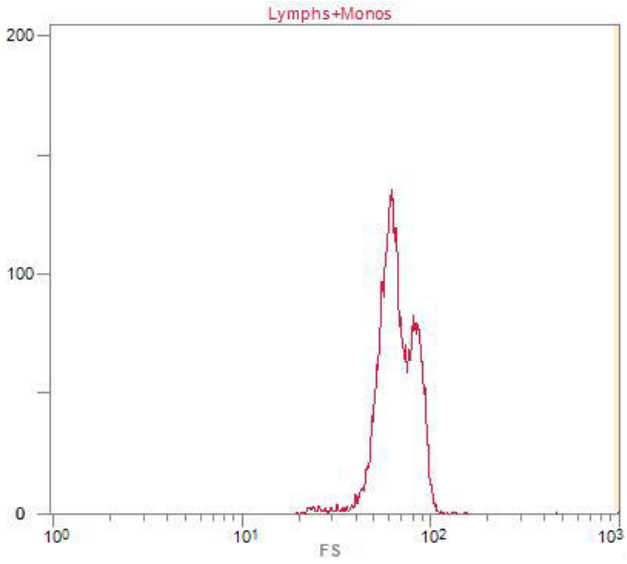


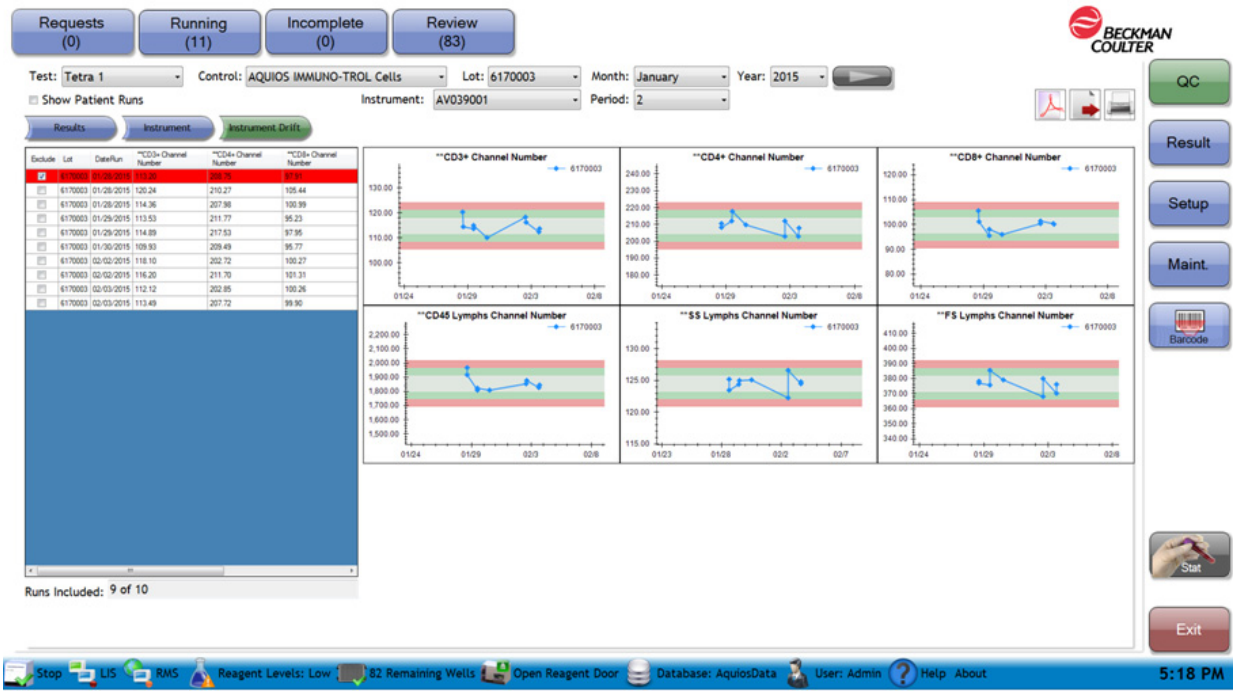
Figure 3.13 Separation Quotient = -4.7 (Poor Separation)



Instrument Drift Tab

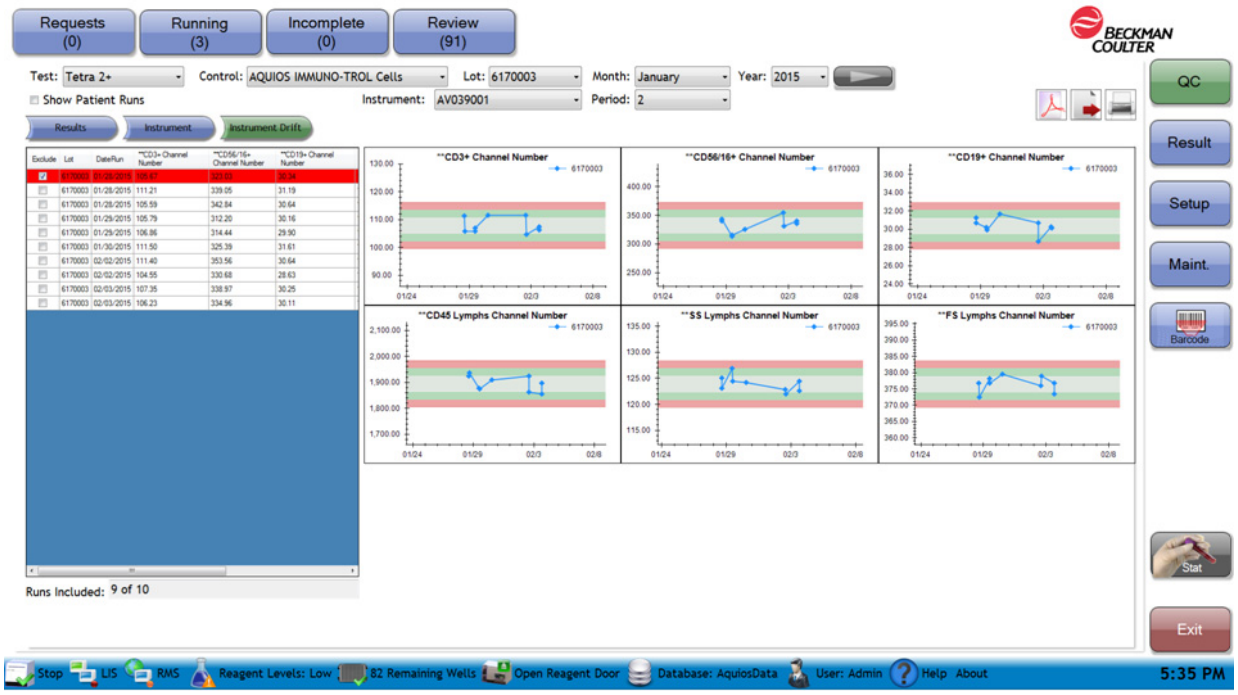
The Instrument Drift Levey-Jennings charts display the sub-population's channel number fluctuation for the AQUIOS IMMUNO-TROL Cells and AQUIOS IMMUNO-TROL Low Cells. This data reflects the changes in fluorescence intensity over the specified period.

Figure 3.14 Instrument Drift Tab: AQUOS IMMUNOTROL, Tetra-1



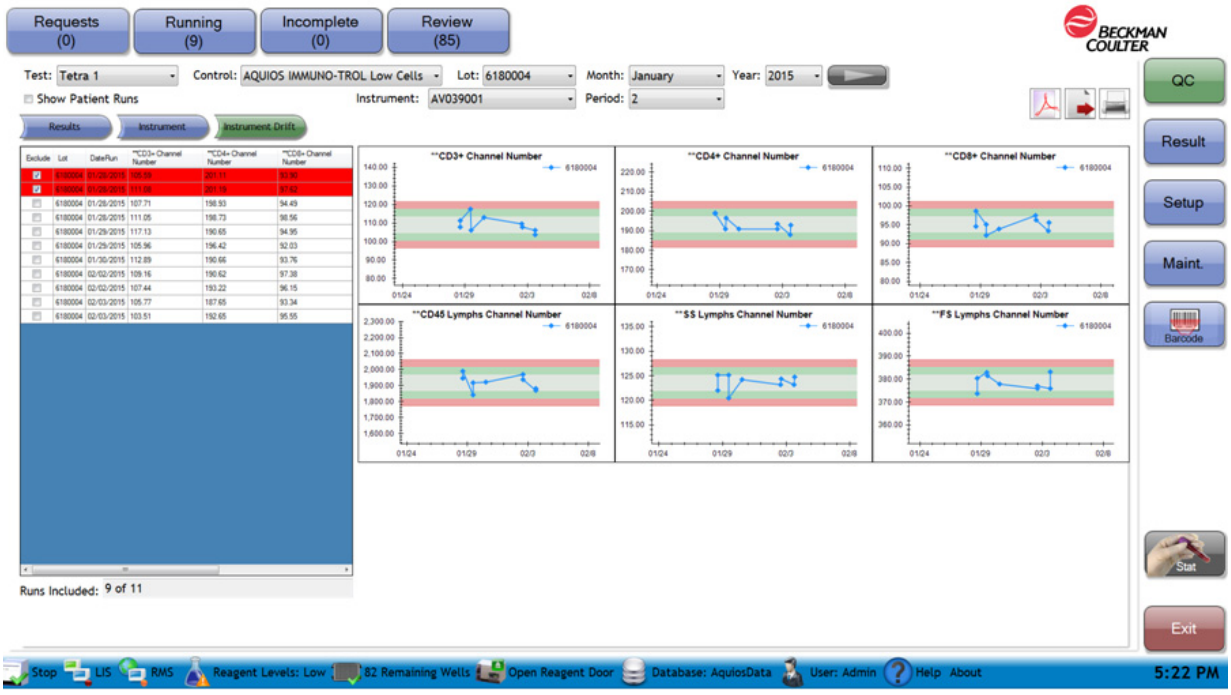
NOTE There is a data table listed on the left of the QC screen with the same information as the data plots. Use the horizontal and vertical scroll bar to access all statistics.

Figure 3.15 Instrument Drift Tab: AQUIOS IMMUNO-TROL, Tetra-2+



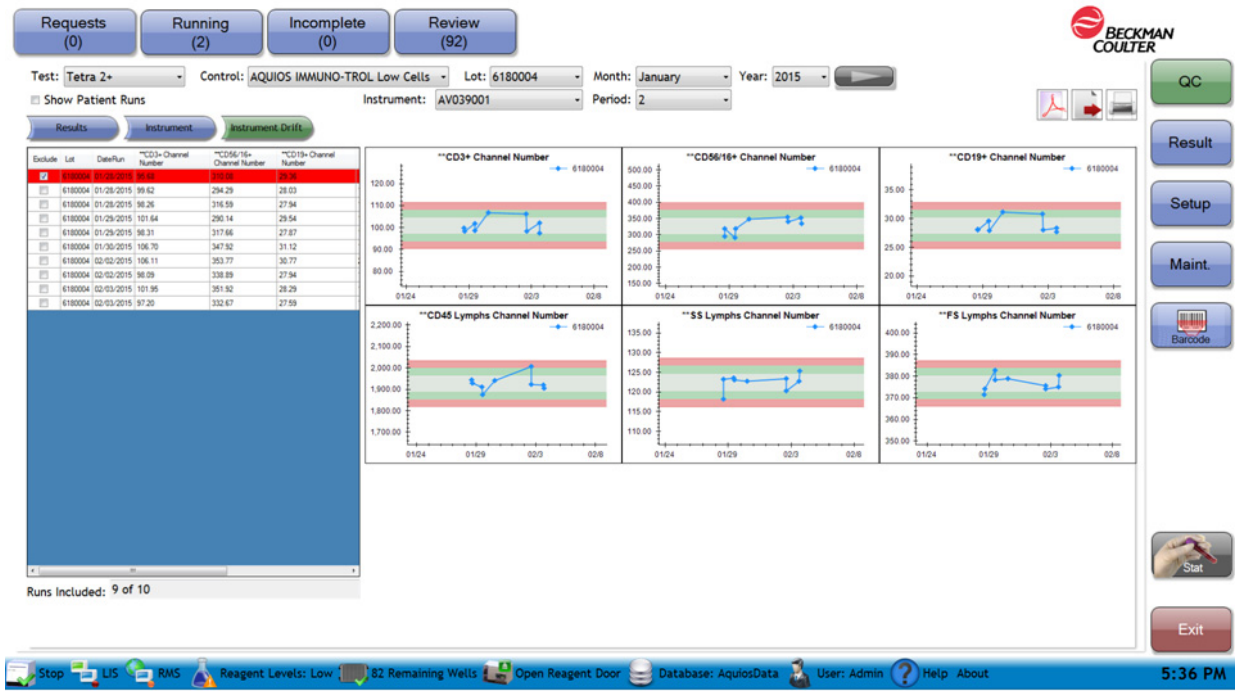
NOTE There is a data table listed on the left of the QC screen with the same information as the data plots. Use the horizontal and vertical scroll bar to access all statistics.

Figure 3.16 Instrument Drift Tab: AQUIOS IMMUNO-TROL Low Cells, Tetra-1



NOTE There is a data table listed on the left of the QC screen with the same information as the data plots. Use the horizontal and vertical scroll bar to access all statistics.

Figure 3.17 Instrument Drift Tab: AQUIOS IMMUNO-TROL Low Cells, Tetra-2+



NOTE There is a data table listed on the left of the QC screen with the same information as the data plots. Use the horizontal and vertical scroll bar to access all statistics.

CHAPTER 4

Sample Analysis

Overview

IMPORTANT Before performing an analysis using the AQUIOS Tetra System application, ensure you are thoroughly familiar with the operating procedures in the AQUIOS CL Flow Cytometer Instructions for Use manual.

This chapter contains the following sections:

Preparation

NOTE Refer to the AQUIOS CL Flow Cytometer Instructions for Use manual, as needed.

Before beginning analysis, make sure:

- Startup is completed and acceptable. See Chapter 3, Daily Startup in the AQUIOS CL Flow Cytometer Instructions for Use manual.

⚠ CAUTION

Risk of instrument damage. For the monoclonal antibody reagents, ensure the shipping cap has been replaced with the pierceable cap provided with the reagent packaging, otherwise the probe can be damaged.

⚠ CAUTION

Risk of losing on-board samples.Replacing reagents and opening the reagent door while operating the Cytometer puts on-board samples at risk. Replacing reagents while the system is running should be performed as quickly as possible to avoid flagged results due to overlysing and overincubation.

If you have not already done so:

- Place the AQUIOS Tetra-1 Panel and AQUIOS Tetra-2+ Panel monoclonal antibody reagents in the carousel. Make sure reagent levels are suitable for the number of samples to be run. If needed, additional vials of AQUIOS Tetra-1 Panel or AQUIOS Tetra-2+ Panel monoclonal antibody reagents should be added before starting the workload.
- Mount the carousel containing the vials of AQUIOS Tetra-1 Panel or AQUIOS Tetra-2+ Panel monoclonal antibody reagents on the carousel platform inside the reagent compartment.
- Remove the caps and place the AQUIOS Lysing Reagent A and Reagent B bottles in any of the four available positions in the reagent compartment. Make sure the bar code labels face outward.
- Insert an AQUIOS 96-deep well plate. (Discard old plate and insert a new plate at the beginning of the day to ensure proper tracking.)

- Quality Control checks were completed and acceptable using AQUIOS IMMUNO-TROL Cells and AQUIOS IMMUNO-TROL Low Cells. See Chapter 4, Quality Control in the AQUIOS CL Flow Cytometer Instructions for Use manual as needed.
- Retain specimens in blood collection tubes at room temperature, between 18°C and 26°C (64.4°F and 78.8°F). Do not refrigerate specimens. Refrigerated specimens may give aberrant results.
- Ensure each whole blood specimen is analyzed within 24 hours of collection. If the specimen has been stored for more than 24 hours, reject the specimen.

⚠ CAUTION

Risk of result misidentification. Ensure that the sample ID is a unique number. If you reuse the sample ID numbers, be sure to archive the old results to avoid duplicate numbers.

- Make sure each patient specimen tube to be loaded complies with the minimum running volume. If running samples on the Autoloader, also ensure that the specimen tube has a barcode on it. See Table 4.1.

Table 4.1 Minimum Blood Volume Required per Specimen Tube

Specimen Tube Size	Minimum Volume
13 mm x 75 mm	750 µL
13 mm x 100 mm	750 µL
16 mm x 100 mm	1000 µL
10.25 mm x 50 mm	650 µL
Sarstedt 13 mm x 75 mm	750 µL

Table 4.1 Minimum Blood Volume Required per Specimen Tube

Specimen Tube Size	Minimum Volume
Sarstedt 13 mm x 90 mm	750 µL
Sarstedt 11 mm x 66 mm	750 µL
Sarstedt 15 mm x 92 mm	1500 µL
Sarstedt 13 mm x 65 mm	750 µL

AQUIOS Tetra Application Workflow

When running samples using the AQUIOS Tetra application, each test goes through four stages. These stages include:

Preparing The whole blood sample and the monoclonal antibodies are aspirated, dispensed and then mixed in the designated well of the 96-deep well plate. See Table 4.2.

Incubating An approximate 15-minute incubation allows proper staining of the whole blood sample by the monoclonal antibodies. See Table 4.2.

Lysing The two-step lysing system is dispensed into the well containing the stained whole blood sample to lyse the RBCs and preserve the WBCs. See Table 4.2.

Analyzing The prepared sample is passed through the flow cell to obtain the sample results. See Table 4.2.

Table 4.2 Processing Samples Using the Tetra Application

PREPPING	INCUBATING	LYSING	ANALYSING
1. User loads specimen[a]. **2.** BLOOD is mixed and aspirated from the collection tube. **3.** BLOOD is dispensed into a microtiter plate well. **4.** ANTIBODY is added to the well. **5.** Solution is mixed.	**1.** About 15 minutes.	**1.** LYSING REAGENT A is added to the well. **2.** Solution is mixed. **3.** LYSING REAGENT B is added to the well. **4.** Solution is mixed.	**1.** Solution is mixed. **2.** Sample is aspirated. **3.** Sample is analyzed.

a. When using the autoloader, samples are automatically mixed. When using the Single-tube Loader, mix the collection tube as instructed before loading the specimen tube. (See the AQUIOS CL Flow Cytometer Instructions for Use manual for details.)

Running Screen

The Running screen (Figure 4.1) tracks any samples currently being processed by the Cytometer. The sample workflow is displayed on the screen (**Run Status**) as the sample progresses from one stage to the next (from Preparing to Analyzing). The **Details** portion of the screen provides visibility and the specifics of each stage, including timing.

Figure 4.1 Running Screen

For more information about the Running Screen, refer to the AQUIOS CL Flow Cytometer Instructions for Use manual.

Options for Running Specimens

⚠ WARNING

Risk of personal injury or contamination:

- **If not properly shielded while using the instrument, it is possible to become injured or contaminated. To prevent possible injury or biological contamination, you must wear proper laboratory attire, including gloves, a laboratory coat, and eye protection.**
- **Operating the instrument while the waste level sensor is disconnected can cause biological contamination. To prevent biological contamination, do not operate the instrument while the waste level sensor is disconnected.**

⚠ CAUTION

Risk of clogging or erroneous results if samples contain clots, fibrin strands, or cellular debris, or if samples are lipemic. Use good laboratory practices and follow your laboratory procedures for specimen handling and/or inspection prior to placing on the system.

Specimen tubes can be loaded in the autoloader or the Single-tube Loader. The Single-tube Loader functions as a stat loader. A higher priority is given to a specimen tube loaded into the Single-tube Loader; therefore, it will be processed ahead of the specimen tubes loaded in an autoloader cassette.

The autoloader has continuous loading capability with up to 40 specimens at once. The specimens are automatically processed according to the unprocessed pile cassette position (bottom to top) at the top of the autoloader holder and according to the sample position within a cassette (position 1 to 5). Place the cassette that you want to run first at the bottom of the unprocessed pile and place the specimen you want to run first in cassette position one (1). See Running Samples Using the Autoloader in Chapter 5, Sample Processing in the AQUIOS CL Flow Cytometer Instructions for Use manual.

Logging In Controls

For detailed information on Logging In Controls, refer to Chapter 11, Replacement Procedures in the AQUIOS CL Flow Cytometer Instructions for Use manual.

Running Samples Using the Autoloader

For detailed information on Running Samples Using the Autoloader, refer to Chapter 5, Sample Processing, in the AQUIOS CL Flow Cytometer Instructions for Use manual.

Running Samples Using the Single-tube Loader

For detailed information on Running Samples Using the Single-tube Loader, refer to Chapter 5, Sample Processing in the AQUIOS CL Flow Cytometer Instructions for Use manual.

CHAPTER 5

Data Review

Overview

This chapter contains information about:

The AQUIOS Workstation provides the user with the ability to review both QC data and completed patient tests.

The review process provides the user with the ability to review data based on the Review/Editor and Administrator rights function to edit completed runs. The following are the items which can be edited:

- Sample information
- Gate and Region Placement
- Compensation

Review Screen

IMPORTANT There is a risk of reporting incorrect results. Data displays used to arrive at the test result for light scatter patterns, antibody staining profiles, and all gates and boundaries should be reviewed by a laboratory professional when interpreting the data. If results are suspect, follow your laboratory's procedures to resolve.

For a more detailed explanation of this screen and its components, see Chapter 6, Data Review in the AQUIOS CL Flow Cytometer Instructions for Use manual.

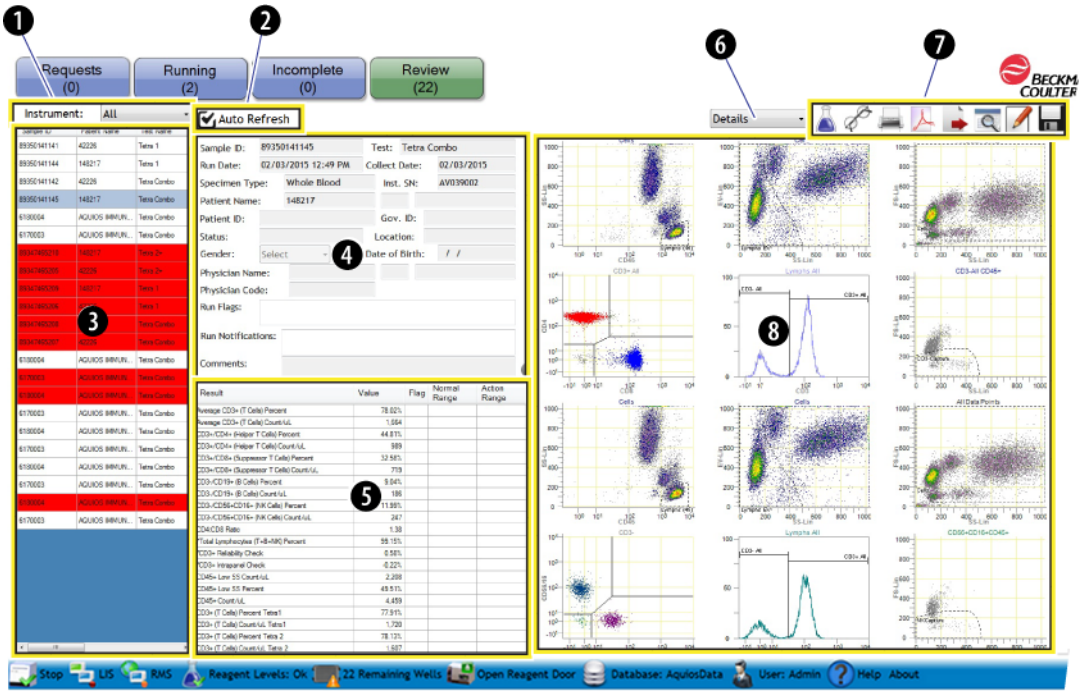
The Review screen displays analyzed samples that were previously run and that are currently pending review and/or transmission. The Review screen can be customized with four pre-defined graph combinations: Histograms, DotPlots, Details, or Results. These screens allow you to review, edit, and save changes to regions, gates, compensation and/or patient information prior to transmitting results to the LIS. For easy editing, you can zoom any of these displays as needed. Also, the results can be printed or exported as a PDF, as a Flow Cytometry Standard listmode (.lmd) or native format (.crd) file. Both sample history changes and reagent information are available for review. All the displays can be zoomed in for easier editing.

The following Review screen (Figure 5.1) illustrates the different display areas associated with a typical Review screen. This is an example; individual results vary.

NOTE The Review Screen can be enabled to automatically refresh to display the last completed run.

NOTE If results for a run are auto-transmitted, they will not appear under the Review Tab. In this case, you will need to reference these results under the Results Tab. See Results Screen.

Figure 5.1 Review Screen: Display Areas



1. Instrument Designation
2. Auto Refresh
3. Completed Run List
4. Sample Identifiers and Run Information Area
5. Result Table Area
6. Graphic Options Selection (drop-down box)
7. Action Toolbar
8. Selected Graphic Display

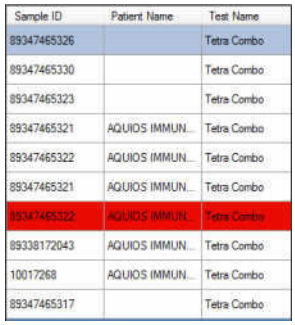
NOTE When a sample is selected for review, the default report selected appears for that sample. Select the Results, Histograms, or DotPlots option from the drop-down box.

Review Screen Display Areas (left to right)

Allows for selection of data based on the instrument that was used to collect the data. This is important when multiple instruments are networked. This feature allows the Operator to view data generated by other instruments from any networked Workstation.

Automatically refreshes to display the last completed run.

Figure 5.2 Review Screen: Completed Runs List



This area presents completed runs that are awaiting review and release. The last run completed is located at the top of the list. The runs are identified by:

- Sample ID
- Patient Name
- Test Name
- Run Date
- Patient ID
- ID

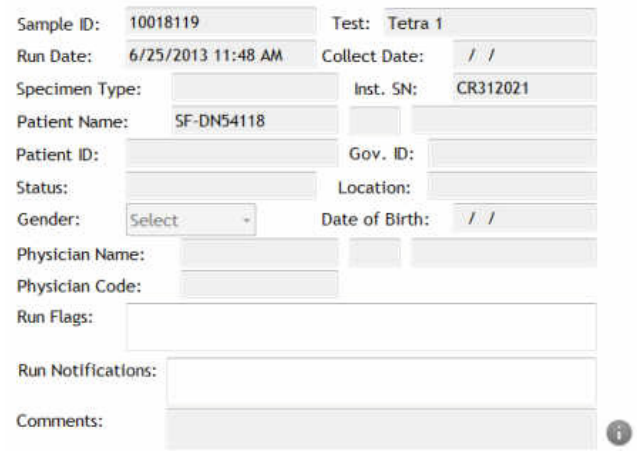
Any test that generated one or more Notification is highlighted in orange.

Any test that resulted in the generation of one or more Flags is highlighted in red.

Samples not highlighted have no flags or notifications.

NOTE Use the horizontal scroll bar to see all fields.

Figure 5.3 Review Screen: Sample Identifiers and Run Information Area



This area provides the sample information that was entered when the test request was generated. It also provides the information regarding when the test was run and displays any Flag or Notification messages generated during the run. See Flags And Notifications.

Next to the comments field is  . Select this to see details about your sample. If a result is modified, the comment field is available for documentation regarding why the modification was performed.

Figure 5.4 Review Screen: Result Table Area (for AQUIOS Tetra Combo)

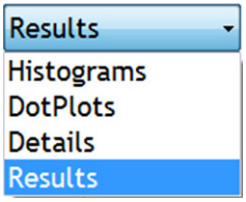
Result	Value	Flag	Normal Range	Action Range
Average CD3+ (T Cells) Percent	78.02%			
Average CD3+ (T Cells) Count/uL	1,664			
CD3+/CD4+ (Helper T Cells) Percent	44.81%			
CD3+/CD4+ (Helper T Cells) Count/uL	989			
CD3+/CD8+ (Suppressor T Cells) Percent	32.58%			
CD3+/CD8+ (Suppressor T Cells) Count/uL	719			
CD3-/CD19+ (B Cells) Percent	9.04%			
CD3-/CD19+ (B Cells) Count/uL	186			
CD3-/CD56+CD16+ (NK Cells) Percent	11.99%			
CD3-/CD56+CD16+ (NK Cells) Count/uL	247			
CD4:CD8 Ratio	1.38			
*Total Lymphocytes (T+B+NK) Percent	99.15%			
*CD3+ Reliability Check	0.58%			
*CD3+ Intrapanel Check	-0.22%			
CD45+ Low SS Count/uL	2,208			
CD45+ Low SS Percent	49.51%			
CD45+ Count/uL	4,459			
CD3+ (T Cells) Percent Tetra1	77.91%			
CD3+ (T Cells) Count/uL Tetra1	1,720			
CD3+ (T Cells) Percent Tetra 2	78.13%			
CD3+ (T Cells) Count/uL Tetra 2	1,607			

This area provides the results generated from the test run, in tabular format. See Quality Control Checks for information on how results are rounded.

NOTE Parameters listed depend on the panel requested: AQUIOS Tetra-1, AQUIOS Tetra-2+, or AQUIOS Tetra Combo (as shown in Figure 5.4).

NOTE Results can be shown or hidden. See Test Setup in Chapter 8 Setup in the AQUIOS CL Flow Cytometer Instructions For Use manual.

Figure 5.5 Review Screen: Graphic Options Selection Menu



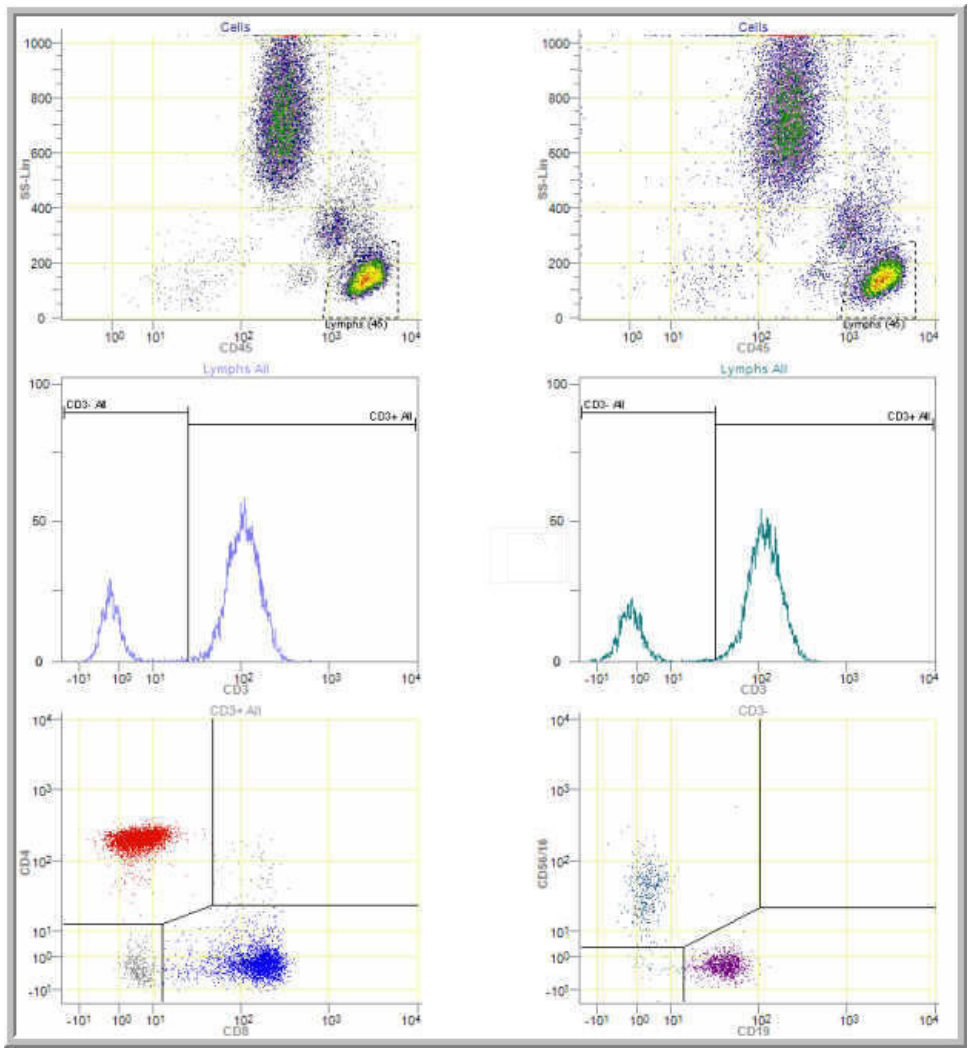
Graphic options are accessed by selecting the desired view from a drop-down menu.

The graphic display on the Review screen can be customized by selecting one of four predefined graph combinations:

- Histograms
- DotPlots
- Details
- Results

In Figures 5.5 and 5.6, the graphic options drop-down box identifies **Results** was selected as the graphic display option.

Figure 5.6 Review Screen: Results Area Display (AQUIOS Tetra Combo)



NOTE When a graphic option is selected, the graphs that are displayed depend on the test that was requested and completed: AQUIOS Tetra-1, AQUIOS Tetra-2+, or AQUIOS Tetra Combo (as shown in Figure 5.6).

Graphic Options

The following Review screens illustrate the different graphic options available for each individual patient result. These are examples only. Results may vary.

NOTE When a sample is selected for review, the Details Review screen appears for that sample. Select the Results, Histograms, or DotPlots option from the drop-down box.

- Figure 5.7, Review Screen: AQUIOS Tetra Combo Details
- Figure 5.8, Review Screen: AQUIOS Tetra Combo Results
- Figure 5.9, Review Screen: AQUIOS Tetra Combo Histograms
- Figure 5.10, Review Screen: AQUIOS Tetra Combo DotPlots

Figure 5.7 Review Screen: AQUIOS Tetra Combo Details

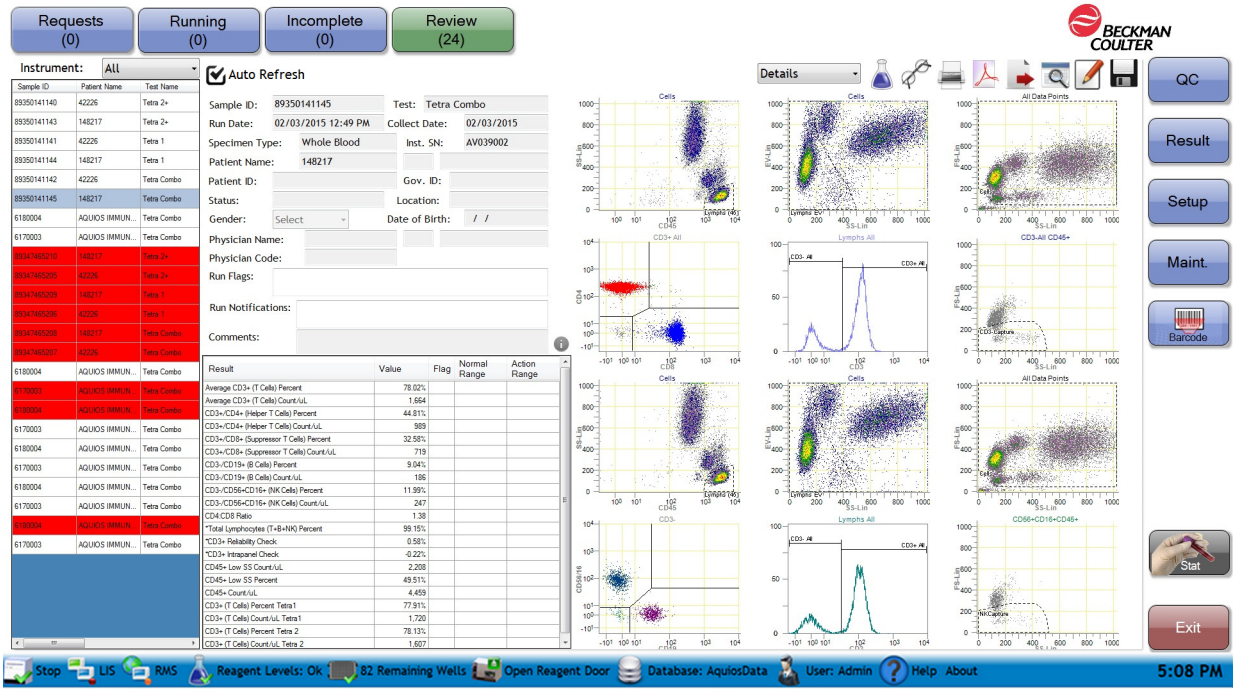


Figure 5.8 Review Screen: AQUIOS Tetra Combo Results

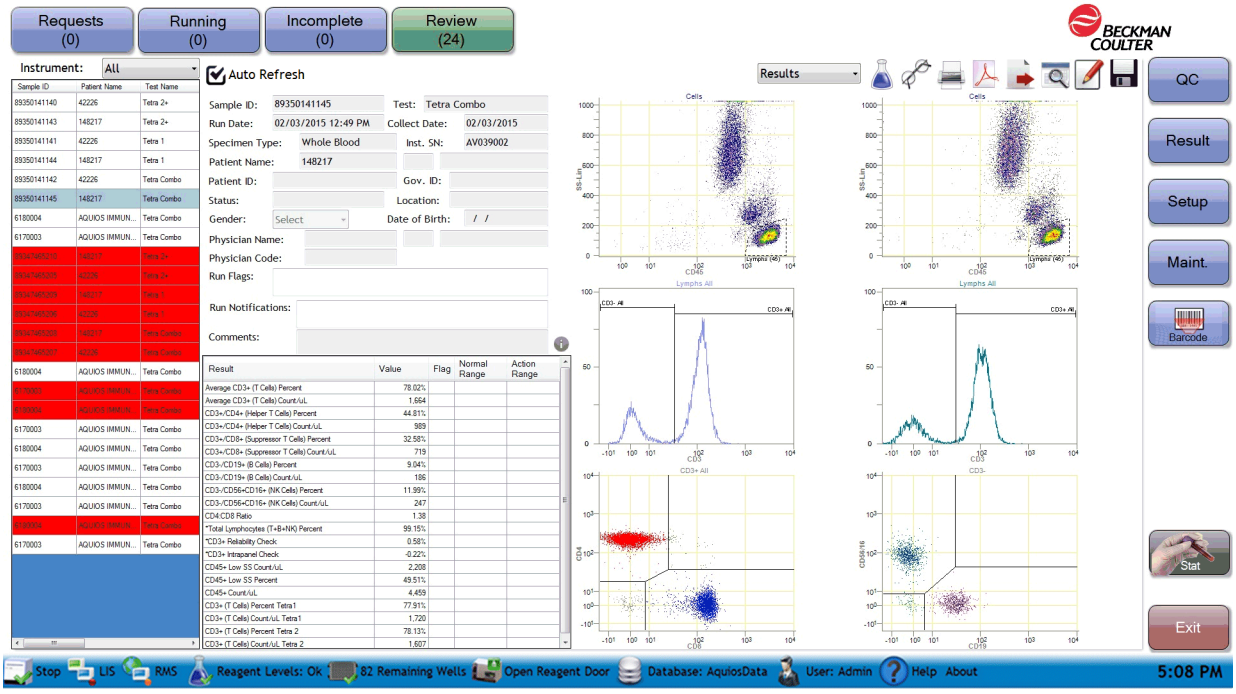


Figure 5.9 Review Screen: AQUIOS Tetra Combo Histograms

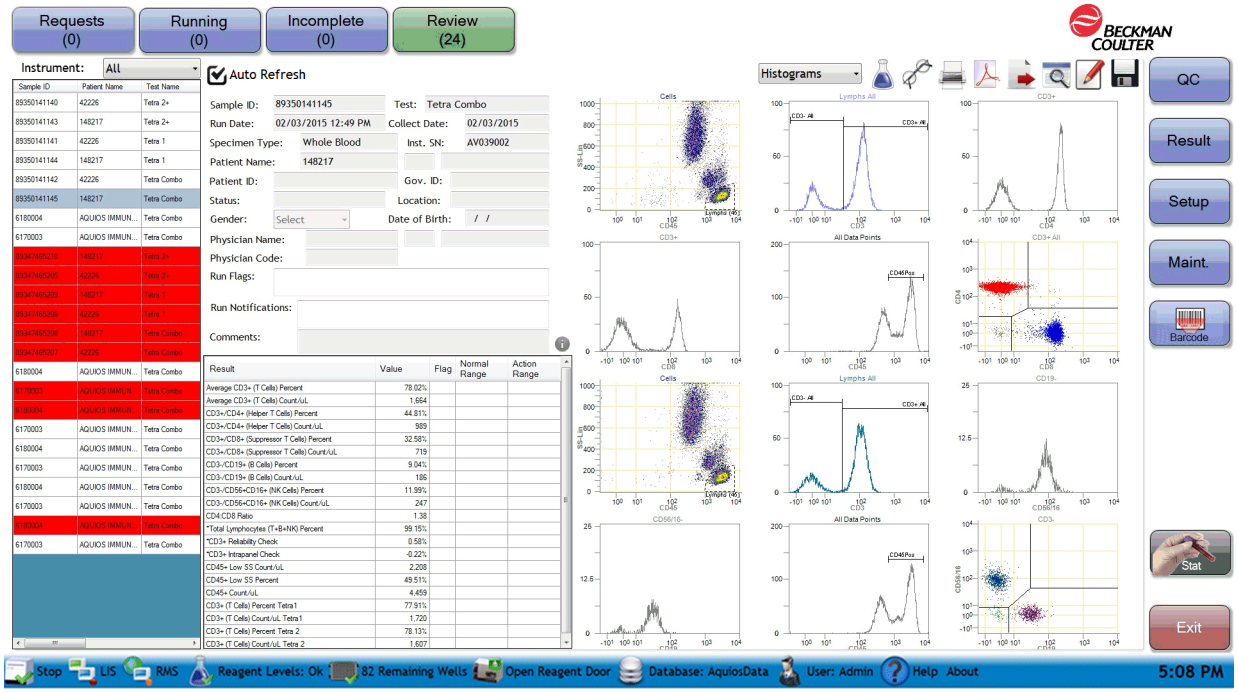
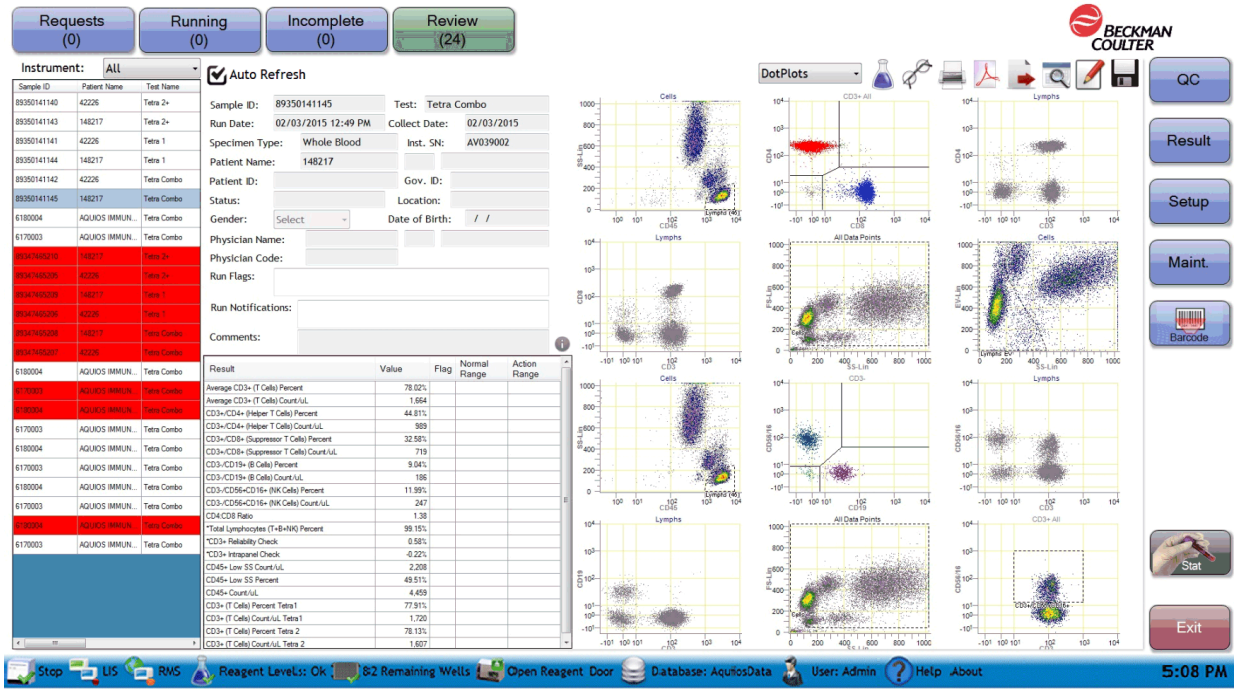


Figure 5.10 Review Screen: AQUIOS Tetra Combo DotPlots



Action Toolbox

Use the Action Toolbox (Figure 5.11) on the Review (64) screen to review, edit, and save changes to both regions and/or patient information prior to transmitting results to the LIS. Also, the results can be printed or exported in a PDF or CRD or LMD format (CRD and LMD files can be exported from the Review screen only). Both the sample history changes and the reagent information are available for review. All the displays can be zoomed in for easier editing. See the Chapter 6, Data Review in the AQUIOS CL FLow Cytometer Instructions for Use manual.

Figure 5.11 Action Toolbox

Data Review

Review Data Displays

⚠ CAUTION

Risk of misleading results if the lymphocyte gate is contaminated with other cell subpopulations. It is strongly recommended to review the collected specimen data prior to releasing sample results to ensure that monocytes or basophils are not contaminating the lymphocyte gate. Refer to Table 5.2 for specific examples of the acceptable and unacceptable placement of gates and the corresponding instructions for adjustment of gating.

IMPORTANT There is a risk of reporting incorrect results. Data displays used to arrive at the test result for light scatter patterns, antibody staining profiles, and all gates and boundaries should be reviewed by a laboratory professional when interpreting the data. If results are suspect, follow your laboratory's procedures to resolve.

Data review should include the following (refer to Tables 5.2 through 5.6 for the cell population identity and specific examples of acceptable and unacceptable placement of gates and regions):

1. Review the light scatter and EV pattern.
   a. Verify any population of interest is above the discriminator to ensure no cell loss.
   b. Verify any population of interest is resolved as much as possible from other populations that are present (within biological constraints).
2. Review the antibody staining patterns.
   a. Verify overall expected staining patterns are observed (the fluorescence staining correlates to the antibody used in sample preparation).
   b. Verify abnormally dim or negative sample staining patterns are reviewed to ensure agreement with clinical presentation and other diagnostic data.

3. Review Gate and Region boundaries.
   a. Verify gates and regions encompass any populations of interest and exclude any undesired events.
   b. All red blood cells may not lyse under the following conditions: presence of nucleated red blood cells, abnormal protein concentration, or hemoglobinopathies. Use of CD45 as a gating condition for lymphocytes ensures that RBCs, as CD45 negative events, are not included in the lymph gate.

Analytic Reliability Checks

For verification of gating accuracy, review gating results according to the following reliability check criteria. Refer to Tables 5.2 through 5.6 for the cell population identity and specific examples of acceptable and unacceptable placement of gates and regions.

1 For the AQUIOS Tetra Combo (AQUIOS Tetra-1 Panel and AQUIOS Tetra-2+ Panel) results:

CD3+ Intrapanel Check:

a. For CD3+ Intrapanel Check, variability between AQUIOS Tetra-1 and AQUIOS Tetra-2+ for CD3 percent positive results serves as an internal control.[10]
b. Differences between replicate CD3 percent positive results should be ±3.5%.
c. Refer to Step 4 for actions to take for analyses that exceed the ±3.5% acceptance criteria.

2 For the AQUIOS Tetra-2+ Panel CD45-FITC/(CD56+CD16)-RD1/CD19-ECD/CD3-PC5 results:

Total Lymphocytes (T+B+NK) Percent:

a. This reliability check is also known as LymphoSum.
b. Total lymphocyte percentage should be determined using the following formula: Total Lymphocyte Percentage (%) = %CD3+(T) +%CD19+(B) + %CD3-(CD56+CD16+)(NK).[7, 8]
c. Optimally, LymphoSum should be 95-105%.
d. Refer to Step 4 for actions to take for analyses that exceed the 95-105% acceptance criteria.

3 For the AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 results:

CD3+ Reliability Check:

a. Optimally, the sum of the percentages of CD3+CD4+ and CD3+CD8+ cells should equal the total percentage of CD3+ cells ±5%.
b. For specimens containing a considerable number of dual negative CD4-CD8- cells and/or dual positive CD4+CD8+ T-cells, this reliability check may exceed the maximum variability and thus is not indicative of a system failure and the result may therefore be reported if there are no other flags or notifications.[6,7,8,9]

c. Refer to Step 4 for actions to take for analyses that exceed the ±5% acceptance criteria and where specimens do not contain a considerable number of dual negative CD4-CD8- cells and/or dual positive CD4+CD8+ T-cells.

d. Refer to Limitations in CHAPTER 1, Use and Function for additional information about CD3+ Reliability Check acceptance criteria. (See Limitation 14.)

4 If results fail to meet the acceptance criteria,

a. Evaluate specimen conditions.

b. Review gate and region boundaries; to adjust regions see Edit Regions for a Review Sample. If this does not correct the analytic reliability check failure, proceed to step c.

c. Run a Bleach Cycle.

d. Re-run sample; Re-Request from the Review Options menu.

e. If the problem persists across multiple samples, contact your local Beckman Coulter Representative.

Population Statistics

The statistics presented in the Table 5.1 below are used as indicators of pre-analytical sample handling issues, instrument performance and to assist in troubleshooting instrument performance issues.

Table 5.1 Population Statistics Recommended Use

Population Statistics	Recommended Use
**CD3+/CD4-/CD8- Percent **CD3+/CD4-/CD8- Count/uL **CD3+/CD4+/CD8+ Percent **CD3+/CD4+/CD8+ Count/uL **CD3+/CD4+/CD8- Percent **CD3+/CD4+/CD8- Count/uL **CD3+/CD4-/CD8+ Percent **CD3+/CD4-/CD8+ Count/uL	Can be used as additional parameters in troubleshooting for CD3+ Reliability Check (The sum of CD3+/CD4+ % and CD3+/CD8+ % should be equal to CD3+ % ± 5%) For specimens containing a considerable number of dual negatives T-cells (≥ 5%), the reliability check may exceed the maximum variability. Information on additional parameters are provided in this table to assess the presence of dual negative CD3+/CD4-/CD8-, and dual positive CD3+/CD4+/CD8+ T cell populations.
**CD3-/CD56+CD16+/CD19+ Percent **CD3-/CD56+CD16+/CD19+ Count/uL **CD3-/CD56-CD16-/CD19- Percent **CD3-/CD56-CD16-/CD19- Count/uL	Can be used as additional parameters in troubleshooting for Total Lymphocytes (T+B+NK), also referred to as Lymphosum reliability QC (The sum of CD3+ %, CD3-/CD56+CD16+ % and CD3-/CD19+ % shall be 100 ±5%). The presence of dual positive (CD3-/(CD56+CD16+)/CD19+) cells and triple negative (CD3-/(CD56-CD16-)/CD19-) cells is used to assess the failure of Lymphosum reliability QC. Presence of triple negative cells may be indicative of intruding cells in the lymphocyte gate.

Table 5.1 Population Statistics Recommended Use

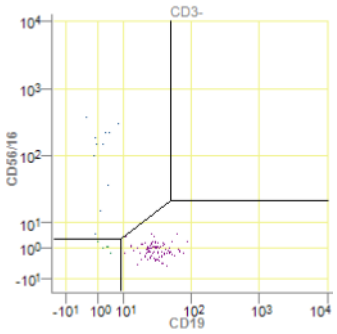
Population Statistics	Recommended Use
**CD3+/CD56+CD16+ Percent **CD3+/CD56+CD16+ Count/uL	Can be used as additional internal control of sample staining and preparation. Review of CD3+/CD56+CD16+ cells can be indicative if CD56/CD16 staining is adequate in case of very low levels of CD3-CD56+/CD16+ populations. Example of specimen with very low level of CD3-CD56+/CD16+ populations is provided below:  Statistic — Value CD3-/CD56+CD16+ (NK Cells) Percent — 1.05% CD3-/CD56+CD16+ (NK Cells) Count/uL — 3 **CD3+/CD56+CD16+ Percent — 17.29% **CD3+/CD56+CD16+ Count/uL — 44
**CD4 Separation **CD8 Separation **CD56/16 Separation **CD19 Separation **CD3 Separation **CD45 Lymph/High SS Cells Separation **SS Lymph/High SS Cells Separation **FS Lymph/High SS Cells Separation	These values are reported as QC parameters when running AQUIOS IMMUNO-TROL and IMMUNO-TROL Low controls. Refer to the AQUIOS CL IFU, Chapter 4 and AQUIOS Tetra System Guide Chapter 3 for further information on separation values. When reported for specimens, these values can be used to assess population resolution. The separation value is an indication of how well resolved two cellular populations are. Separation ranges and examples are provided in AQUIOS Tetra System Guide Chapter 3.
**CD4+ Channel Number **CD8+ Channel Number **CD56/16+ Channel Number **CD19+ Channel Number **CD45 Lymphs Channel Number **SS Lymphs Channel Number **FS Lymphs Channel Number **CD3+ Channel Number	Provides numeric aid to visual inspection of the results. Values are tracked for AQUIOS IMMUNO-TROL and IMMUNO-TROL Low controls. Refer to the AQUIOS CL IFU Chapter 4 and AQUIOS Tetra System Guide Chapter 3 for further information. Can be used to assess the fluorescence intensity of the population. Significant change may be an indication of potential reagent or sample flow cytometry analysis issues.
**CD4- Channel Number **CD8- Channel Number **CD56/16- Channel Number **CD19- Channel Number **CD3- Channel Number	Provides numeric aid to visual inspection of the results. Refer to the AQUIOS CL IFU Chapter 4 and AQUIOS Tetra System Guide Chapter 3 for further information. Can be used to assess the fluorescence intensity of the population. Significant change may be an indication of potential reagent or sample flow cytometry analysis issues.

Table 5.1 Population Statistics Recommended Use

Population Statistics	Recommended Use
**CD45+ Low SS Events	This statistic provides the number of acquired Lymphocyte cell events. Can be used to ensure that the acquired number of cells is adequate to provide an accurate analysis of all lymphocyte subsets. May be used in conjunction with Total Count parameter to assess ratio of RBC and Lymphocyte populations in case of underlysed samples.
**Total Count	Can be used as a troubleshooting parameter to assess total acquired events and assess underlysing.

Edit Regions for a Review Sample

IMPORTANT Do NOT move the regions for control runs.

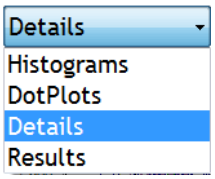
IMPORTANT Sample ID, Test, Run Date, and Serial # cannot be edited.

IMPORTANT Editing or adjusting regions is not a routine adjustment. Regions must only be adjusted by an Operator with special access, such as an Administrator or Reviewer/Editor.

1 Select 

.

2 Select the sample of interest from the Review Screen Completed Run List. See Figure 5.2. Select the view menu option from the menu below.



3 Select  . The Edit action toolbox appears, and the fields for Sample ID, Test and Run Date and Instrument Serial Number turn grey to indicate that this is information that cannot be changed.

4 Select any other field to add or change patient information.

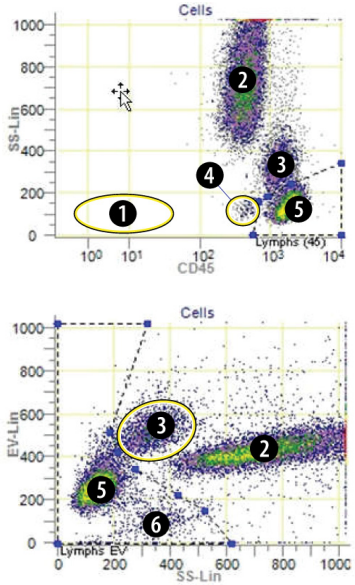
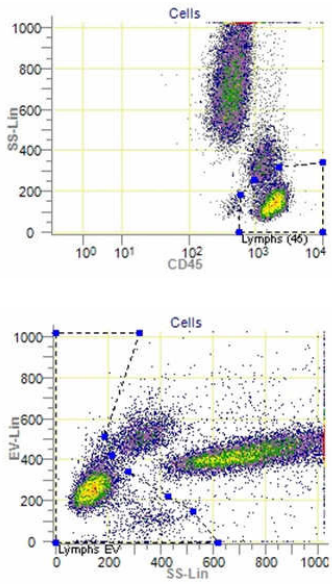
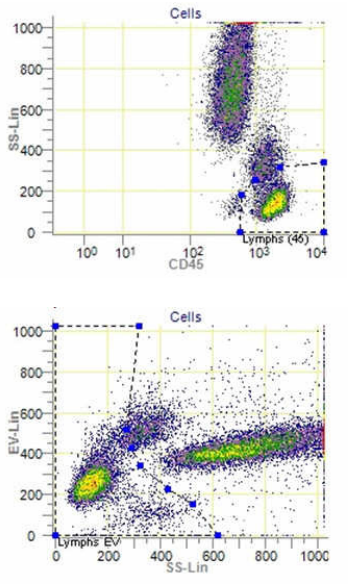
5 Select the plot that you want to edit.

a. Double-click the graph to enlarge.

b. Click on the region you want to edit.

Table 5.2 Editing Regions on Detail Plots for AQUIOS Tetra-1 and AQUIOS Tetra-2+

DETAIL PLOTS
AQUIOS Tetra-1 and AQUIOS Tetra-2+ CD45 vs SS-Lin + AQUIOS Tetra-1 and AQUIOS Tetra-2+&2 SS-Lin vs EV-Lin • Lymph gate is defined from the following two plots: SS-Lin versus CD45 and EV versus SS-Lin. • Drag or reshape the region using the mouse to edit gate Lymphs (45) to include the lymphocytes which have bright CD45+FITC fluorescence and low SS (see Acceptable example). In this analysis, Lymphocytes are included while monocytes (lower CD45 expression and intermediate SS) and basophils (lower CD45 and low SS) are excluded as much as possible. • Drag the pointer using the mouse to edit gate Lymphs (EV) gate to include the lymphocytes that have low and medium SS (see Acceptable example). In this analysis, Lymphocytes are included while monos and baso are excluded as much as possible. Refer to Table 5.3 to identify the numbered cell populations.

Acceptable	Acceptable	**Unacceptable**
		

NOTE Occasionally, the CD45 gate is loosely set, but this can be acceptable as long as the EV versus SS region is drawn correctly.

Table 5.3 Cell Populations

1	Debris / unlysed RBC
2	Granulocytes
3	Monocytes
4	Basophils
5	Lymphocytes
6	Fallen Lymphocytes

Table 5.4 Editing Regions on Detail Plots for Tetra-1

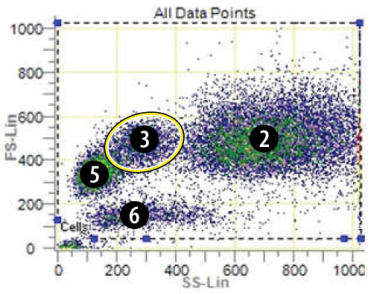
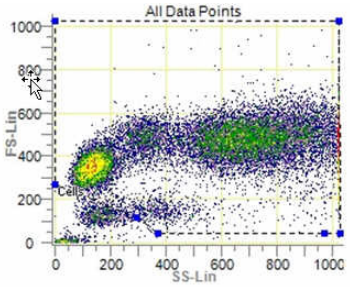
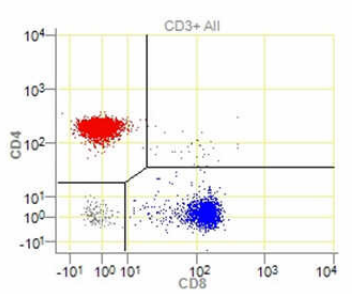
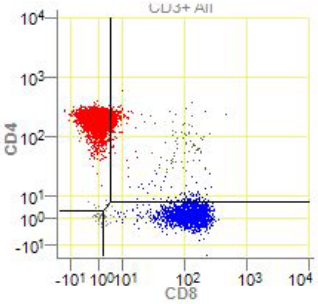
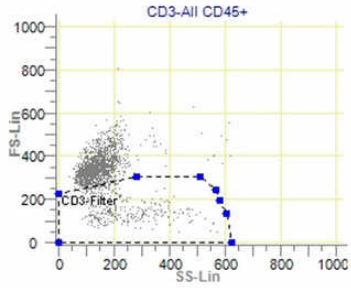
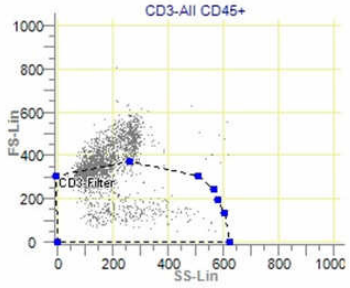
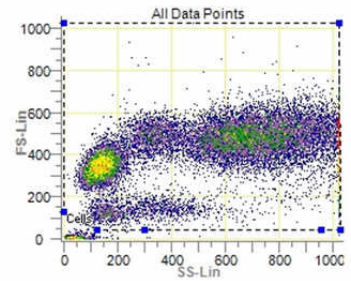
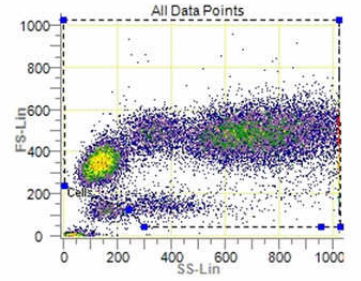
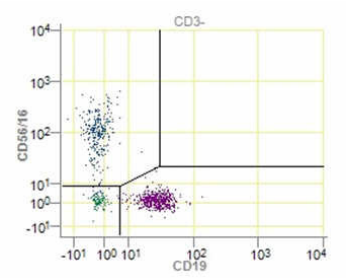
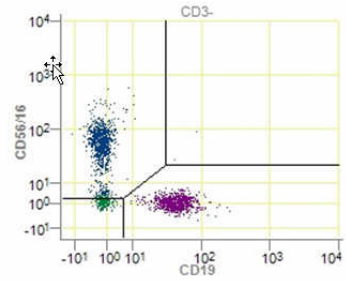
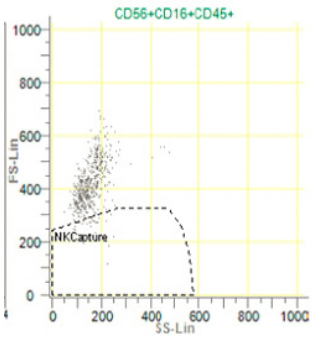
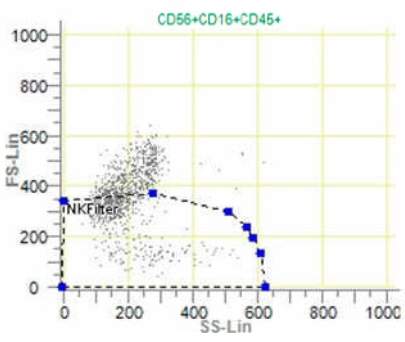
DETAIL PLOT	
AQUIOS Tetra-1 FS-Lin vs SS-Lin (All Data Points) • Drag or reshape the region to edit **Cells gate** to include all white blood cells: lymphocytes, fallen lymphocytes, monocytes, granulocytes (see Acceptable example). Refer to Table 5.3 to identify the numbered cell populations.	
Acceptable	**Unacceptable**
	
AQUIOS Tetra-1 CD3+ All CD4 vs CD8 • Set region boundaries on the two parameter histograms to bracket the double negative populations (CD3+CD4- and CD3+CD8-) and to encompass the CD3+CD4+ and CD3+CD8- populations as illustrated in the Acceptable example.	
Acceptable	**Unacceptable**
	
AQUIOS Tetra-1 CD3-All CD45+ FS-Lin vs SS-Lin • Set CD3-Capture region boundaries to tightly encompass the lymphocytes with low FS and low SS expression.	
Acceptable	**Unacceptable**
	

Table 5.5 Editing Regions on Detail Plots for Tetra-2+

DETAIL PLOT	
AQUIOS Tetra-2+ FS-Lin vs SS-Lin (All Data Points) • Drag or reshape the region to edit **Cells gate** to include all white blood cells: lymphocytes, fallen lymphocytes, monocytes, granulocytes (see Acceptable example).	
Acceptable	**Unacceptable**
	
AQUIOS Tetra-2+ CD56/16 vs CD19 • Set region boundaries on the two parameter histograms to bracket the double negative population (CD3-CD19- and CD3-CD56-) and to encompass the CD3-CD56+CD16+ and the CD3-C19+ populations as illustrated in the Acceptable example. **NOTE** The divider that separates negative from positive CD56/16, is by default, close to the negatives. This region should not be adjusted if not necessary.	
Acceptable	**Unacceptable**
	
AQUIOS Tetra-2+ CD56+CD45+ FS-Lin vs SS-Lin • Set region boundaries for NK Capture to encompass the low FS and low SS CD56+16+ CD45+ cells as illustrated in the Acceptable example.	
Acceptable	**Unacceptable**
	

6 Double-click the enlarged graph to return to standard view.

7 Select  to refresh the region statistics.

8 If you need to correct your modification, select  and then  to reject the change. Repeat the steps above.

9 Select  to save the changes to the file.

Edit Regions for a Review Sample (Histograms View)

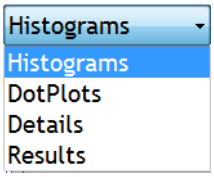
IMPORTANT Editing or adjusting regions is not a routine adjustment. Regions must only be adjusted by an Operator with special access, such as an Administrator or Reviewer/Editor.

NOTE Sample ID, Test, Run Date, and Serial # cannot be edited.

1 Select 

.

2 Select the sample of interest from the Review Details List.



3 Select  . The Edit action toolbox appears, and the fields for Sample ID, Test and Run Date turn grey to indicate that this information can no longer be edited.

4 Select any other field to add or change patient information.

5 Select the histogram that you want to edit. See Table 5.6.

a. Double-click the graph to enlarge.

b. Choose the region you want to modify.

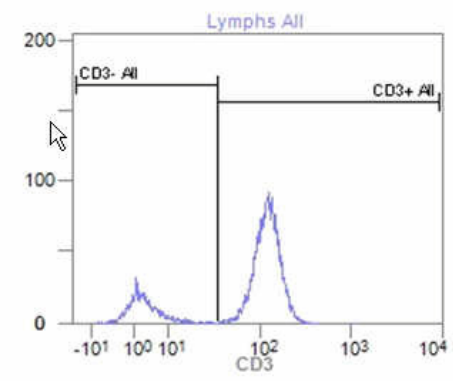
Table 5.6 Editing Regions on Detail Histograms

DETAIL HISTOGRAM

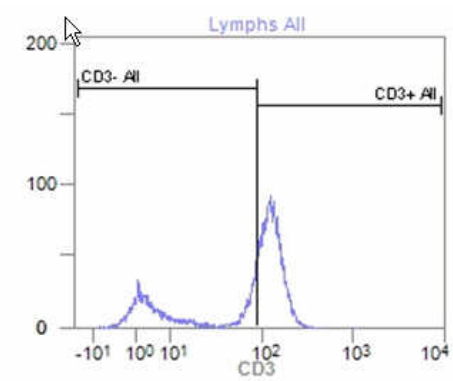
AQUIOS Tetra-1 Lymphs All CD3 Histogram

- Set a region boundary on the Total CD3+ histogram to encompass the CD3+ population as illustrated in the Acceptable example.

Acceptable



Unacceptable



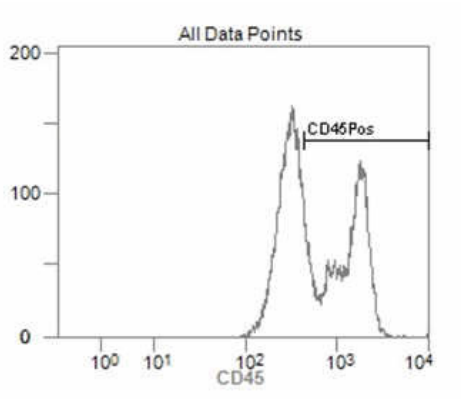
AQUIOS Tetra-1 All Data Points CD45 Histogram

- Set a region boundary on the Total CD45+ histogram to encompass the CD45+ population as illustrated in the Acceptable example.

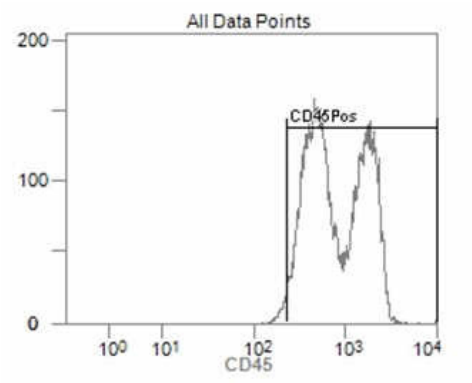
Table 5.6 Editing Regions on Detail Histograms

DETAIL HISTOGRAM

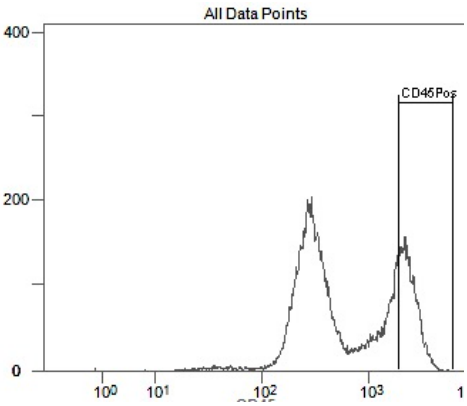
Acceptable



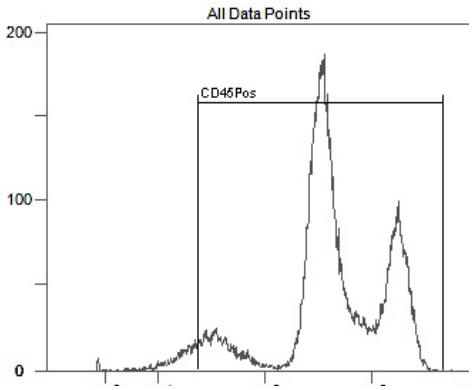
Acceptable



Unacceptable



Unacceptable



6 Double-click the enlarged histogram to return to standard view.

7 Select  to refresh the region statistics.

8 If you need to correct your modification, select  and then  to reject the change. Repeat the steps above.

9 Select  to save the changes to the file.

Adjust Compensation Manually

IMPORTANT Adjustment to compensation may be required infrequently. Circumstances may include:

- At installation when compensation is performed the first time.
- After service is performed.
- The operator determines that compensation needs to be adjusted (a check with controls is recommended).

IMPORTANT Compensation can only be adjusted by an Operator with special access, such as an Administrator or Reviewer/Editor. The user has the ability to adjust the compensation, however, new compensation settings will only be applied to future runs, and not any prior run(s) which prompted the adjustment. New compensation values must be verified by running a control after adjusting the compensation matrix.

Compensation can be adjusted as follows:

1 Select 

.

2 Select the sample of interest from the Review Details List.

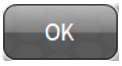
3 Select  . The Edit action toolbox appears.

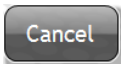
4 Select  .

5 If an AQUIOS Tetra Combo sample is selected, the message *which run do you wish to edit*? appears:

- Select Type ***1*** for Tetra-1 run of the selected Tetra Combo
- Select Type ***2*** for Tetra-2+ run of the selected Tetra Combo

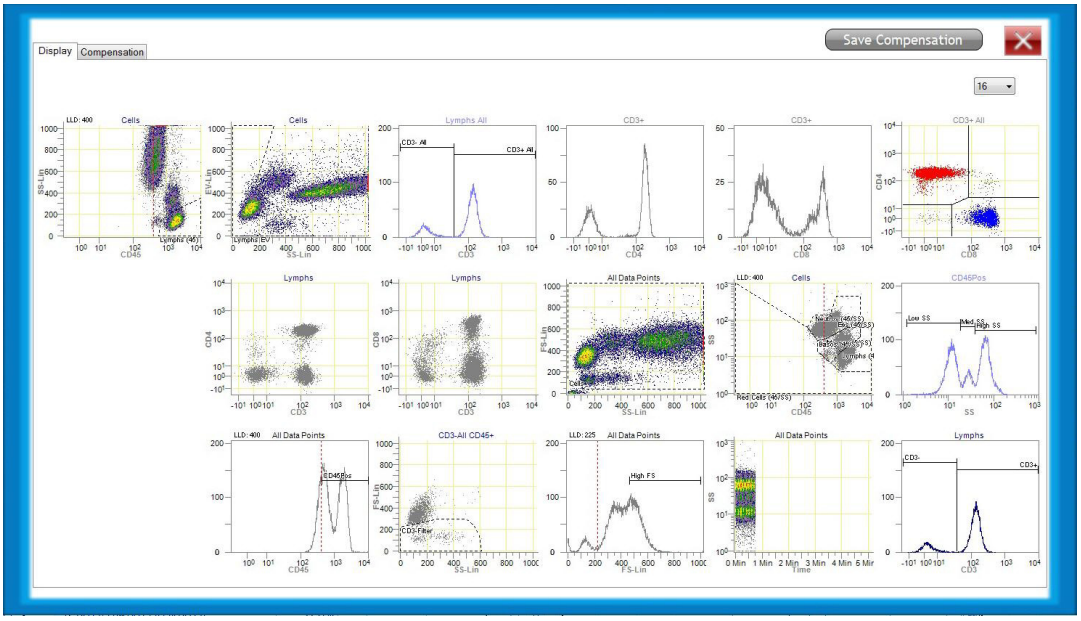
NOTE When adjusting compensation in a combo run, be aware that adjusting compensation to future runs from an already run Combo Tetra does not affect Tetra 2+ and vice versa. If you apply "adjust compensation to future runs" in a run where compensation was not adjusted, the original values will be applied (no adjustment) to future runs.

6 Select 

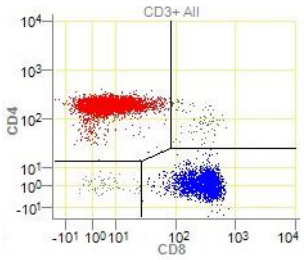
to edit compensation or select 

to cancel the adjust compensation action.

7 Adjust from Display tab as show below:



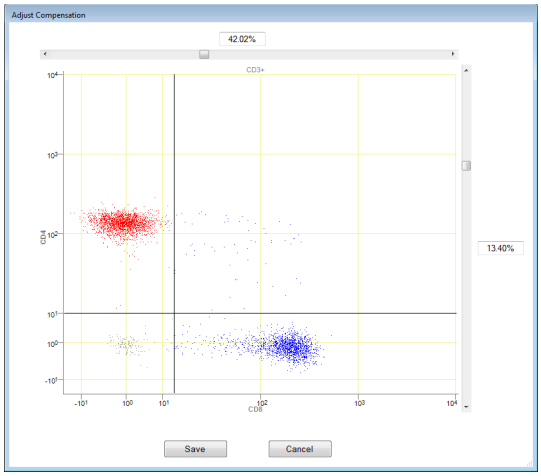
a. Right click on the Dual Fluorescent parameters plot you wish to adjust as shown below. Only fluorescence plots may be adjusted.



b. Select **Adjust Compensation**.

c. Use slider bars to adjust compensation manually so that the populations are properly displayed. The compensation should be adjusted as shown in Figure 5.12.

Figure 5.12 Adjust Compensation



1. Scroll bars - Manually adjust compensation
2. Save - Record changes in compensation.
3. Cancel - Close the adjust compensation dialog without saving changes.

NOTE Do not over compensate the sample as shown in Figure 5.13 or under compensate the sample as shown in Figure 5.14.

Figure 5.13 Over Compensation

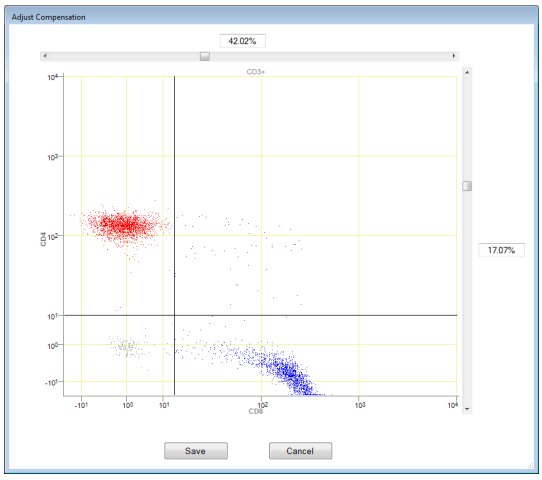
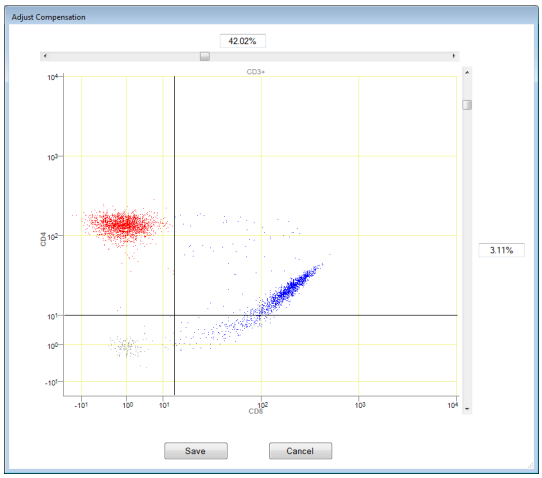


Figure 5.14 Under Compensation



d. Select Save.

---

8 Select Save Compensation, select Yes to save the changes to Future Runs or Cancel to cancel the changes.

NOTE After saving to Future Runs, re-run of controls is recommended. See Run QC Samples in CHAPTER 3, Quality Control.

9 Select Save.

NOTE Compensation changes are recorded in the Maintenance Log. See Maintenance Screen in Chapter 8, Setup in the AQUIOS CL Flow Cytometer Instructions for Use manual.

NOTE To revert to the previous compensation, go to the previous run and resave the compensation.

Result Screen

NOTE For a more detailed explanation of this screen and its components, see Chapter 6, Data Review in the AQUIOS CL Flow Cytometer Instructions for Use manual.

The Result screen provides a search menu to navigate through the results database. The Search criteria is entered under Search and the results for the query are displayed under View Results.

NOTE Results can also be accessed and viewed through Today or Last 7 Days.

To review the status of a sample, see the **Review Option** icons below (under the action toolbox).

NOTE Once the Review Option is selected, the sample is removed from the Review screen but it continues to be accessible through the Result screen.

 indicates that the sample was reviewed.

 indicates that the sample was reviewed and rejected.

 indicates that the sample was not reviewed.

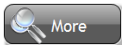
Using the Result Screen to Select Data for Review

Once you have selected 

, you will have access to the following options:

- Search results by the following criteria:
  - Sample ID
  - Patient ID
  - Gov. ID
  - Patient Name
  - Test
  - Instrument Start Date
  - Instrument End Date
  - QC Results
  - Reviewed Only
- Clear search criteria.
- Export results to a .crd, .lmd, .pdf or .csv file.

  NOTE LMD files can be exported as FCS2.0 Low Res Listmode Files or as FCS3.1 High Res Listmode Files.

- Do advanced searches using 

.
- Fields to be displayed as columns in View Results screen.
- Exclude run from View Results screen.
- Load a Query request.
- Append to a current search.
- Save a query result.

For detailed instructions for using any of these options, see Chapter 6 in the AQUIOS CL Flow Cytometer Instructions manual.

Example Result Screens and Associated Reports

The following review screens illustrate the different display options available for an individual patient result. These are examples only. Results may vary.

NOTE When a sample is selected for review, the Details Review screen appears for that sample. Select the Results, Histograms, or DotPlots option from the drop-down box.

- Figure 5.15, Result Screen: AQUIOS Tetra Combo Details
- Figure 5.16, Results Screen: AQUIOS Tetra Combo Results
- Figure 5.17, Results Screen: AQUIOS Tetra Combo Histograms
- Figure 5.18, Results Screen: AQUIOS Tetra Combo DotPlots

Figure 5.15 Result Screen: AQUIOS Tetra Combo Details

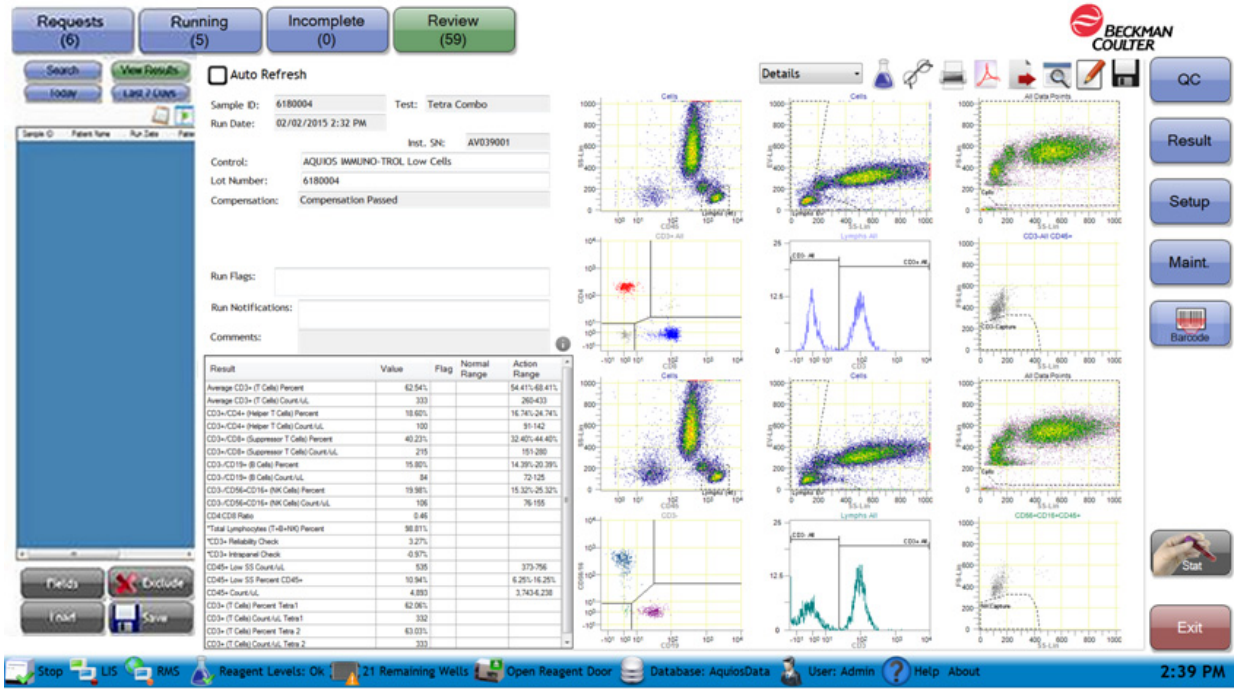


Figure 5.16 Results Screen: AQUIOS Tetra Combo Results

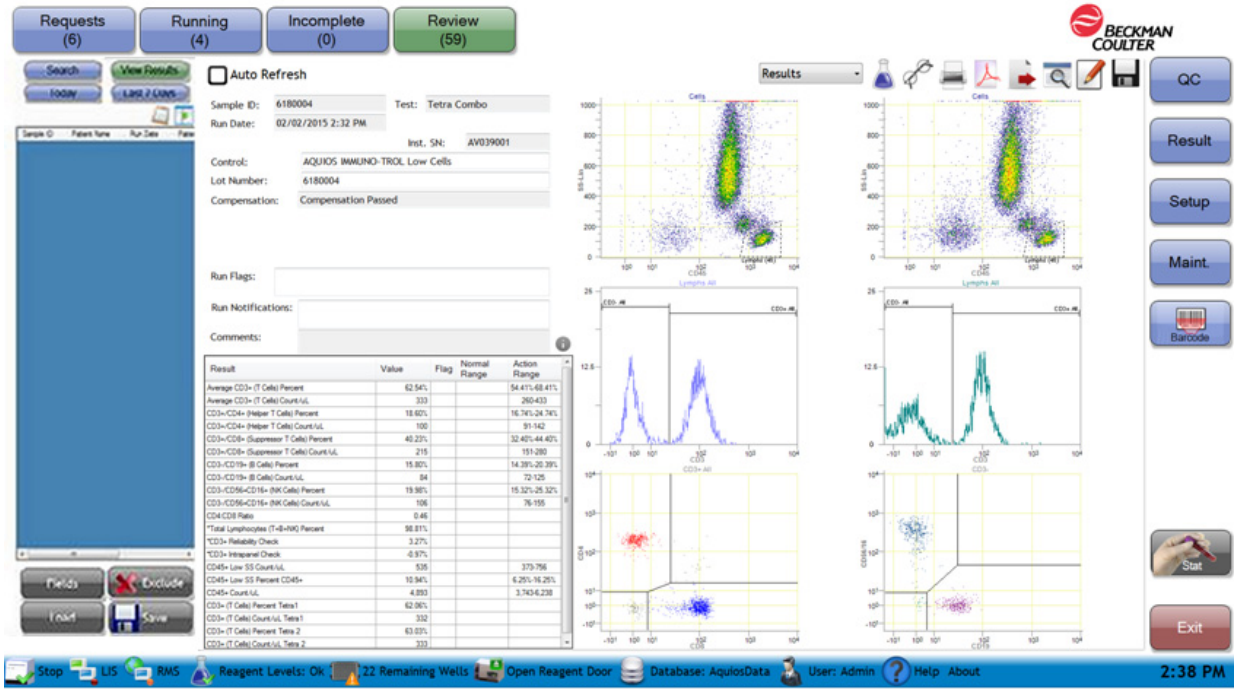
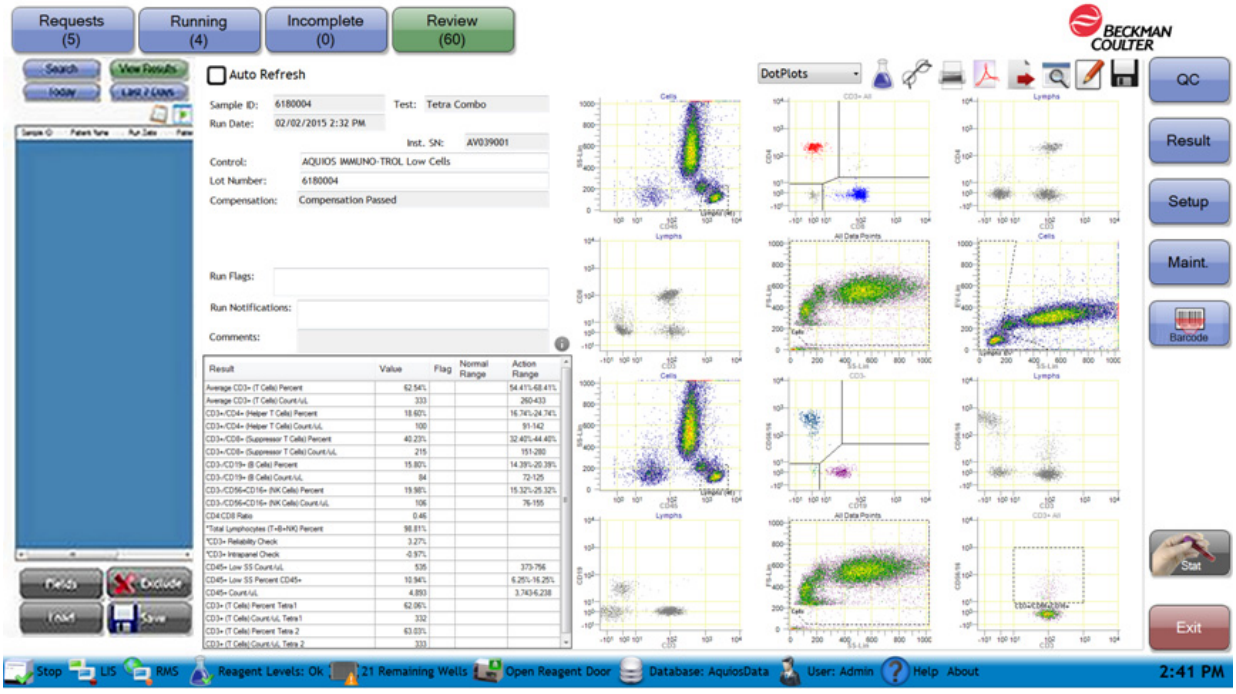


Figure 5.17 Results Screen: AQUIOS Tetra Combo Histograms

Figure 5.18 Results Screen: AQUIOS Tetra Combo DotPlots



Flags And Notifications

⚠ CAUTION

Beckman Coulter Inc. does not claim to identify every abnormality in all samples. Beckman Coulter suggests using all available options to optimize the sensitivity of instrument results. Options include:

- **Flags**
- **Notifications**
- **Reference range limits**

The AQUIOS System Software generates a set of algorithm flags and notifications. The AQUIOS Tetra Tests also generates flags and notifications that are specific for the AQUIOS Tetra application.

The flag and notification messages appear on the Review and Result screen. Samples with flags are highlighted in red, while samples with notifications are highlighted in orange.

All results with flags or notifications are automatically sent to the review tab for further review. Only samples with no flags or notifications can be setup for automatic transmission. A laboratory professional should review all results, regardless of Flags and Notifications, prior to data release. To setup automatic transmission, see the **Auto Transmit** heading in Chapter 8 in the AQUIOS CL Flow Cytometer Instructions for Use manual.

- For the list of AQUIOS System Software flags, notifications, and specific result notifications, see Chapter 6 in the AQUIOS CL Flow Cytometer Instructions for Use manual.
- For the list of AQUIOS Tetra specific flags and criteria, see Table 5.7. For AQUIOS Tetra specific notifications and criteria, see Table 5.8.

Flags

A Flag indicates an issue that affects the results, follow the Recommended User Action in the table below.

Table 5.7 AQUIOS Tetra Specific Flags

Flag	Definition	Recommended User Action
Insufficient Lysing	Too many red cells measured in the sample.	**1.** Ensure Lyse reagent volume is sufficient. **2.** Reject, Re-Request from the Review Options menu. **3.** If the problem persists, change the Lyse reagent set. **4.** If the problem persists, contact your local Beckman Coulter Representative.
Insufficient Lymphs	Too few Lymphs measured.	**1.** Review the sample results from the Review screen. This flag could be donor dependent or may be resolved with manual re-adjustment of the gating. **2.** If the condition affects the results, then select Reject, Re-Request from the Review Options menu. **3.** If the problem persists across different samples, contact your local Beckman Coulter Representative.
No Events	Not enough data points in the sample.	**1.** Run a Bleach Cycle. **2.** Reject, Re-Request from the Review Options menu. **3.** If the problem persists, contact your local Beckman Coulter Representative.
Compensation Flag (Control Only)	Populations do not line up as expected.	**1.** Review results to confirm compensation failure. **2.** Repeat Quality Control. See Run QC Samples in CHAPTER 3, Quality Control. **3.** Repeat Quality Control with new vials of control cells and new monoclonal reagents. **4.** Adjust compensation. See Adjust Compensation Manually. **5.** If the problem persists, contact your local Beckman Coulter Representative.
Inter Panel Count Error	Tetra-1 to Tetra-2+ CD3+ and CD45+ Low SS counts are inconsistent.	**1.** Run a Bleach Cycle. **2.** Reject, Re-Request from the Review Options menu. **3.** If the problem persists, contact your local Beckman Coulter Representative.

Notifications

Notifications are generated to convey an abnormal cell distribution or population which may be specimen specific. Follow the Recommended User Action in the table below.

Table 5.8 AQUIOS Tetra Specific Notifications

Flag	Definition	Recommended User Action
Under Lysed	A large number of red cells measured in the sample.	**1.** All red blood cells may not lyse under the following conditions: presence of nucleated red blood cells, abnormal protein concentration, or hemoglobinopathies. Higher underlysis is also observed for controls or samples analyzed at the lower limit of ambient temperature range. Use of CD45 as a gating condition for lymphocytes ensures that RBCs, as CD45 negative events, are not included in the lymph gate, refer to Figure 5.19 and Figure 5.20. **2.** Review that the integrity of the lymph gate on the EV display is preserved. **3.** If the condition exceeds the expected performance or affects the integrity of the lymph gate on the EV display, then select Reject, Re-Request from the Review Options menu. **4.** If the problem persists across different samples, contact your local Beckman Coulter Representative.
Potential sample or gating issue	Potential for a gate misplacement or a sample anomaly that has the potential to affect the results.	This notification might be triggered by several conditions **1.** Review light scatter and EV patterns. If light scatter or EV patterns are disrupted, Reject and Re-request sample. Refer to Figure 5.21, Figure 5.22 and Figure 5.23. **2.** Review gate and region boundaries; to adjust regions see Edit Regions for a Review Sample. **3.** Review QC Reliability Checks; to verify reliability checks see Analytic Reliability Checks. **4.** Review sample compensation to verify compensation. Adjust Compensation Manually only if the problem occurs across different samples. **5.** If the problem persists across different samples, contact your local Beckman Coulter Representative.
Low CD8 Positives	CD8 positive region has a low number of events.	**1.** Review the sample results from the Review screen. This notification could be donor dependent or may be resolved with manual re-adjustment of the gating. **2.** If step 1 does not resolve the condition, then select Reject, Re-Request from the Review Options menu. **3.** If the problem persists across different samples, contact your local Beckman Coulter Representative.

Table 5.8 AQUIOS Tetra Specific Notifications

Flag	Definition	Recommended User Action
Low CD3 Positives	CD3 positive region has a low number of events.	**1.** Review the sample results from the Review screen. This notification could be donor dependent or may be resolved with manual re-adjustment of the gating. **2.** If step 1 does not resolve the condition, then select Reject, Re-Request from the Review Options menu. **3.** Verify reagent levels. **4.** If the problem persists across different samples, contact your local Beckman Coulter Representative.
Low CD3 Negatives	CD3 negative region has a low number of events.	**1.** Review the sample results from the Review screen. This notification could be donor dependent or may be resolved with manual re-adjustment of the gating. **2.** If step 1 does not resolve the condition, then select Reject, Re-Request from the Review Options menu. **3.** Verify reagent levels. **4.** If the problem persists across different samples, contact your local Beckman Coulter Representative.

Figure 5.19 Underlysed Specimen

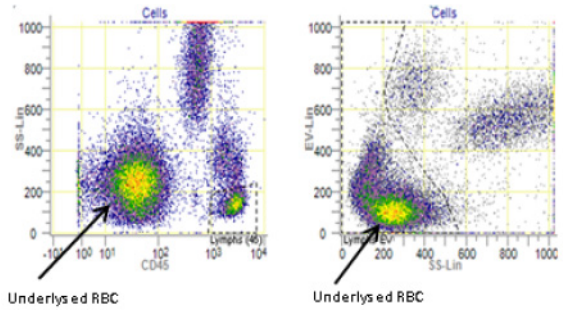


Figure 5.20 Underlysed IMMUNO-TROL

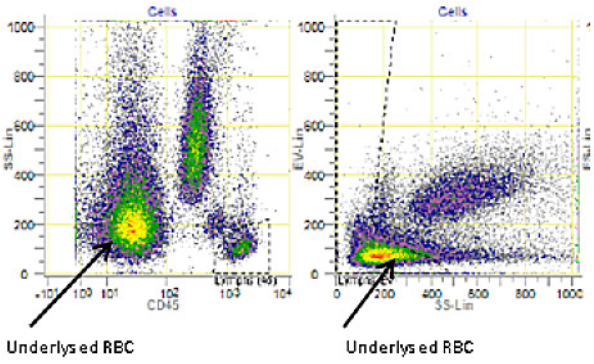


Figure 5.21 EV pattern disruption

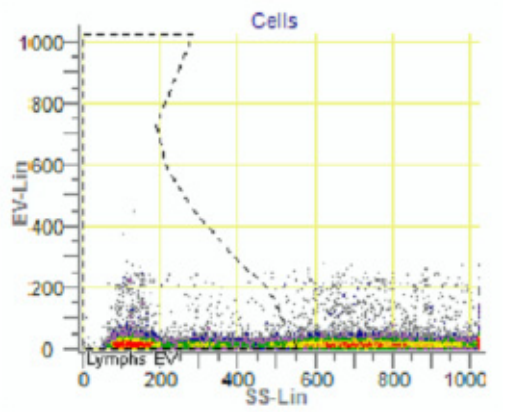


Figure 5.22 EV pattern disruption

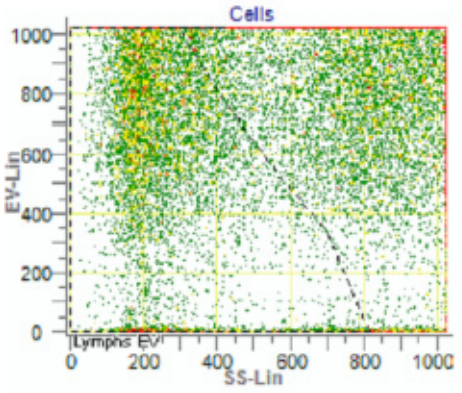
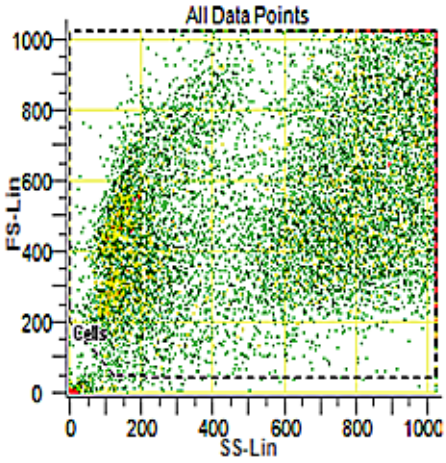


Figure 5.23 Light scatter pattern disruption



CHAPTER 6

Performance Characteristics

Overview

The performance characteristics presented below were collected on the AQUIOS CL Flow Cytometry System with AQUIOS System software and AQUIOS Tetra Combo Test with no modification of the algorithm generated results.

This chapter contains the following information:

Reference Ranges

A study of apparently healthy adults was conducted to assess the Reference Ranges for the AQUIOS Tetra System. Whole blood specimens were collected from donors (males and females) from three geographically diverse sites. The selection of donors was consistent with guidelines stated in CLSI EP28-A3C Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory. Representative normal values are presented in Table 6.1. Each laboratory should establish its own reference intervals.

Samples were stained with AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and AQUIOS Tetra-2+ Panel CD45-FITC/(CD56+CD16)-RD1/CD19-ECD/CD3-PC5 monoclonal antibody reagents and analyzed using the Tetra Combo analytical mode. Values determined on AQUIOS CL Flow Cytometers with AQUIOS system software represent CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/CD19+, CD3-/(CD56+ CD16+), CD45+Low SS, and CD45+ cells and are provided in Table 6.1 and Table 6.2. The AQUIOS System uses a syringe-based methodology for determining absolute counts. Values are expressed as percentage (%) of the total lymphocyte or CD45+ count and as absolute counts (cells/µL).

95% confidence was used to calculate the reference limits.

These values are intended to be representative only. Each laboratory must establish its own reference ranges from the local population of normal donors.

Table 6.1 Normal Whole Blood: CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 AQUIOS Tetra-1 Panel

Parameter	Units	n	Mean	Lower Limit	Upper Limit
% CD3+	% +Lymphocytes	161	72.67	57.97	83.98
CD3+	Absolute Count (cells/uL)	161	1386	856	2237
% CD3+/CD4+	% +Lymphocytes	161	47.41	33.62	64.83
CD3+/CD4+	Absolute Count (cells/uL)	161	904	518	1472
% CD3+/CD8+	% +Lymphocytes	161	23.02	13.01	37.57
CD3+/CD8+	Absolute Count (cells/uL)	161	439	205	924
CD45+	Absolute Count (cells/uL)	161	6555	3897	9997
% CD45+ Low SS	%	161	29.96	17.57	42.54
CD45+ Low SS	Absolute Count (cells/uL)	161	1903	1198	2856

Table 6.2 Normal Whole Blood: CD45-FITC/(CD56+CD16)-RD1/CD19-ECD/CD3-PC5 AQUIOS Tetra-2+ Panel

Parameter	Units	n	Mean	Lower Limit	Upper Limit
% CD3+	% +Lymphocytes	161	72.83	57.72	84.42
CD3+	Absolute Count (cells/uL)	161	1371	857	2253
% CD3-/CD19+	% +Lymphocytes	161	12.90	5.71	24.91
CD3-/CD19+	Absolute Count (cells/uL)	161	244	87	507
% CD3-/CD56+ CD16+	% +Lymphocytes	161	13.02	4.26	26.58
CD3-/CD56+ CD16+	Absolute Count (cells/uL)	161	241	74	562

Linearity

Three replicate measurements were made at each of 19 independent dilutions (CD3+, CD3+/CD4+, CD3+/CD8+) and (CD3-/CD19+, CD3-/(CD56+ CD16+), AQUIOS Tetra-1 Panel and AQUIOS Tetra-2+ Panel representing a high and low range, of a concentrated AQUIOS IMMUNO-TROL Cells sample. Ranges for CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/CD19+, CD3-/(CD56+ CD16+), CD45+, and CD45+ Low SS concentrations were established by staining with AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 or AQUIOS Tetra-2+ Panel CD45-FITC/(CD56+CD16)-RD1/CD19-ECD/CD3-PC5 monoclonal antibody reagent, and analyzing by flow cytometry using AQUIOS system software. Statistical determination of linearity was performed using the guideline CLSI EP06-A3, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach. See Figure 6.1 through Figure 6.8. Values are expressed in terms of absolute count (cells/µL).

AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5

Figure 6.1 Linearity: CD3+ Cells

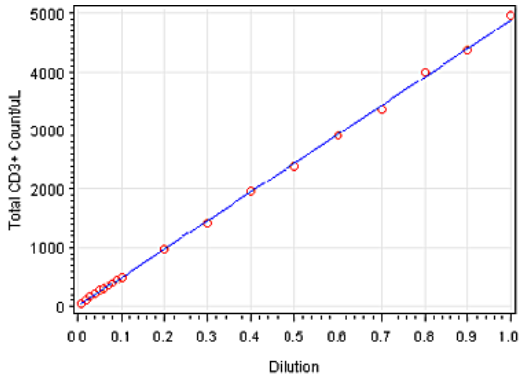


Figure 6.2 Linearity: CD3+/CD4+ Cells

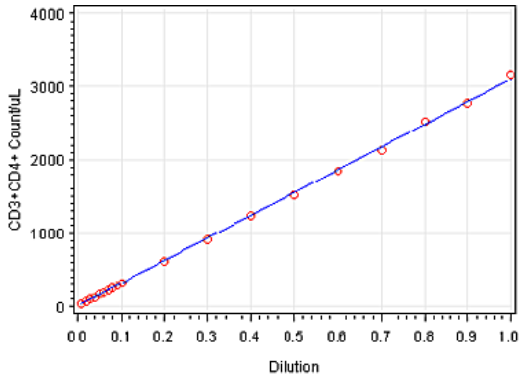


Figure 6.3 Linearity: CD3+/CD8+ Cells

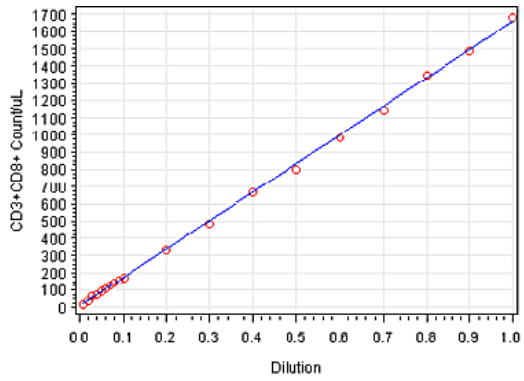


Figure 6.4 Linearity: CD45+ Cells

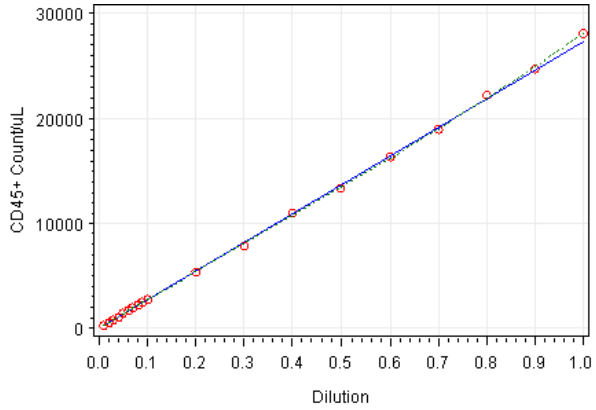
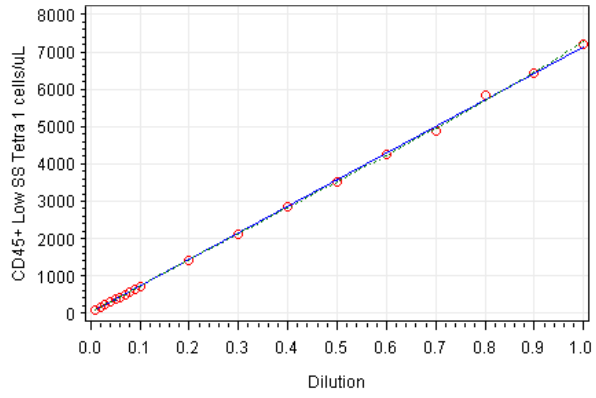


Figure 6.5 Linearity: CD45+ Low SS Cells



AQUIOS Tetra-2+ Panel CD45-FITC/(CD56+CD16)-RD1/CD19-ECD/CD3-PC5

Figure 6.6 Linearity: CD3+ Cells

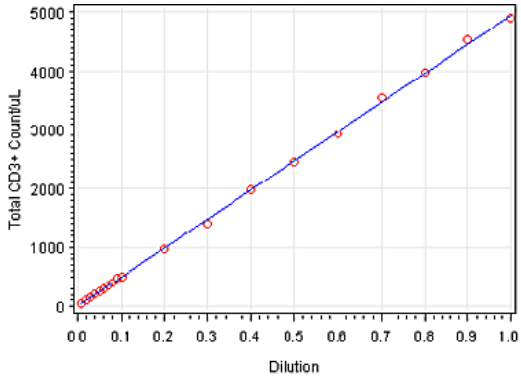


Figure 6.7 Linearity: CD3-/CD19+ Cells

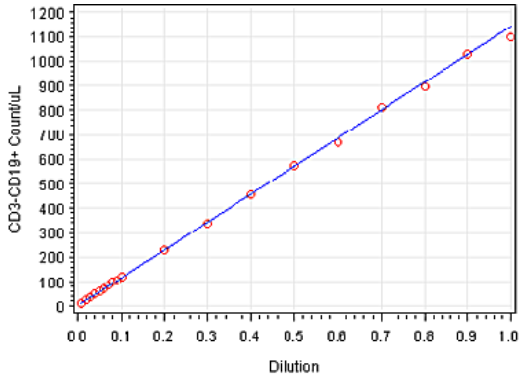
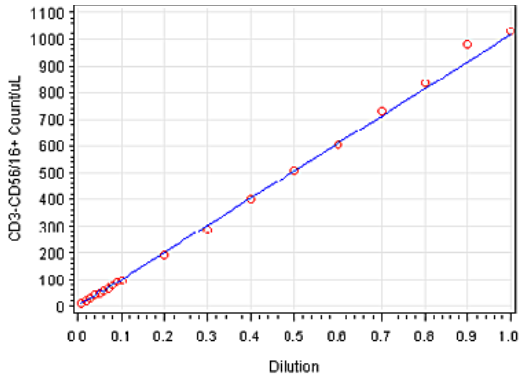


Figure 6.8 Linearity: CD3-/CD56+ CD16+ Cells



Method Comparison

The comparability of the AQUIOS Tetra System was assessed by comparing the results with the predicate method using the estimation of difference as described in CLSI EP9-A3 Method Comparison and Bias Estimation Using Patient Samples. For CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/(CD56+CD16+), and CD3-CD19+, the comparator method is samples stained with MultiTEST CD3-FITC/CD8-PE/CD45-PerCP/CD4-APC and MultiTEST CD3-FITC/CD16+CD56+PE/CD45-PerCP/CD19-APC monoclonal antibody reagents run on a BD FACSCalibur flow cytometer with MultiTEST 4-color TBNK software. For CD45+ and CD45+ Low SS, the comparator method is specimens run on a UniCel DxH 800 hematololgy analyzer. Both method comparisons are comprised of 78% clinical specimens and 22% normal specimens. The data provided in Table 6.3 and Figure 6.9 through Figure 6.16, supports the premise that the systems are equivalent in their performance enumerating mature T, B, and NK lymphocytes in peripheral whole blood. Values are expressed in terms of percentage (%) of the total lymphocyte count and as absolute count (cells/uL) for CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/CD19+, and CD3-/CD56+ CD16+ cells as well as percentage (%) of CD45+ Low SS and absolute count (cells/uL) for CD45+ and CD45+Low SS when analyzed using Tetra Combo analytical mode.

Table 6.3 Method Comparison - Whole Blood

Parameter	Units	Measuring Range	Difference
% CD3+	% + Lymphocytes	All Ranges	±2.5 percentage points
%CD3+/CD4+			
%CD3+/CD8+			
%CD3-/CD19+			
%CD3-/CD56+CD16+			
% CD45+ Low SS	%	All Ranges	±3 percentage points or ±10% whichever is greater

CD3+ CD3+/CD4+ CD3+/CD8+	Absolute Count (cells/µL)	0-300	±40 cells/µL
		>300	±13%
CD3-/CD19+ CD3-/CD56+CD16+	Absolute Count (cells/µL)	0-300	±65 cells/µL
		>300	±22%
CD45+	Absolute Count (cells/µL)	All Ranges	±13%
CD45+ Low SS	Absolute Count (cells/µL)	All Ranges	±13%

Table 6.4 Method Comparison: AQUIOS Tetra vs. BD FACSCalibur

AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 (n= 443)					
	Mean BD FACSCalibur	**Mean AQUIOS Tetra**	**Correlation**	**Slope**	**Intercept**
% + **Lymphocytes**					
CD3+	71.64	71.75	0.986	0.963	2.786
CD3+/CD4+	31.11	31.26	0.996	0.997	0.228
CD3+/CD8+	38.59	38.16	0.995	0.983	0.210
cells/µL					
CD3+	1146	1118	0.977	0.978	10.453
CD3+/CD4+	514	499	0.986	0.984	2.657
CD3+/CD8+	603	582	0.982	0.965	4.689

AQUIOS Tetra-2+ Panel CD45-FITC/CD56+CD16-RD1/CD19-ECD/CD3-PC5 (n=443)					
% + Lymphocytes					
CD3+	71.65	71.93	0.987	0.970	2.433
CD3-/CD19+	14.01	13.61	0.987	0.990	-0.260
CD3-/CD56+ CD16+	12.93	13.18	0.986	0.998	0.278
cells/µL					
CD3+	1166	1106	0.973	0.950	9.643
CD3-/CD19+	226	207	0.975	0.873	9.665
CD3-/CD56+ CD16+	190	181	0.979	0.966	2.756

Table 6.5 Method Comparison: AQUIOS Tetra vs. UniCel DxH 800

AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 (n= 445)					
	Mean UniCel DxH 800	**Mean AQUIOS Tetra**	**Correlation**	**Slope**	**Intercept**
%					
CD45+ Low SS	27.60	27.49	0.995	0.999	-0.084
cells/µL					
CD45+	6131	5991	0.992	0.985	-51.430
CD45+ Low SS	1583	1537	0.993	0.977	-6.156

AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5

Figure 6.9 Regression Analysis: CD3+ Cells AQUIOS Tetra vs BD FACSCalibur

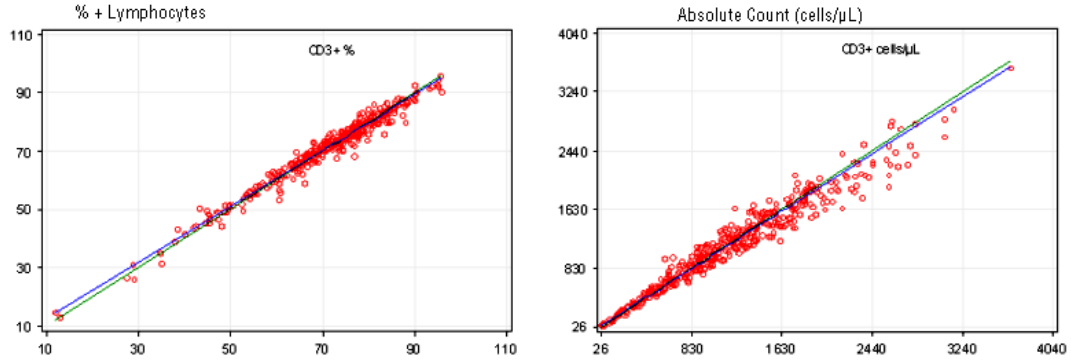


Figure 6.10 Regression Analysis: CD3+/CD4+ Cells AQUIOS Tetra vs BD FACSCalibur

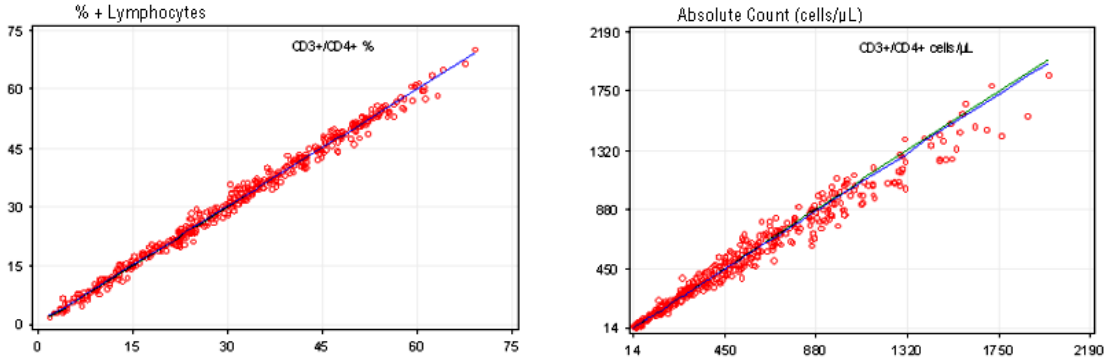


Figure 6.11 Regression Analysis: CD3+/CD8+ Cells AQUIOS Tetra vs BD FACSCalibur

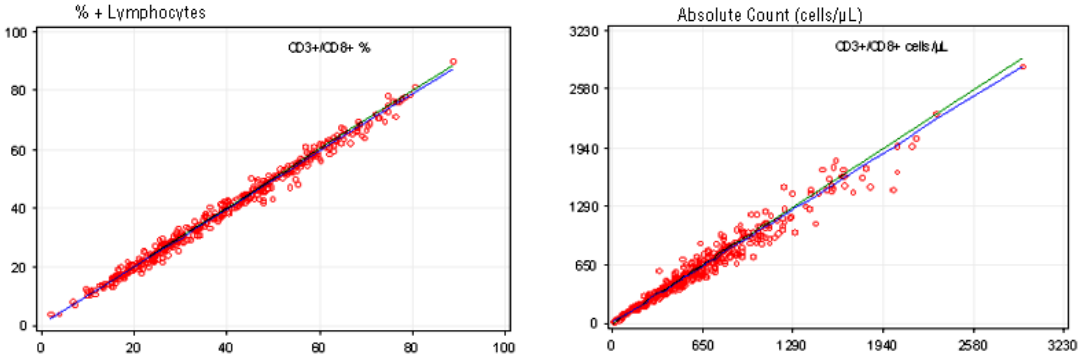


Figure 6.12 Regression Analysis: CD45+ Cells AQUIOS Tetra vs. UniCel DxH 800

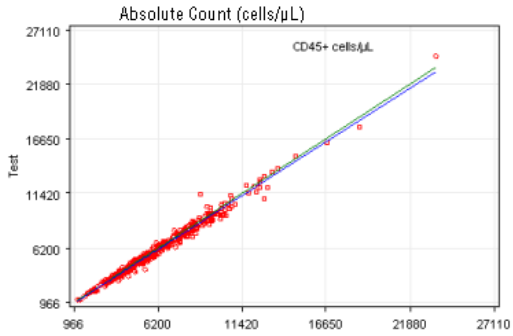
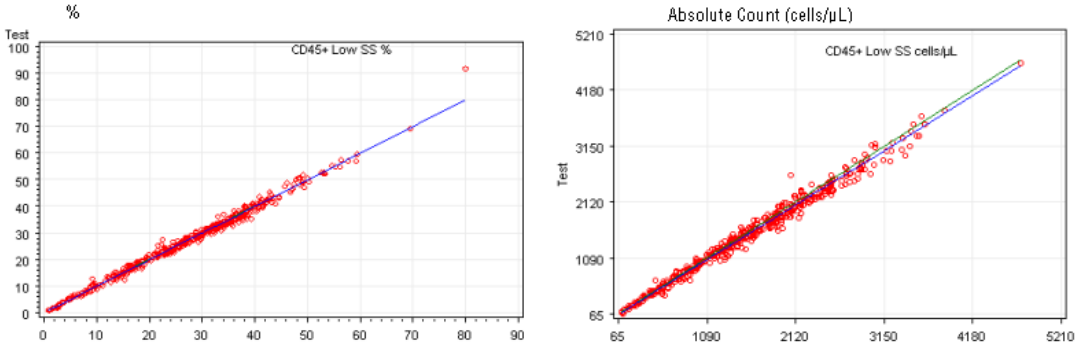


Figure 6.13 Regression Analysis: CD45+ Low SS Cells AQUIOS Tetra vs. UniCel DxH 800



AQUIOS Tetra-2+ Panel CD45-FITC/(CD56+CD16)-RD1/CD19-ECD/CD3-PC5

Figure 6.14 Regression Analysis: CD3+ Cells AQUIOS Tetra vs BD FACSCalibur

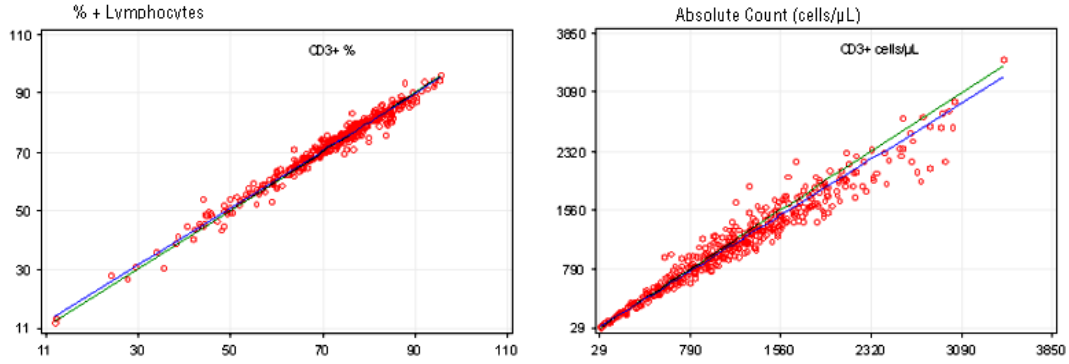


Figure 6.15 Regression Analysis: CD3-/CD19+ Cells AQUIOS Tetra vs BD FACSCalibur

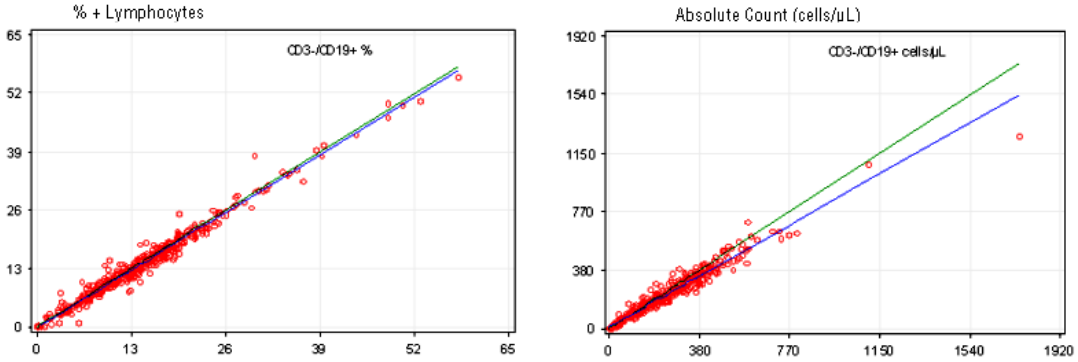
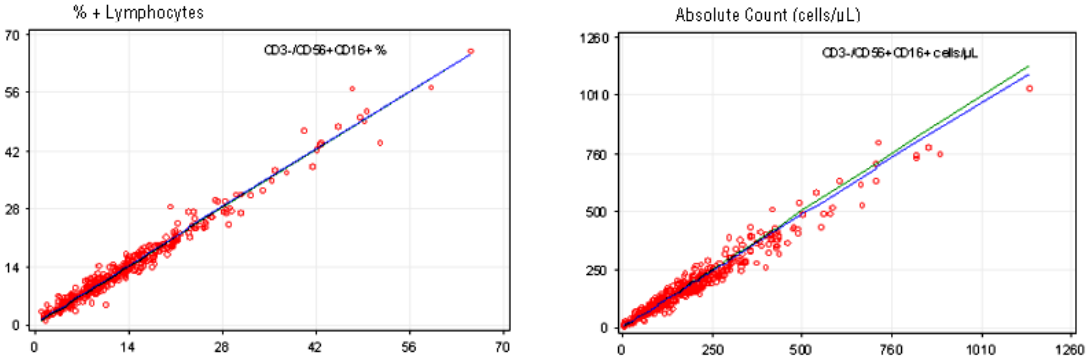


Figure 6.16 Regression Analysis: CD3-/CD56+ CD16+ Cells AQUIOS Tetra vs BD FACSCalibur



Precision

The percent positive and absolute count values were determined using AQUIOS IMMUNO-TROL Cells and AQUIOS IMMUNO-TROL Low Cells, run in duplicate, twice each day for a minimum of 20 days at three geographically diverse sites using AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and AQUIOS Tetra-2+ Panel CD45-FITC/(CD56+CD16)-RD1/CD19-ECD/CD3-PC5 Monoclonal Antibody Reagents analyzed using Tetra Combo analytical mode. Measurements (% positive and absolute counts) for CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/CD19+, and CD3-/(CD56+ CD16+) populations, as well as CD45+ absolute count and CD45+Low SS percent and absolute count, were within assay ranges as reported in the control package inserts. Analysis was conducted using the CLSI method EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods. Repeatability for all sites combined is presented. See Table 6.7 through Table 6.8.

Table 6.6 Repeatability Specification: Whole Blood, IMMUNO-TROL Cells and IMMUNO-TROL Low Cells

Parameter	Units	Measuring Range	Limit (%CV)
% CD3+	% + Lymphocytes	≤ 20	≤ 10%
%CD3+/CD4+			
%CD3+/CD8+			
%CD3-/CD19+		> 20	≤ 5%
%CD3-/CD56+CD16+			
% CD45+ Low SS	%	≤ 5 > 5 - < 25 25 - 50 > 50	≤ 20% ≤ 10% ≤ 5% ≤ 3.5%
CD3+	Absolute Count (cells/µL)	< 300	≤ 10%
CD3+/CD4+			
CD3+/CD8+			
CD3-/CD19+		≥ 300	≤ 5%
CD3-/CD56+CD16+			
CD45+	Absolute Count (cells/µL)	≤ 5000	≤ 5%
		> 5000	≤ 3%
CD45+ Low SS		≤ 300 >300 - <1300 1300 - 2600 > 2600	≤ 20% ≤ 10% ≤ 5% ≤ 3.5%

Table 6.7 Repeatability Results - AQUIOS IMMUNO-TROL Cells

Reagent	Parameter	Units	Mean	%CV
AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 (n=311)	CD3+	% + Lymphocytes	72.40	1.23
		Absolute Count (cells/µL)	831	2.66
	CD3+/CD4+	% + Lymphocytes	48.37	1.76
		Absolute Count (cells/µL)	555	2.94
	CD3+/CD8+	% + Lymphocytes	22.16	3.11
		Absolute Count (cells/µL)	254	3.93
	CD45+	Absolute Count (cells/µL)	4845	2.21
	CD45+ Low SS	%	23.69	1.57
		Absolute Count (cells/µL)	1147	2.87
AQUIOS Tetra-2+ Panel CD45-FITC/(CD56+CD16)-RD1/CD19-ECD/CD3-PC5 (n=311)	CD3+	% + Lymphocytes	72.00	1.37
		Absolute Count (cells/µL)	833	2.81
	CD3-/CD19+	% + Lymphocytes	13.65	3.39
		Absolute Count (cells/µL)	158	4.38
	CD3-/CD56+ CD16+	% + Lymphocytes	12.30	5.28
		Absolute Count (cells/µL)	142	6.69

Table 6.8 Repeatability: Results - AQUIOS IMMUNO-TROL Low Cells

Reagent	Parameter	Units	Mean	%CV
AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 (n=311)	CD3+	% + Lymphocytes	58.00	2.07
		Absolute Count (cells/µL)	388	3.09
	CD3+/CD4+	% + Lymphocytes	18.37	3.84
		Absolute Count (cells/µL)	123	4.52
	CD3+/CD8+	% + Lymphocytes	35.60	3.06
		Absolute Count (cells/µL)	238	3.92
	CD45+	Absolute Count (cells/µL)	5046	2.26
	CD45+ Low SS	%	13.22	2.23
		Absolute Count (cells/µL)	669	3.21
AQUIOS Tetra-2+ Panel CD45-FITC/(CD56+CD16)-RD1/CD19-ECD/CD3-PC5 (n= 311)	CD3+	% + Lymphocytes	57.45	2.06
		Absolute Count (cells/µL)	387	2.89
	CD3-/CD19+	% + Lymphocytes	17.33	4.46
		Absolute Count (cells/µL)	117	5.10
	CD3-/CD56+ CD16+	% + Lymphocytes	23.65	4.25
		Absolute Count (cells/µL)	159	5.78

Analytical Measuring Ranges

The Analytical Measuring Ranges provided indicate the AQUIOS Tetra System's analytical capabilities to enumerate lymphocyte subsets. The AQUIOS Tetra Analytical Measuring Ranges (see Table 6.9) are derived from Linearity and Lower Limit of Quantitation values:

- **Linearity:** Ranges over which results are directly proportional to the amount of analyte in a sample, per the CLSI EP06-A2 guideline, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach.
- **Lower Limit of Quantitation:** The lowest amount of analyte in a sample that can be quantitatively determined with stated acceptable precision and trueness, under stated experimental conditions per the CLSI EP17-A3 guideline, Protocols for Determination of Limits of Detection and Limits of Quantitation.

Table 6.9 Analytical Measuring Ranges

Parameters	Units	AQUIOS Tetra Analytical Measuring Ranges
CD3+	cells/µL	55-4700
CD3+/CD4+	cells/µL	35-3000
CD3+/CD8+	cells/µL	45-1600
CD3-/CD19	cells/µL	25-1000
CD3-/CD56+ CD16+	cells/µL	20-1000
CD45+	cells/µL	350-26500
CD45+ Low SS	cells/µL	80-6500

Specificity

Reagent Specificity

The CD45 antigen is expressed on every type of hematopoietic cell except mature erythrocytes and their immediate progenitors.[11, 12] It has not been detected in differentiated nonhematopoietic tissue.[11, 12, 13, 14]

The CD3 antigen is normally present on the cell surface of mature thymocytes and resting and activated peripheral blood mature T lymphocytes (both helper and suppressor/cytotoxic populations).[15, 16, 17]

The CD4 antigen is present on thymoctyes and the helper T lymphocyte population in peripheral blood.[16, 17] It is also expressed at low density on some monocytes.[18]

The CD8 antigen is normally present on approximately 80% of thymocytes and approximately 30-35% of peripheral blood T lymphocytes and some natural killer cells.[19, 20]

The CD16 antigen is the low-affinity receptor for IgG (FcγRIII) that binds immune complexes, but not monomeric IgG. The CD16 antigen exists in two different forms encoded by two different genes: FcγRIIIA (or III-2) and FcγRIIIB (or III-1). The genetic heterogeneity of CD16 generates alternative membrane-anchored molecules. One is a transmembrane form (FcγRIIIA, 50 - 65 kDa) expressed on NK cells, monocytes and macrophages. The other is a glycosylphosphatidylinositol (GPI)-anchored form (FcγRIIIB, 48 kDa) only expressed on neutrophils.[21, 22] It has been shown that the CD16 antigen can be non-covalently associated within the membrane of NK cells, to the 16 kDa CD3ζ chain,[23] or to the dimeric FcRγ chain.[24] The 3G8 monoclonal antibody (mAb) binds to FcγRIIIA as well as to FcγRIIIB (strongly). It was shown to block almost completely the binding of IgG dimers to FcγRIIIB.[24] Experiments where amino acid mutations were made to the FcγRIIIB molecule showed that the 3G8 mAb is affected by Lys162 and Val164 substitutions in the FG loop of the membrane-proximal Ig-like domain of the molecule.[25, 27] The 3G8 mAb has been assigned to the CD16 cluster of differentiation at the Fifth International Workshop on Human Leucocyte Differentiation Antigens held in Boston, USA, in 1993.[26]

The CD19 antigen is expressed on all B-cells, including early progenitor B-cells.[28] It can also be found on follicular dendritic cells and myelomonocyte lineage progenitor cells, but is not expressed on T-cells, monocytes or granulocytes.[29, 30, 31]

The CD56 antigen is expressed on a subpopulation of lymphocytes that demonstrates natural killer activity (and also on various types of non-circulating cells of neural and/neuroendocrine origin). This subpopulation consists of both natural killer cells (CD3-(CD56+) and a subset of T-cells (CD3+CD56+).[29, 30, 32, 34] CD3-CD56+ cells capable of mediating non-TCR cytotoxicity in peripheral blood.[32, 33] CD56 is not expressed on other T or B lymphocyte, monocyte, granulocyte, or erythrocyte populations.[34, 35, 36]

The antigen specificity of the CD45, CD3, CD4 and CD8 monoclonal antibodies comprising the AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 monoclonal antibody reagent has been previously established by the First (CD4, CD8 and CD3) and the Fifth (CD45) International Workshop for Leukocyte Typing.[29, 37]

The antigen specificity of the CD45, CD3, CD19, CD56, and CD16 monoclonal antibodies comprising the AQUIOS Tetra-2+ Panel CD45-FITC/(CD56+CD16)-RD1/CD19-ECD/CD3-PC5 monoclonal antibody reagent has been previously established by the First (CD3), Fourth (CD16, CD19, and CD56), and Fifth (CD45) International Workshop for Leukocyte Typing.[29, 37, 38]

To assess cellular cross-reactivity, the CD3, CD4, CD8, CD19, and CD56 monoclonal antibodies comprising the AQUIOS Tetra-1 Panel CD45 FITC/ CD4-RD1/CD8 ECD/CD3-PC5 and AQUIOS Tetra-2+ Panel CD45 FITC/(CD56+CD16) RD1/ CD19 ECD/ CD3-PC5 monoclonal antibody reagents were screened on normal human adult donor blood samples. Results consistently demonstrated that the CD3, CD4, CD8, CD19, and CD3 (CD56+CD16) RD1 antibodies reacted specifically with the appropriate lymphocyte populations. Monocytes were dimly stained with CD4 monoclonal antibody.

Quality Control

Ensure that controls are run according to manufacturer's recommendations as listed in the instrument manuals. Target value ranges are provided for each lot of AQUIOS IMMUNO-TROL Cells and AQUIOS IMMUNO-TROL Low Cells to optimize light scatter and fluorescence instrument settings for analyzing samples stained with AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5, and AQUIOS Tetra-2+ Panel CD45-FITC/(CD56+CD16)-RD1/CD19-ECD/CD3-PC5.

The phycoerythrin-Cy5 (PC5), phycoerythrin (RD1), phycoerythrin-Texas Red-X (ECD), and fluorochromes fluorescein isothiocyanate (FITC) emit at different wavelengths, but they do have some spectral overlap that must be corrected by software compensation. Fluorescence compensation settings are verified by the system when controls are run.

Monocytes and granulocytes in a sample can be excluded by proper gating on lymphocytes on the flow cytometer.[5] To ensure method specificity, the AQUIOS System software is designed to automatically identify and optimize the lymphocyte gate based on CD45 bright positive (vs. side scatter), EV, and forward and side scatter characteristics. To further ensure method specificity, the AQUIOS system software monitors non-specific antibody binding to lymphocytes by automatically placing cursors based on the separation of positive and negative peaks. This eliminates the need for an isotypic control.

Before samples are analyzed, assayed controls (AQUIOS IMMUNO-TROL Cells and AQUIOS IMMUNO-TROL Low Cells) need to be run to verify antibody reactivity.

CHAPTER 7

Application Specifications

Overview

This chapter contains the following information:

Storage Conditions and Stability

Whole Blood Specimen

The whole blood specimen is ideally analyzed as soon as possible after the blood is drawn, but no later than 24 hours. The whole blood specimen is stable up to 24 hours if stored at room temperature, between 18°C and 26°C (64.4°F and 78.8°F).

For best staining results, the white blood cell (WBC) counts should be 0.35-26.5 x 10^3 cells/µL.

Reagents

Refer to the appropriate package inserts for information on reagent stability.

- AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and AQUIOS Tetra-2+ Panel CD45-FITC/(CD56+CD16)-RD1/CD19-ECD/CD3-PC5
- AQUIOS Cleaning Agent
- AQUIOS Sodium Hypochlorite Solution
- AQUIOS Sheath Solution
- AQUIOS Lysing Reagent Kit
- AQUIOS IMMUNO-TROL Cells
- AQUIOS IMMUNO-TROL Low Cells

AQUIOS Tetra Preparation

All samples are prepared and analyzed onboard using a 96-deep well plate.

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 in AQUIOS Tetra-1 Or AQUIOS Tetra-2+ Sample Preparation.

AQUIOS Tetra Throughput

Time from loading a blood specimen to result for the first sample is approximately 20 minutes including sample preparation. Subsequent sample results (including sample preparation) are reported at a rate of 25 samples per hour for up to a full 96-well microplate (assuming no interruptions) for each test of Tetra Combo.

APPENDIX A

Reports

Overview

This appendix contains the following information:

- Reports
- QC Report

Reports

Reports can be printed or saved (PDF) from the Review or the Result screens.

NOTE The displays and statistics on the report are assay dependant. Also, the report can be customized under System Setup to display the name, address, and facility logo.

- Example report formats for AQUIOS Tetra-1
  - Figure A.1, Report: Tetra-1 Results
  - Figure A.2, Report: Tetra-1 Details
  - Figure A.3, Report: Tetra-1 DotPlots
  - Figure A.4, Report: Tetra-1 Histograms
- Example report formats for Tetra-2+
  - Figure A.5, Report: Tetra-2+ Results
  - Figure A.6, Report: Tetra-2+ Details
  - Figure A.7, Report: Tetra-2+ DotPlots
  - Figure A.8, Report: Tetra-2+ Histograms
- Example report formats for Tetra Combo
  - Figure A.9, Report: AQUIOS Tetra Combo Results (Page 1)
  - Figure A.11, Report: AQUIOS Tetra Combo Details
  - Figure A.13, Report: AQUIOS Tetra Combo DotPlots
  - Figure A.15, Report: AQUIOS Tetra Combo Histograms

Also see Report Description and QC Report Description.

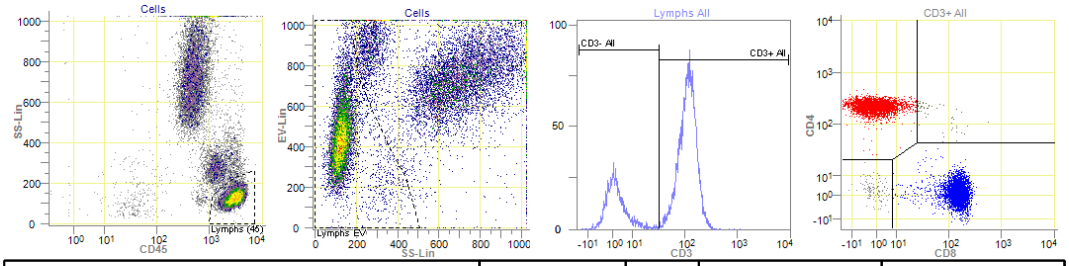
Figure A.1 Report: Tetra-1 Results

Sample ID:	89350141144	Test:	Tetra 1 v1.1
Run Date:	03 Feb 2015 12:57:08	Analysis Date:	03 Feb 2015 12:57:08
User:	Admin	Collect Date:	03 Feb 2015
Specimen Type:	Whole Blood	Patient Name:	148217
Patient ID:		Gov. ID:	
Status:		Location:	
Gender:		Date Of Birth:	
Physician:		Physician Code:	
Instrument Serial #:	AV039002		

Run Flags:

Run Notifications:

Comments:



Result	Value	Flag	Normal Range	Action Range
CD3+ (T Cells) Percent	77.19%			
CD3+ (T Cells) Count/uL	1,876			
CD3+/CD4+ (Helper T Cells) Percent	44.02%			
CD3+/CD4+ (Helper T Cells) Count/uL	1,070			
CD3+/CD8+ (Suppressor T Cells) Percent	32.62%			
CD3+/CD8+ (Suppressor T Cells) Count/uL	793			
CD4:CD8 Ratio	1.35			
*CD3+ Reliability Check	0.58%			
CD45+ Low SS Count/uL	2,431			
CD45+ Low SS Percent	50.12%			
CD45+ Count/uL	4,851			

* For Analytical QC purposes only | ** Population Statistics 03 Feb 2015 13:12:02 Page #1 of 1 Aquios v1.1.0.15026

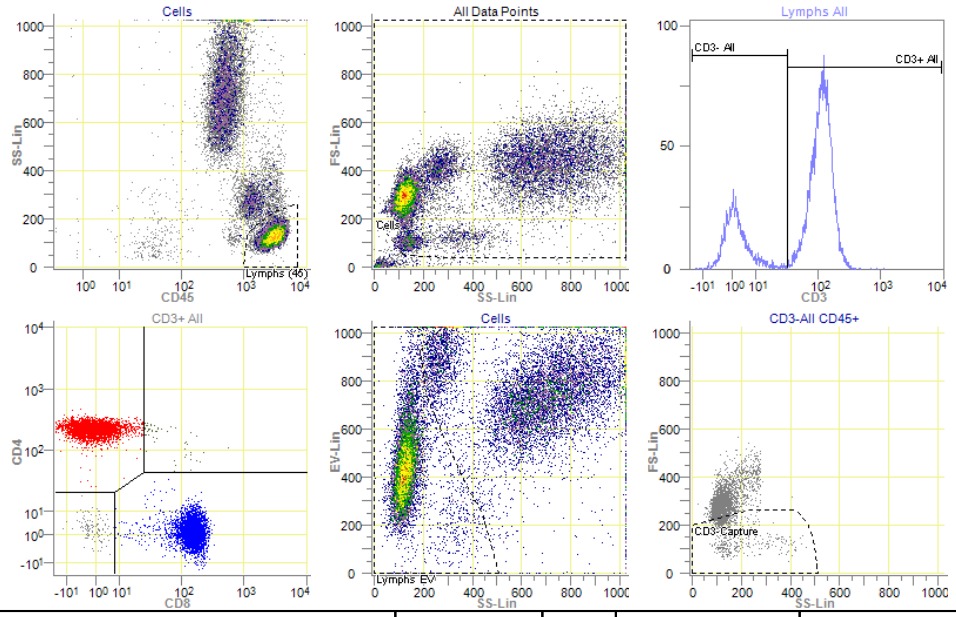
Figure A.2 Report: Tetra-1 Details

Sample ID:	89350141144	Test:	Tetra 1 v1.1
Run Date:	03 Feb 2015 12:57:08	Analysis Date:	03 Feb 2015 12:57:08
User:	Admin	Collect Date:	03 Feb 2015
Specimen Type:	Whole Blood	Patient Name:	148217
Patient ID:		Gov. ID:	
Status:		Location:	
Gender:		Date Of Birth:	
Physician:		Physician Code:	
Instrument Serial #:	AV039002		

Run Flags:

Run Notifications:

Comments:



Result	Value	Flag	Normal Range	Action Range
CD3+ (T Cells) Percent	77.19%			
CD3+ (T Cells) Count/uL	1,876			
CD3+/CD4+ (Helper T Cells) Percent	44.02%			
CD3+/CD4+ (Helper T Cells) Count/uL	1,070			
CD3+/CD8+ (Suppressor T Cells) Percent	32.62%			
CD3+/CD8+ (Suppressor T Cells) Count/uL	793			
CD4:CD8 Ratio	1.35			
*CD3+ Reliability Check	0.58%			
CD45+ Low SS Count/uL	2,431			
CD45+ Low SS Percent	50.12%			
CD45+ Count/uL	4,851			

* For Analytical QC purposes only | ** Population Statistics 03 Feb 2015 13:13:31 Page #1 of 1 Aquios v1.1.0.15026

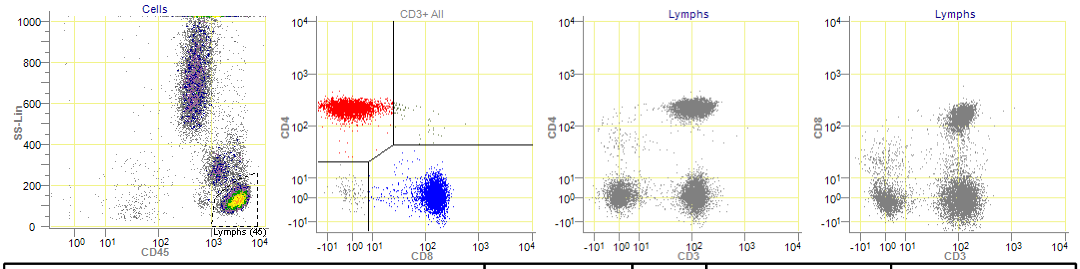
Figure A.3 Report: Tetra-1 DotPlots

Sample ID:	89350141144	Test:	Tetra 1 v1.1
Run Date:	03 Feb 2015 12:57:08	Analysis Date:	03 Feb 2015 12:57:08
User:	Admin	Collect Date:	03 Feb 2015
Specimen Type:	Whole Blood	Patient Name:	148217
Patient ID:		Gov. ID:	
Status:		Location:	
Gender:		Date Of Birth:	
Physician:		Physician Code:	
Instrument Serial #:	AV039002		

Run Flags:

Run Notifications:

Comments:



Result	Value	Flag	Normal Range	Action Range
CD3+ (T Cells) Percent	77.19%			
CD3+ (T Cells) Count/uL	1,876			
CD3+/CD4+ (Helper T Cells) Percent	44.02%			
CD3+/CD4+ (Helper T Cells) Count/uL	1,070			
CD3+/CD8+ (Suppressor T Cells) Percent	32.62%			
CD3+/CD8+ (Suppressor T Cells) Count/uL	793			
CD4:CD8 Ratio	1.35			
*CD3+ Reliability Check	0.58%			
CD45+ Low SS Count/uL	2,431			
CD45+ Low SS Percent	50.12%			
CD45+ Count/uL	4,851			

* For Analytical QC purposes only | ** Population Statistics 03 Feb 2015 13:14:08 Page #1 of 1 Aquios v1.1.0.15026

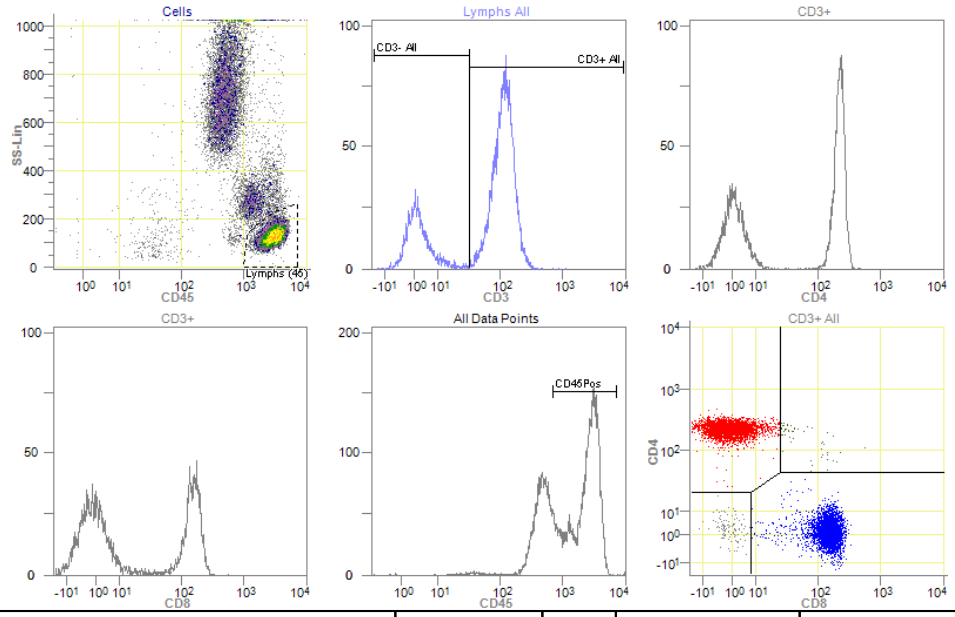
Figure A.4 Report: Tetra-1 Histograms

Sample ID:	89350141144	Test:	Tetra 1 v1.1
Run Date:	03 Feb 2015 12:57:08	Analysis Date:	03 Feb 2015 12:57:08
User:	Admin	Collect Date:	03 Feb 2015
Specimen Type:	Whole Blood	Patient Name:	148217
Patient ID:		Gov. ID:	
Status:		Location:	
Gender:		Date Of Birth:	
Physician:		Physician Code:	
Instrument Serial #:	AV039002		

Run Flags:

Run Notifications:

Comments:

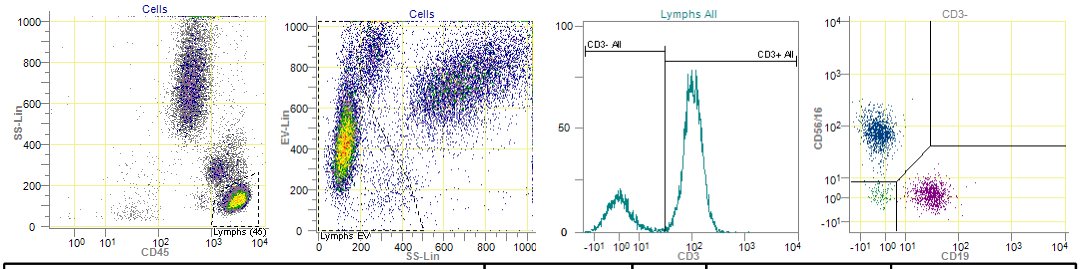


Result	Value	Flag	Normal Range	Action Range
CD3+ (T Cells) Percent	77.19%			
CD3+ (T Cells) Count/uL	1,876			
CD3+/CD4+ (Helper T Cells) Percent	44.02%			
CD3+/CD4+ (Helper T Cells) Count/uL	1,070			
CD3+/CD8+ (Suppressor T Cells) Percent	32.62%			
CD3+/CD8+ (Suppressor T Cells) Count/uL	793			
CD4:CD8 Ratio	1.35			
*CD3+ Reliability Check	0.58%			
CD45+ Low SS Count/uL	2,431			
CD45+ Low SS Percent	50.12%			
CD45+ Count/uL	4,851			

* For Analytical QC purposes only | ** Population Statistics 03 Feb 2015 13:15:39 Page #1 of 1 Aquios v1.1.0.15026

Figure A.5 Report: Tetra-2+ Results

Sample ID:	89350141143	Test:	Tetra 2+ v1.1
Run Date:	03 Feb 2015 13:01:00	Analysis Date:	03 Feb 2015 13:01:00
User:	Admin	Collect Date:	03 Feb 2015
Specimen Type:	Whole Blood	Patient Name:	148217
Patient ID:		Gov. ID:	
Status:		Location:	
Gender:		Date Of Birth:	
Physician:		Physician Code:	
Instrument Serial #:	AV039002		
Run Flags:			
Run Notifications:			
Comments:			

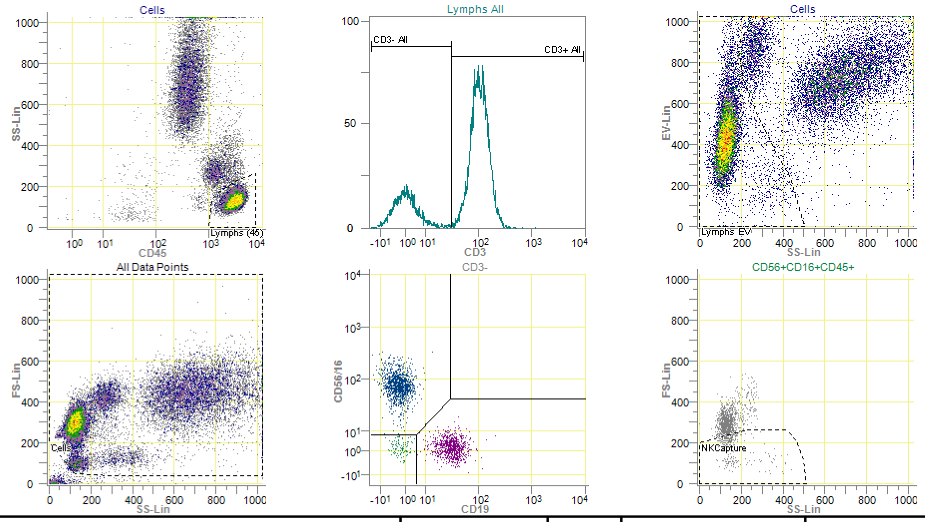


Result	Value	Flag	Normal Range	Action Range
CD3+ (T Cells) Percent	78.67%			
CD3+ (T Cells) Count/uL	1,914			
CD3-/CD19+ (B Cells) Percent	8.87%			
CD3-/CD19+ (B Cells) Count/uL	216			
CD3-/CD56+CD16+ (NK Cells) Percent	11.51%			
CD3-/CD56+CD16+ (NK Cells) Count/uL	280			
*% Total Lymphocytes (T+B+NK) Percent	99.05%			
CD45+ Low SS Count/uL	2,433			
CD45+ Low SS Percent	50.71%			
CD45+ Count/uL	4,797			

* For Analytical QC purposes only | ** Population Statistics 03 Feb 2015 13:16:29 Page #1 of 1 Aquios v1.1.0.15026

Figure A.6 Report: Tetra-2+ Details

Sample ID:	89350141143	Test:	Tetra 2+ v1.1
Run Date:	03 Feb 2015 13:01:00	Analysis Date:	03 Feb 2015 13:01:00
User:	Admin	Collect Date:	03 Feb 2015
Specimen Type:	Whole Blood	Patient Name:	148217
Patient ID:		Gov. ID:	
Status:		Location:	
Gender:		Date Of Birth:	
Physician:		Physician Code:	
Instrument Serial #:	AV039002		
Run Flags:			
Run Notifications:			
Comments:			

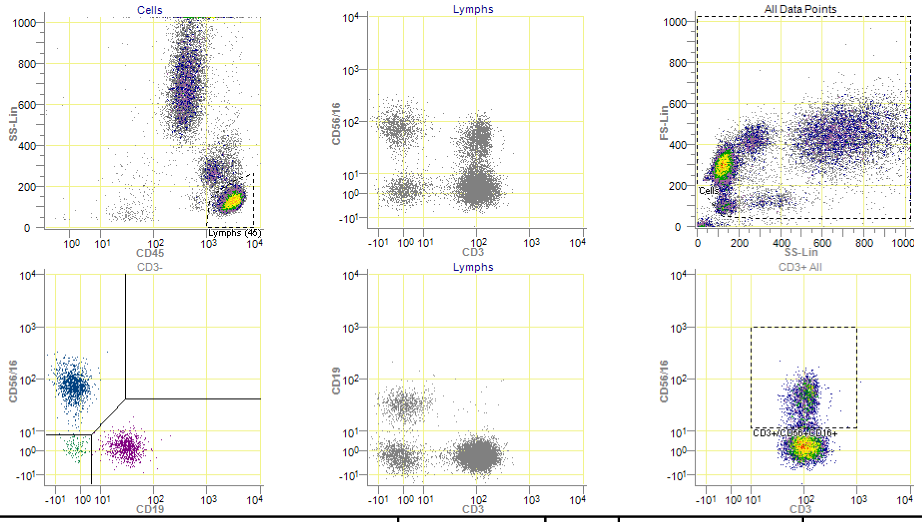


Result	Value	Flag	Normal Range	Action Range
CD3+ (T Cells) Percent	78.67%			
CD3+ (T Cells) Count/uL	1,914			
CD3-/CD19+ (B Cells) Percent	8.87%			
CD3-/CD19+ (B Cells) Count/uL	216			
CD3-/CD56+CD16+ (NK Cells) Percent	11.51%			
CD3-/CD56+CD16+ (NK Cells) Count/uL	280			
*% Total Lymphocytes (T+B+NK) Percent	99.05%			
CD45+ Low SS Count/uL	2,433			
CD45+ Low SS Percent	50.71%			
CD45+ Count/uL	4,797			

* For Analytical QC purposes only | ** Population Statistics 03 Feb 2015 13:17:06 Page #1 of 1 Aquios v1.1.0.15026

Figure A.7 Report: Tetra-2+ DotPlots

Sample ID:	89350141143	Test:	Tetra 2+ v1.1
Run Date:	03 Feb 2015 13:01:00	Analysis Date:	03 Feb 2015 13:01:00
User:	Admin	Collect Date:	03 Feb 2015
Specimen Type:	Whole Blood	Patient Name:	148217
Patient ID:		Gov. ID:	
Status:		Location:	
Gender:		Date Of Birth:	
Physician:		Physician Code:	
Instrument Serial #:	AV039002		
Run Flags:			
Run Notifications:			
Comments:			

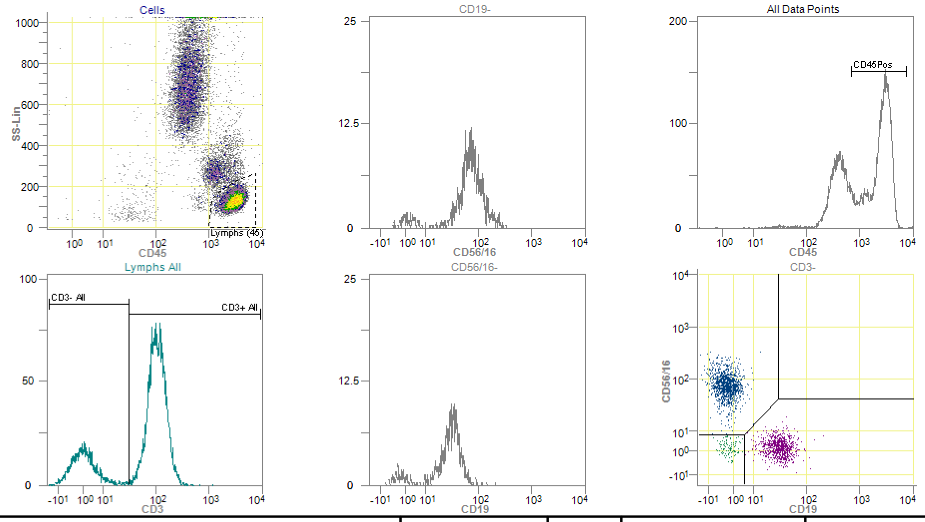


Result	Value	Flag	Normal Range	Action Range
CD3+ (T Cells) Percent	78.67%			
CD3+ (T Cells) Count/uL	1,914			
CD3-/CD19+ (B Cells) Percent	8.87%			
CD3-/CD19+ (B Cells) Count/uL	216			
CD3-/CD56+CD16+ (NK Cells) Percent	11.51%			
CD3-/CD56+CD16+ (NK Cells) Count/uL	280			
*% Total Lymphocytes (T+B+NK) Percent	99.05%			
CD45+ Low SS Count/uL	2,433			
CD45+ Low SS Percent	50.71%			
CD45+ Count/uL	4,797			

* For Analytical QC purposes only | ** Population Statistics 03 Feb 2015 13:17:49 Page #1 of 1 Aquios v1.1.0.15026

Figure A.8 Report: Tetra-2+ Histograms

Sample ID:	89350141143	Test:	Tetra 2+ v1.1
Run Date:	03 Feb 2015 13:01:00	Analysis Date:	03 Feb 2015 13:01:00
User:	Admin	Collect Date:	03 Feb 2015
Specimen Type:	Whole Blood	Patient Name:	148217
Patient ID:		Gov. ID:	
Status:		Location:	
Gender:		Date Of Birth:	
Physician:		Physician Code:	
Instrument Serial #:	AV039002		
Run Flags:			
Run Notifications:			
Comments:			

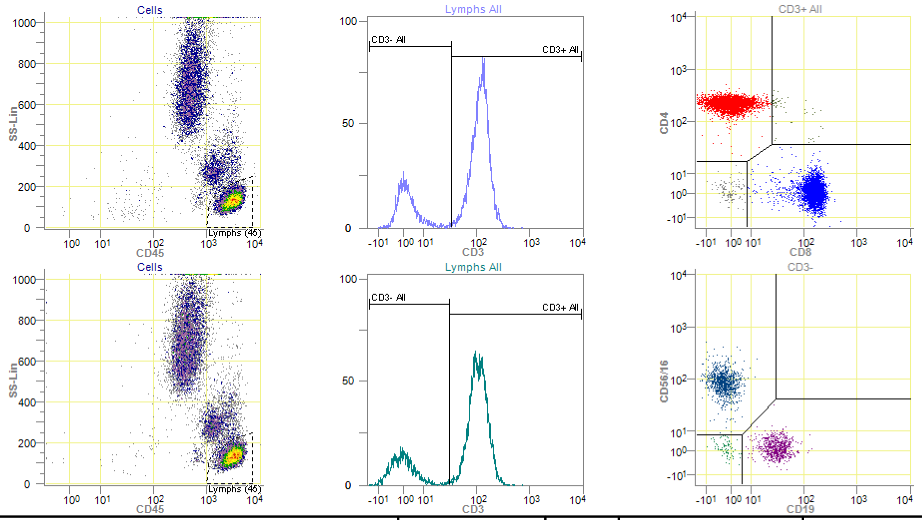


Result	Value	Flag	Normal Range	Action Range
CD3+ (T Cells) Percent	78.67%			
CD3+ (T Cells) Count/uL	1,914			
CD3-/CD19+ (B Cells) Percent	8.87%			
CD3-/CD19+ (B Cells) Count/uL	216			
CD3-/CD56+CD16+ (NK Cells) Percent	11.51%			
CD3-/CD56+CD16+ (NK Cells) Count/uL	280			
*% Total Lymphocytes (T+B+NK) Percent	99.05%			
CD45+ Low SS Count/uL	2,433			
CD45+ Low SS Percent	50.71%			
CD45+ Count/uL	4,797			

* For Analytical QC purposes only | ** Population Statistics 03 Feb 2015 13:18:19 Page #1 of 1 Aquios v1.1.0.15026

Figure A.9 Report: AQUIOS Tetra Combo Results (Page 1)

Sample ID:	89350141145	Test:	Tetra Combo v1.1
Run Date:	03 Feb 2015 12:49:31	Analysis Date:	03 Feb 2015 12:50:55
User:	Admin	Collect Date:	03 Feb 2015
Specimen Type:	Whole Blood	Patient Name:	148217
Patient ID:		Gov. ID:	
Status:		Location:	
Gender:		Date Of Birth:	
Physician:		Physician Code:	
Instrument Serial #:	AV039002		
Run Flags:			
Run Notifications:			
Comments:			



Result	Value	Flag	Normal Range	Action Range
Average CD3+ (T Cells) Percent	78.02%			
Average CD3+ (T Cells) Count/uL	1,664			
CD3+/CD4+ (Helper T Cells) Percent	44.81%			
CD3+/CD4+ (Helper T Cells) Count/uL	989			
CD3+/CD8+ (Suppressor T Cells) Percent	32.58%			
CD3+/CD8+ (Suppressor T Cells) Count/uL	719			
CD3-/CD19+ (B Cells) Percent	9.04%			
CD3-/CD19+ (B Cells) Count/uL	186			
CD3-/CD56+CD16+ (NK Cells) Percent	11.99%			
CD3-/CD56+CD16+ (NK Cells) Count/uL	247			
CD4:CD8 Ratio	1.38			
*Total Lymphocytes (T+B+NK) Percent	99.15%			
*CD3+ Reliability Check	0.58%			
*CD3+ Intrapanel Check	-0.22%			
CD45+ Low SS Count/uL	2,208			
CD45+ Low SS Percent	49.51%			
CD45+ Count/uL	4,459			

* For Analytical QC purposes only | ** Population Statistics 03 Feb 2015 13:19:07 Page #1 of 2 Aquios v1.1.0.15026

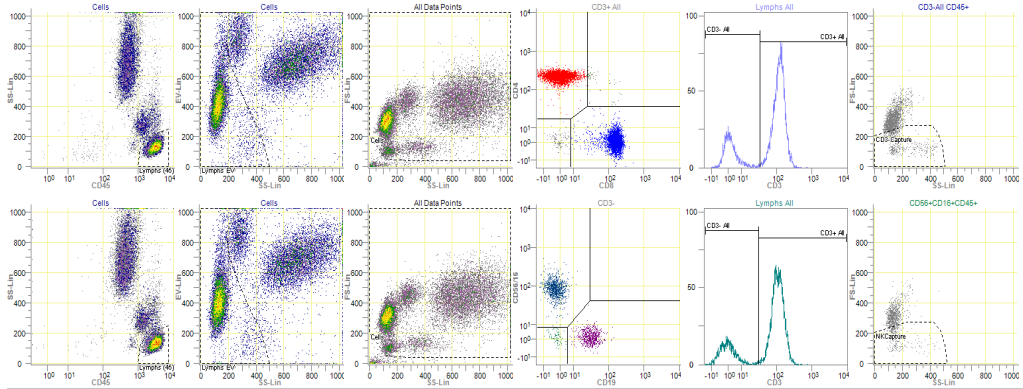
Figure A.10 Report: AQUIOS Tetra Combo Results (Page 2)

Sample ID:	89350141145	Test:	Tetra Combo v1.1
Run Date:	03 Feb 2015 12:49:31	Analysis Date:	03 Feb 2015 12:50:55

Result	Value	Flag	Normal Range	Action Range
CD3+ (T Cells) Percent Tetra1	77.91%			
CD3+ (T Cells) Count/uL Tetra1	1,720			
CD3+ (T Cells) Percent Tetra 2	78.13%			
CD3+ (T Cells) Count/uL Tetra 2	1,607			

Figure A.11 Report: AQUIOS Tetra Combo Details

Sample ID:	89350141145	Test:	Tetra Combo v1.1
Run Date:	03 Feb 2015 12:49:31	Analysis Date:	03 Feb 2015 12:50:55
User:	Admin	Collect Date:	03 Feb 2015
Specimen Type:	Whole Blood	Patient Name:	148217
Patient ID:		Gov. ID:	
Status:		Location:	
Gender:		Date Of Birth:	
Physician:		Physician Code:	
Instrument Serial #:	AV039002		
Run Flags:			
Run Notifications:			
Comments:			



Result	Value	Flag	Normal Range	Action Range
Average CD3+ (T Cells) Percent	78.02%			
Average CD3+ (T Cells) Count/uL	1,664			
CD3+/CD4+ (Helper T Cells) Percent	44.81%			
CD3+/CD4+ (Helper T Cells) Count/uL	989			
CD3+/CD8+ (Suppressor T Cells) Percent	32.58%			
CD3+/CD8+ (Suppressor T Cells) Count/uL	719			
CD3-/CD19+ (B Cells) Percent	9.04%			
CD3-/CD19+ (B Cells) Count/uL	186			
CD3-/CD56+CD16+ (NK Cells) Percent	11.99%			
CD3-/CD56+CD16+ (NK Cells) Count/uL	247			
CD4:CD8 Ratio	1.38			
*Total Lymphocytes (T+B+NK) Percent	99.15%			
*CD3+ Reliability Check	0.58%			
*CD3+ Intrapanel Check	-0.22%			
CD45+ Low SS Count/uL	2,208			
CD45+ Low SS Percent	49.51%			

* For Analytical QC purposes only | ** Population Statistics 03 Feb 2015 13:20:59 Page #1 of 2 Aquios v1.1.0.15026

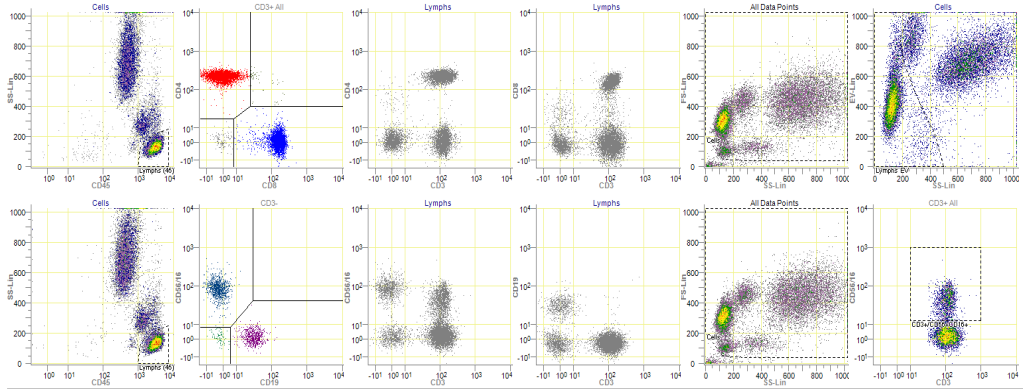
Figure A.12 Report: AQUIOS Tetra Combo Details (Page 2)

Sample ID:	89350141145	Test:	Tetra Combo v1.1
Run Date:	03 Feb 2015 12:49:31	Analysis Date:	03 Feb 2015 12:50:55

Result	Value	Flag	Normal Range	Action Range
CD45+ Count/uL	4,459			
CD3+ (T Cells) Percent Tetra1	77.91%			
CD3+ (T Cells) Count/uL Tetra1	1,720			
CD3+ (T Cells) Percent Tetra 2	78.13%			
CD3+ (T Cells) Count/uL Tetra 2	1,607			

Figure A.13 Report: AQUIOS Tetra Combo DotPlots

Sample ID:	89350141145	Test:	Tetra Combo v1.1
Run Date:	03 Feb 2015 12:49:31	Analysis Date:	03 Feb 2015 12:50:55
User:	Admin	Collect Date:	03 Feb 2015
Specimen Type:	Whole Blood	Patient Name:	148217
Patient ID:		Gov. ID:	
Status:		Location:	
Gender:		Date Of Birth:	
Physician:		Physician Code:	
Instrument Serial #:	AV039002		
Run Flags:			
Run Notifications:			
Comments:			



Result	Value	Flag	Normal Range	Action Range
Average CD3+ (T Cells) Percent	78.02%			
Average CD3+ (T Cells) Count/uL	1,664			
CD3+/CD4+ (Helper T Cells) Percent	44.81%			
CD3+/CD4+ (Helper T Cells) Count/uL	989			
CD3+/CD8+ (Suppressor T Cells) Percent	32.58%			
CD3+/CD8+ (Suppressor T Cells) Count/uL	719			
CD3-/CD19+ (B Cells) Percent	9.04%			
CD3-/CD19+ (B Cells) Count/uL	186			
CD3-/CD56+CD16+ (NK Cells) Percent	11.99%			
CD3-/CD56+CD16+ (NK Cells) Count/uL	247			
CD4:CD8 Ratio	1.38			
*Total Lymphocytes (T+B+NK) Percent	99.15%			
*CD3+ Reliability Check	0.58%			
*CD3+ Intrapanel Check	-0.22%			
CD45+ Low SS Count/uL	2,208			
CD45+ Low SS Percent	49.51%			

* For Analytical QC purposes only | ** Population Statistics 03 Feb 2015 13:21:36 Page #1 of 2 Aquios v1.1.0.15026

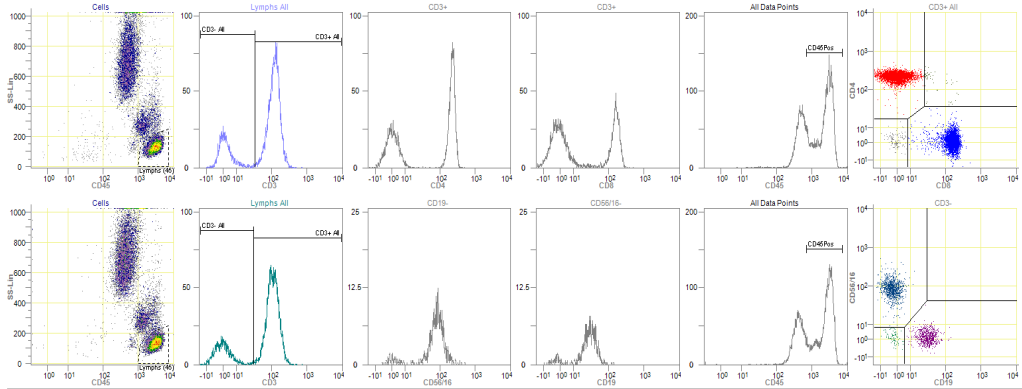
Figure A.14 Report: AQUIOS Tetra Combo DotPlots (Page 2)

Sample ID:	89350141145	Test:	Tetra Combo v1.1
Run Date:	03 Feb 2015 12:49:31	Analysis Date:	03 Feb 2015 12:50:55

Result	Value	Flag	Normal Range	Action Range
CD45+ Count/uL	4,459			
CD3+ (T Cells) Percent Tetra1	77.91%			
CD3+ (T Cells) Count/uL Tetra1	1,720			
CD3+ (T Cells) Percent Tetra 2	78.13%			
CD3+ (T Cells) Count/uL Tetra 2	1,607			

Figure A.15 Report: AQUIOS Tetra Combo Histograms

Sample ID:	89350141145	Test:	Tetra Combo v1.1
Run Date:	03 Feb 2015 12:49:31	Analysis Date:	03 Feb 2015 12:50:55
User:	Admin	Collect Date:	03 Feb 2015
Specimen Type:	Whole Blood	Patient Name:	148217
Patient ID:		Gov. ID:	
Status:		Location:	
Gender:		Date Of Birth:	
Physician:		Physician Code:	
Instrument Serial #:	AV039002		
Run Flags:			
Run Notifications:			
Comments:			



Result	Value	Flag	Normal Range	Action Range
Average CD3+ (T Cells) Percent	78.02%			
Average CD3+ (T Cells) Count/uL	1,664			
CD3+/CD4+ (Helper T Cells) Percent	44.81%			
CD3+/CD4+ (Helper T Cells) Count/uL	989			
CD3+/CD8+ (Suppressor T Cells) Percent	32.58%			
CD3+/CD8+ (Suppressor T Cells) Count/uL	719			
CD3-/CD19+ (B Cells) Percent	9.04%			
CD3-/CD19+ (B Cells) Count/uL	186			
CD3-/CD56+CD16+ (NK Cells) Percent	11.99%			
CD3-/CD56+CD16+ (NK Cells) Count/uL	247			
CD4:CD8 Ratio	1.38			
*Total Lymphocytes (T+B+NK) Percent	99.15%			
*CD3+ Reliability Check	0.58%			
*CD3+ Intrapanel Check	-0.22%			
CD45+ Low SS Count/uL	2,208			
CD45+ Low SS Percent	49.51%			

* For Analytical QC purposes only | ** Population Statistics 03 Feb 2015 13:22:04 Page #1 of 2 Aquios v1.1.0.15026

Figure A.16 Report: AQUIOS Tetra Combo Histograms (Page 2)

Sample ID:	89350141145	Test:	Tetra Combo v1.1
Run Date:	03 Feb 2015 12:49:31	Analysis Date:	03 Feb 2015 12:50:55

Result	Value	Flag	Normal Range	Action Range
CD45+ Count/uL	4,459			
CD3+ (T Cells) Percent Tetra1	77.91%			
CD3+ (T Cells) Count/uL Tetra1	1,720			
CD3+ (T Cells) Percent Tetra 2	78.13%			
CD3+ (T Cells) Count/uL Tetra 2	1,607			

Report Description

Report Heading

For a description of how to set up report headings, see Appendix A, Software Screens in the AQUIOS CL Flow Cytometer Instructions for Use manual.

Displays: Generating Reports

Reports can be generated using the available pre-defined formats for data display. Use the Graph Format drop-down menu under the Review or Result screens to select the display desired for the report.

Result Table Heading

- **Result** - use to view the assay statistics
- **Value** - use to view the patient's result obtained with the AQUIOS system
- **Flag** - Results are flagged as H or L if they are outside the normal ranges, and HH or LL if outside of the action ranges
- **Normal Range** - Reference values defined by the user in the Setup window, Test Settings tab
- **Action Range** - Reference values defined by the user in the Setup window, Test Settings tab

Statistics: AQUIOS Tetra-1 Report

- CD3+ (T-cells) Percent
- CD3+ (T-cells) Count/µL
- CD3+/CD4+ (Helper T-cells) Percent
- CD3+/CD4+ (Helper T-cells) Count/µL
- CD3+/CD8+ (Suppressor T-cells) Percent
- CD3+/CD8+ (Suppressor T-cells) Count/µL
- CD4:CD8 Ratio
- *CD3+ Reliability Check
- CD45+ Count/µL
- CD45+ Low SS Percent
- CD45+ Low SS Count/µL

NOTE QC Results are notated with a "*".

Statistics: AQUIOS Tetra-2+ Report

- CD3+ (T-cells) Percent
- CD3+ (T-cells) Count/µL
- CD3-/CD19+ (B-cells) Percent
- CD3-/CD19+ (B-cells) Count/µL
- CD3-/CD56+16 (NK Cells) Percent
- CD3-/CD56+16 (NK Cells) Count/µL
- *% Total Lymphocytes (T+B+NK)
- CD45+ Count/µL
- CD45+ Low SS Percent
- CD45+ Low SS Count/µL

NOTE QC Results are notated with a "*".

Statistics: AQUIOS Tetra Combo Report

- Average CD3+ (T-cells) Percent
- Average CD3+ (T-cells) Count/µL
- CD3+/CD4+ (Helper T-cells) Percent
- CD3+/CD4+ (Helper T-cells) Count/µL
- CD3+/CD8+ (Suppressor T-cells) Percent
- CD3+/CD8+ (Suppressor T-cells) Count/µL
- CD3-/CD19+ (B-cells) Percent
- CD3-/CD19+ (B-cells) Count/µL
- CD3-/CD56+16 (NK Cells) Percent
- CD3-/CD56+16 (NK Cells) Count/µL
- CD4:CD8 Ratio
- *Total Lymphocytes (T+B+NK) Percent
- *CD3+ Reliability Check
- *CD3+ Intrapanel Check
- CD3+ (T-cells) Percent Tetra 1
- CD3+ (T-cells) Count/µLTetra 1
- CD3+ (T-cells) Percent Tetra 2
- CD3+ (T-cells) Count/µL Tetra 2
- CD45+ Count/µL
- CD45+ Low SS Percent
- CD45+ Low SS Count/µL

NOTE QC Results are notated with a "*".

QC Report

The QC reports can be printed, saved (PDF) or created as a spreadsheet (CSV) file from any of the three QC screens. All three screens generate the same all-inclusive report containing the QC data in the following order: Results, Instrument, and Instrument Drift. Note that the QC report statistics depend on the assay selected under the Test drop-down menu. Also, the report can be customized under System Setup to display the name, address and facility logo. For generating a QC report, see the AQUIOS CL Flow Cytometer Instructions for Use manual.

The QC report can show all lot numbers or a single lot number. QC can be displayed for up to four months using the "Period" option. If lot (All) is selected, available lot numbers under this time frame will be displayed in the same graph identified with a different color.

NOTE Users with Administrator privileges or Reviewer/Editor privileges can exclude/restore data points.

QC Report Description

QC Result Report Heading, QC Instrument Report Heading, QC Instrument Drift Report Heading

- **Facility Name** - location where test was performed.
- **Test** - assay used for the control.
- **Control** - AQUIOS control selected for the report.
- **Lot number** - single lot selected or (All) if plotting all available lots.
- **Date** - month, year, and period selected for the report.

NOTE QC can be displayed for up to four months using the "Period" option.

QC Result Displays & Statistics (Depend on Test Selected)

- CD3+/CD4+ (Helper T-cells) Percent
- CD3+/CD4+ (Helper T-cells) Count/µL
- CD3+/CD8+ (Suppressor T-cells) Percent
- CD3+/CD8+ (Suppressor T-cells) Count/µL
- CD3-/CD19+ (B-cells) Percent
- CD3-/CD19+ (B-cells) Count/µL
- CD3-/CD56+CD16+ (NK Cells) Percent
- CD3-/CD56+CD16+ (NK Cells) Count/µL
- CD3+ (T-cells) Percent Tetra 1
- CD3+ (T-cells) Count/µL Tetra 1
- CD3+ (T-cells) Percent Tetra 2
- CD3+ (T-cells) Count/µL Tetra 2
- CD45+ Count/µL

- CD45+ Low SS Percent
- CD45+ Low SS Count/µL

QC Result First Table Heading

- **Lot** - lot number used for the specified QC run.
- **Average** - average of all listed runs.
- **Min** - lowest value of all listed runs.
- **Max** - highest value of all listed runs.
- **CV** - coefficient of variation of all listed runs (Standard Deviation/Average) x 100
- **StDev** - standard deviation of all listed runs.

QC Result Second Table Heading, QC Instrument Second Table Heading, QC Instrument Drift Second Table Heading

- **Exclude** - data points included or excluded in the report.
- **Lot** - single lot selected or (All) if plotting all available lots.
- **Date run** - date in which control was analyzed.
- **Pass/Fail** - If true, data point passes expected range. If false, data point failed expected range.
- **Assay statistics** - percent or count/µL of selected assay.

QC Instrument Displays (Depends on Test Selected)

- **CD3 Separation
- **CD4 Separation
- **CD8 Separation
- **CD19 Separation
- **CD56/16 Separation
- **CD45 Lymph/High SS Cells Separation
- **SS Lymph/High SS Cells Separation
- **FS Lymph/High SS Cells Separation

NOTE Population Statistics are notated with a "**". When the software says Separation it is referring to Separation Quotient. For a definition of Separation Quotient, see the Instrument Tab in CHAPTER 3, Quality Control.

QC Instrument Drift Displays (Depends on Test Selected)

- **CD3+ Channel Number
- **CD4+ Channel Number
- **CD8+ Channel Number
- **CD19+ Channel Number
- **CD56/16+ and/or CD16 Channel Number

- **CD45 Lymphs Channel Number
- **SS Lymphs Channel Number
- **FS Lymphs Channel Number

NOTE Population Statistics are notated with a "**".

QC Reports

Figure A.17 QC Results: Results View of AQUIOS IMMUNO-TROL, AQUIOS Tetra-1

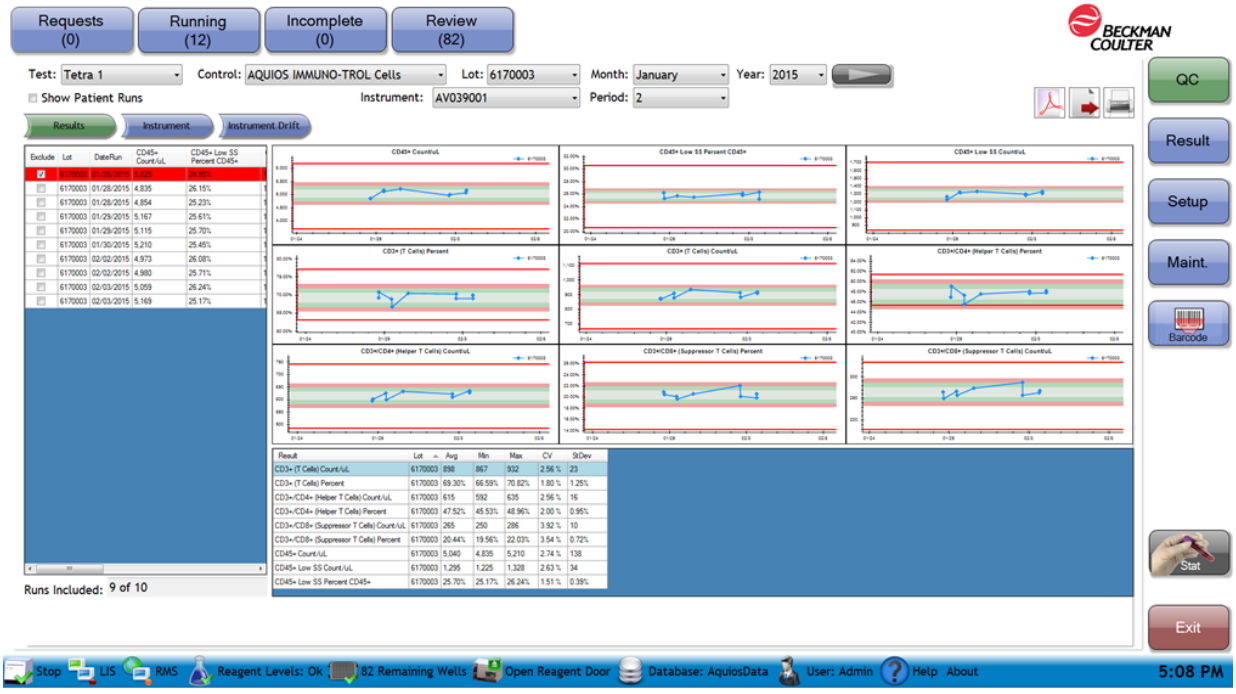


Figure A.18 QC Report: AQUIOS IMMUNO-TROL, AQUIOS Tetra-1 (Page 1)

Instrument SN: All | Test: Tetra 1 | Control: AQUIOS IMMUNO-TROL Cells | Lot: 6170003 | Date: January 2015

Results:

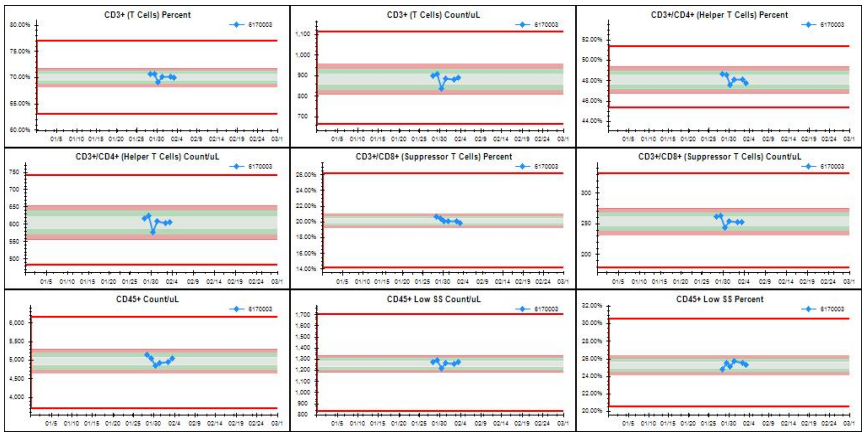


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Figure A.19 QC Report: AQUIOS IMMUNO-TROL, AQUIOS Tetra-1 (Page 2)

Instrument SN: All | Test: Tetra 1 | Control: AQUIOS IMMUNO-TROL Cells | Lot: 6170003 | Date: January 2015

Instrument:

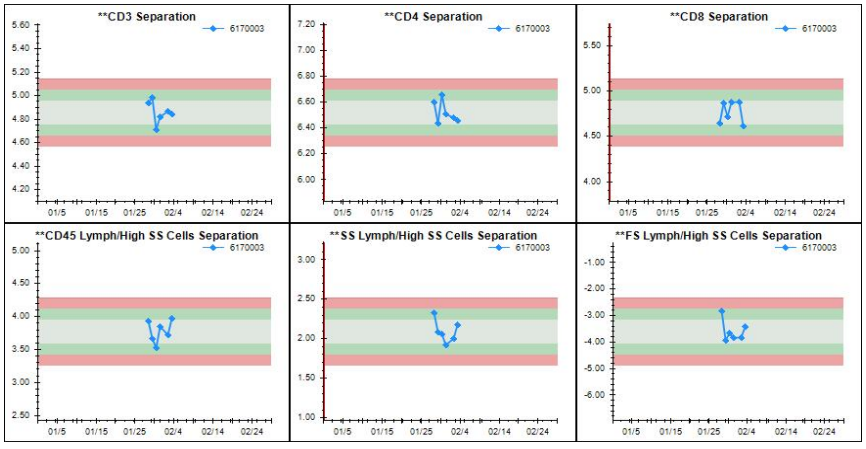

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Figure A.20 QC Report: AQUIOS IMMUNO-TROL, AQUIOS Tetra-1 (Page 3)

Instrument SN: All | Test: Tetra 1 | Control: AQUIOS IMMUNO-TROL Cells | Lot: 6170003 | Date: January 2015

Instrument Drift:

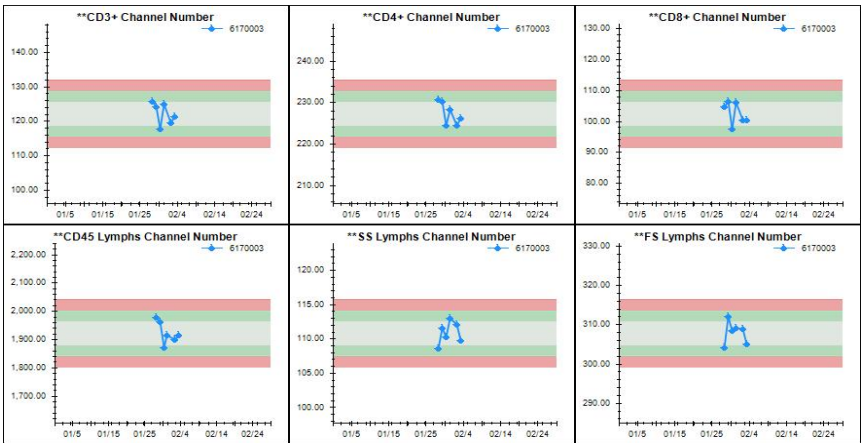

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Figure A.21 QC Report: AQUIOS IMMUNO-TROL, AQUIOS Tetra-1 (Page 4)

Instrument SN: All | Test: Tetra 1 | Control: AQUIOS IMMUNO-TROL Cells | Lot: 6170003 | Date: January 2015

Result	Lot	Avg	Min	Max	CV	StDev
CD3+ (T Cells) Percent	6170003	70.07%	69.09%	70.69%	0.83 %	0.58%
CD3+ (T Cells) Count/uL	6170003	882	837	908	2.76 %	24
CD3+/CD4+ (Helper T Cells) Percent	6170003	48.09%	47.52%	48.64%	0.92 %	0.44%
CD3+/CD4+ (Helper T Cells) Count/uL	6170003	605	575	624	2.73 %	17
CD3+/CD8+ (Suppressor T Cells) Percent	6170003	20.17%	19.84%	20.59%	1.39 %	0.28%
CD3+/CD8+ (Suppressor T Cells) Count/uL	6170003	254	243	263	2.82 %	7
CD45+ Count/uL	6170003	4,979	4,831	5,128	2.15 %	107
CD45+ Low SS Count/uL	6170003	1,259	1,211	1,286	2.03 %	26
CD45+ Low SS Percent	6170003	25.29%	24.71%	25.70%	1.41 %	0.36%

Exclude (Yes/No)	Lot	DateRun	CD3+ (T Cells) Percent	Pass/Fail	CD3+ (T Cells) Count/uL	CD3+/CD4+ (Helper T Cells) Percent	CD3+/CD4+ (Helper T Cells) Count/uL	CD3+/CD8+ (Suppressor T Cells) Percent	CD3+/CD8+ (Suppressor T Cells) Count/uL	CD45+ Count/uL	CD45+ Low SS Count/uL	CD45+ Low SS Percent	**CD3 Separation	**CD4 Separation	**CD8 Separation	**CD45 Lymph/High SS Cells Separation	**SS Lymph/High SS Cells Separation	**FS Lymph/High SS Cells Separation	**CD3+ Channel Number	**CD4+ Channel Number	**CD8+ Channel Number
No	6170003	01/28/2015	70.69%	Pass	896	48.64%	616	20.59%	261	5,128	1,267	24.71%	4.93	6.60	4.64	3.93	2.32	-2.83	125.63	230.56	104.58
No	6170003	01/29/2015	70.61%	Pass	908	48.51%	624	20.42%	263	5,044	1,286	25.50%	4.98	6.43	4.86	3.66	2.08	-3.97	124.20	230.15	106.26
No	6170003	01/30/2015	69.09%	Pass	837	47.52%	575	20.05%	243	4,831	1,211	25.07%	4.71	6.65	4.71	3.52	2.06	-3.68	117.53	224.43	97.47
No	6170003	01/31/2015	70.09%	Pass	885	48.09%	607	20.06%	253	4,913	1,263	25.70%	4.81	6.51	4.88	3.84	1.92	-3.84	124.99	228.15	106.04
No	6170003	02/02/2015	70.08%	Fail	880	48.06%	604	20.05%	252	4,929	1,256	25.48%	4.86	6.48	4.88	3.72	2.00	-3.85	119.49	224.49	100.29
No	6170003	02/03/2015	69.85%	Pass	887	47.68%	606	19.84%	252	5,026	1,271	25.28%	4.84	6.45	4.61	3.96	2.17	-3.45	121.29	226.07	100.22

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Figure A.22 QC Results: Results View of AQUIOS IMMUNO-TROL Low, AQUIOS Tetra-1

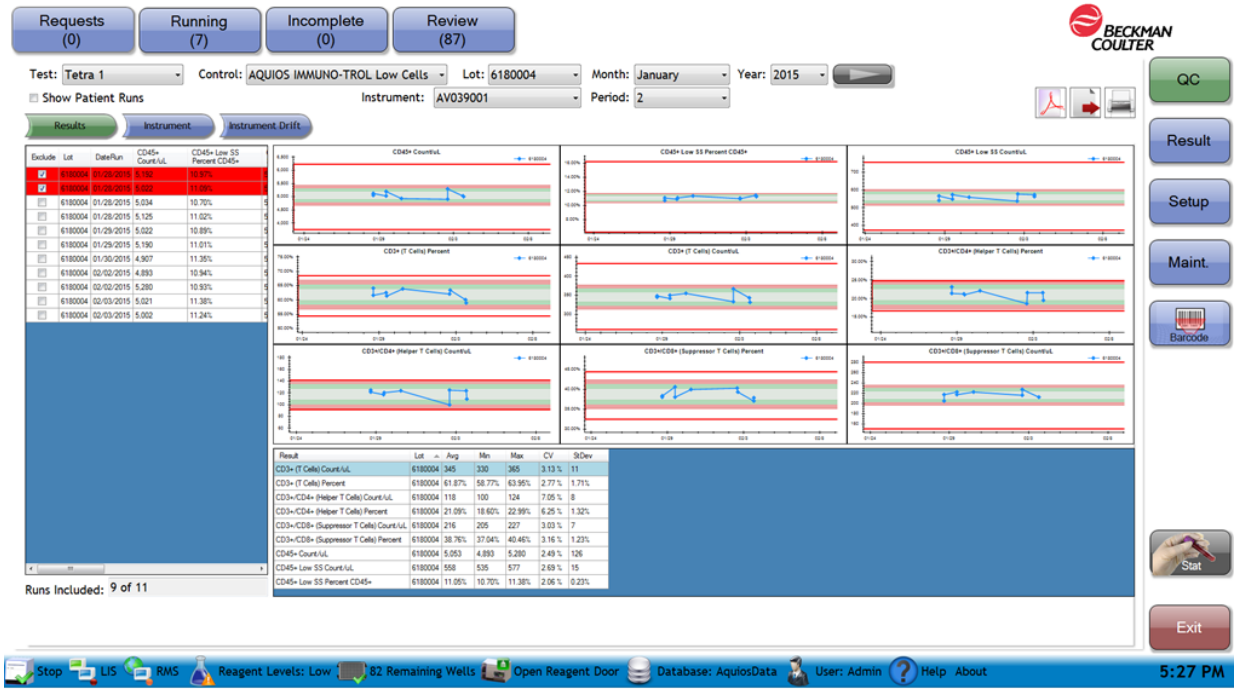

Figure A.23 QC Report: AQUIOS IMMUNO-TROL Low, AQUIOS Tetra-1 (Page 1)

Instrument SN: All | Test: Tetra 1 | Control: AQUIOS IMMUNO-TROL Low Cells | Lot: 6180004 | Date: January 2015

Results:

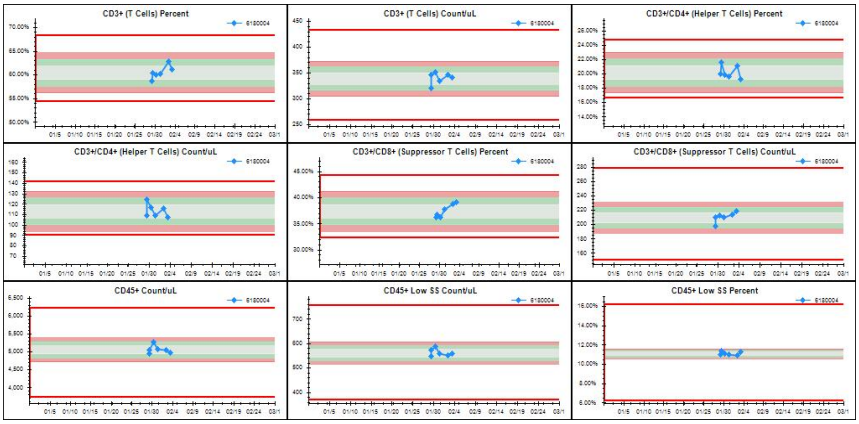

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Figure A.24 QC Report: AQUIOS IMMUNO-TROL Low, AQUIOS Tetra-1 (Page 2)

Instrument SN: All | Test: Tetra 1 | Control: AQUIOS IMMUNO-TROL Low Cells | Lot: 6180004 | Date: January 2015

Instrument:

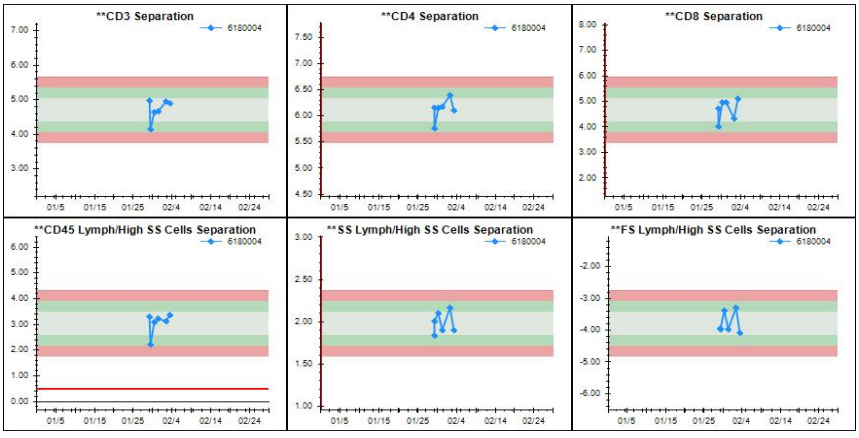

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Figure A.25 QC Report: AQUIOS IMMUNO-TROL Low, AQUIOS Tetra-1 (Page 3)

Instrument SN: All | Test: Tetra 1 | Control: AQUIOS IMMUNO-TROL Low Cells | Lot: 6180004 | Date: January 2015

Instrument Drift:

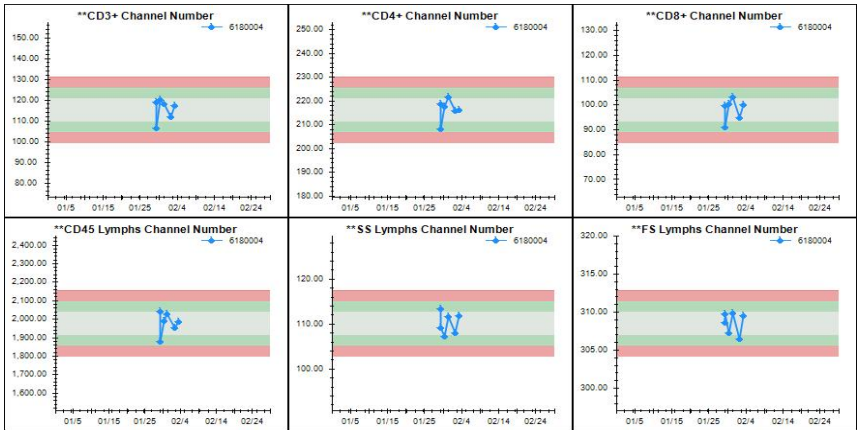

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Figure A.26 QC Report: AQUIOS IMMUNO-TROL Low, AQUIOS Tetra-1 (Page 4)

Instrument SN: All | Test: Tetra 1 | Control: AQUIOS IMMUNO-TROL Low Cells | Lot: 6180004 | Date: January 2015

Result	Lot	Avg	Min	Max	CV	StDev
CD3+ (T Cells) Percent	6180004	60.43%	58.57%	62.82%	2.33 %	1.41%
CD3+ (T Cells) Count/uL	6180004	339	319	350	3.33 %	11
CD3+/CD4+ (Helper T Cells) Percent	6180004	20.18%	19.13%	21.58%	4.65 %	0.94%
CD3+/CD4+ (Helper T Cells) Count/uL	6180004	113	107	124	5.68 %	6
CD3+/CD8+ (Suppressor T Cells) Percent	6180004	37.40%	36.12%	39.10%	3.43 %	1.28%
CD3+/CD8+ (Suppressor T Cells) Count/uL	6180004	210	197	218	3.42 %	7
CD45+ Count/uL	6180004	5,053	4,946	5,264	2.24 %	113
CD45+ Low SS Count/uL	6180004	561	545	585	2.69 %	15
CD45+ Low SS Percent	6180004	11.10%	10.92%	11.35%	1.50 %	0.17%

Exclude (Yes/No)	Lot	DateRun	CD3+ (T Cells) Percent	Pass/Fail	CD3+ (T Cells) Count/uL	CD3+/CD4+ (Helper T Cells) Percent	CD3+/CD4+ (Helper T Cells) Count/uL	CD3+/CD8+ (Suppressor T Cells) Percent	CD3+/CD8+ (Suppressor T Cells) Count/uL	CD45+ Count/uL	CD45+ Low SS Count/uL	CD45+ Low SS Percent	**CD3 Separation	**CD4 Separation	**CD8 Separation	**CD45 Lymph/High SS Cells Separation	**SS Lymph/High SS Cells Separation	**FS Lymph/High SS Cells Separation	**CD3+ Channel Number	**CD4+ Channel Number	**CD8+ Channel Number
No	6180004	01/29/2015	58.57%	Fail	319	19.88%	108	36.12%	197	4,946	545	11.01%	4.98	6.14	4.72	3.28	1.84	-3.96	118.91	218.69	99.44
No	6180004	01/29/2015	60.21%	Pass	345	21.58%	124	36.62%	210	5,048	573	11.35%	4.14	5.75	4.01	2.20	2.00	-3.97	106.18	207.97	91.03
No	6180004	01/30/2015	59.92%	Pass	350	19.83%	116	36.22%	212	5,264	585	11.11%	4.63	6.15	4.95	3.09	2.09	-3.39	120.07	217.51	100.19
No	6180004	01/31/2015	60.06%	Fail	334	19.59%	109	37.64%	209	5,059	555	10.98%	4.66	6.16	4.94	3.20	1.90	-3.98	117.79	221.71	103.16
No	6180004	02/02/2015	62.82%	Pass	345	21.08%	116	38.69%	213	5,035	550	10.92%	4.95	6.39	4.31	3.12	2.16	-3.30	111.89	215.91	94.58
No	6180004	02/03/2015	60.99%	Pass	341	19.13%	107	39.10%	218	4,966	559	11.25%	4.90	6.10	5.10	3.36	1.89	-4.09	117.00	216.06	99.93

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Figure A.27 QC Results: Results View of AQUIOS IMMUNO-TROL, AQUIOS Tetra-2+

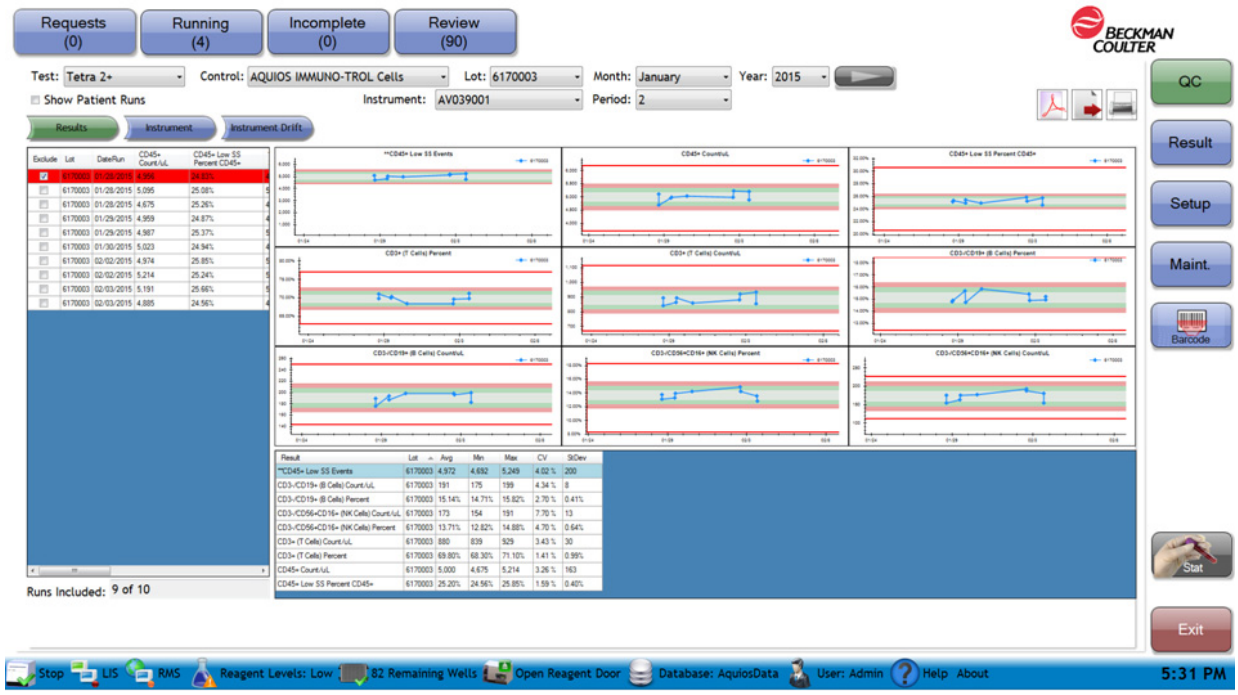

Figure A.28 QC Report: AQUIOS IMMUNO-TROL, AQUIOS Tetra-2+ (Page 1)

Instrument SN: All | Test: Tetra 2+ | Control: AQUIOS IMMUNO-TROL Cells | Lot: 6170003 | Date: January 2015

Results:

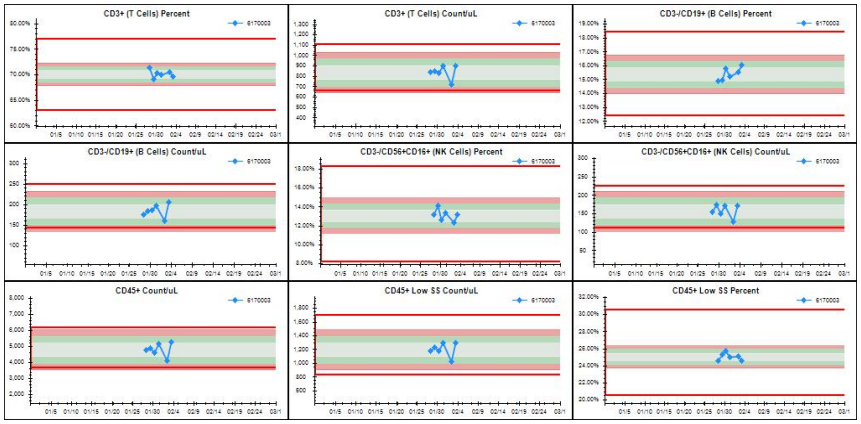

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Figure A.29 QC Report: AQUIOS IMMUNO-TROL, AQUIOS Tetra-2+ (Page 2)

Instrument SN: All | Test: Tetra 2+ | Control: AQUIOS IMMUNO-TROL Cells | Lot: 6170003 | Date: January 2015

Instrument:

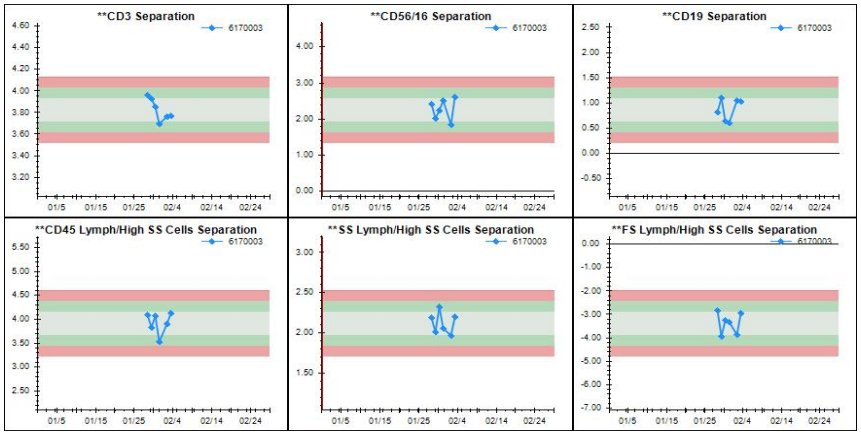

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Figure A.30 QC Report: AQUIOS IMMUNO-TROL, AQUIOS Tetra-2+ (Page 3)

Instrument SN: All | Test: Tetra 2+ | Control: AQUIOS IMMUNO-TROL Cells | Lot: 6170003 | Date: January 2015

Instrument Drift:

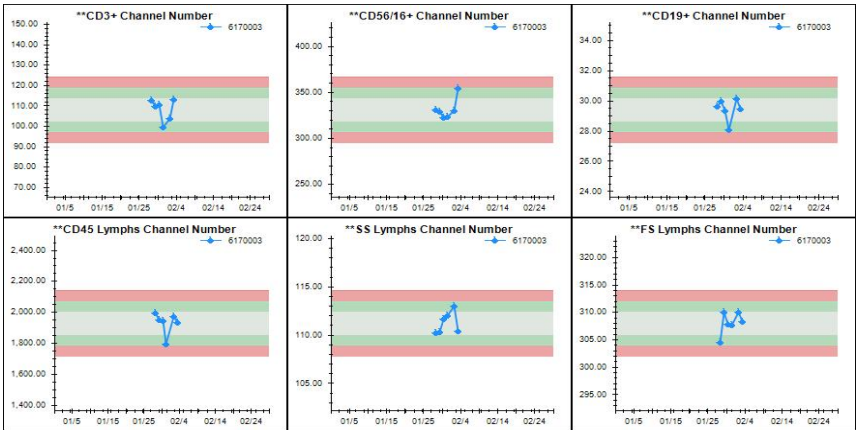

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Figure A.31 QC Report: AQUIOS IMMUNO-TROL, AQUIOS Tetra-2+ (Page 4)

Instrument SN: All | Test: Tetra 2+ | Control: AQUIOS IMMUNO-TROL Cells | Lot: 6170003 | Date: January 2015

Result	Lot	Avg	Min	Max	CV	StDev
CD3+ (T Cells) Percent	6170003	70.06%	69.10%	71.22%	1.05 %	0.73%
CD3+ (T Cells) Count/uL	6170003	837	721	900	7.73 %	65
CD3-/CD19+ (B Cells) Percent	6170003	15.37%	14.85%	16.00%	3.03 %	0.47%
CD3-/CD19+ (B Cells) Count/uL	6170003	184	159	206	8.90 %	16
CD3-/CD56+CD16+ (NK Cells) Percent	6170003	13.07%	12.28%	14.04%	4.71 %	0.62%
CD3-/CD56+CD16+ (NK Cells) Count/uL	6170003	157	126	172	11.65 %	18
CD45+ Count/uL	6170003	4,774	4,079	5,229	8.80 %	420
CD45+ Low SS Count/uL	6170003	1,195	1,024	1,286	8.17 %	98
CD45+ Low SS Percent	6170003	25.04%	24.56%	25.71%	1.76 %	0.44%

Exclude (Yes/No)	Lot	DateRun	CD3+ (T Cells) Percent	Pass/Fail	CD3+ (T Cells) Count/uL	CD3 -/CD19+ (B Cells) Percent	CD3 -/CD19+ (B Cells) Count/uL	CD3 -/CD56+CD16+ (NK Cells) Percent	CD3 -/CD56+CD16+ (NK Cells) Count/uL	CD45+ Count/uL	CD45+ Low SS Count/uL	CD45+ Low SS Percent	**CD3 Separation	**CD56/16 Separation	**CD19 Separation	**CD45 Lymph/High SS Cells Separation	**SS Lymph/High SS Cells Separation	**FS Lymph/High SS Cells Separation	**CD3+ Channel Number	**CD56/16+ Channel Number	**CD19+ Channel Number
No	6170003	01/28/2015	71.22%	Pass	833	14.85%	174	13.11%	153	4,763	1,170	24.56%	3.96	2.40	0.80	4.09	2.19	-2.84	112.41	330.24	29.58
No	6170003	01/29/2015	69.10%	Pass	849	14.92%	183	14.04%	172	4,855	1,228	25.30%	3.92	2.00	1.10	3.82	2.01	-3.98	109.53	328.37	29.93
No	6170003	01/30/2015	70.19%	Pass	824	15.76%	185	12.55%	147	4,568	1,174	25.71%	3.85	2.22	0.64	4.06	2.32	-3.26	110.51	321.69	29.33
No	6170003	01/31/2015	69.96%	Pass	900	15.17%	195	13.30%	171	5,152	1,286	24.97%	3.70	2.50	0.60	3.52	2.05	-3.35	99.53	322.46	28.07
No	6170003	02/02/2015	70.36%	Fail	721	15.49%	159	12.28%	126	4,079	1,024	25.11%	3.76	1.82	1.04	3.89	1.96	-3.88	103.57	329.05	30.13
No	6170003	02/03/2015	69.51%	Pass	893	16.00%	206	13.17%	169	5,229	1,285	24.57%	3.77	2.60	1.02	4.12	2.20	-2.97	113.04	354.23	29.41

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Figure A.32 QC Results: Results View of AQUIOS IMMUNO-TROL Low, AQUIOS Tetra-2+

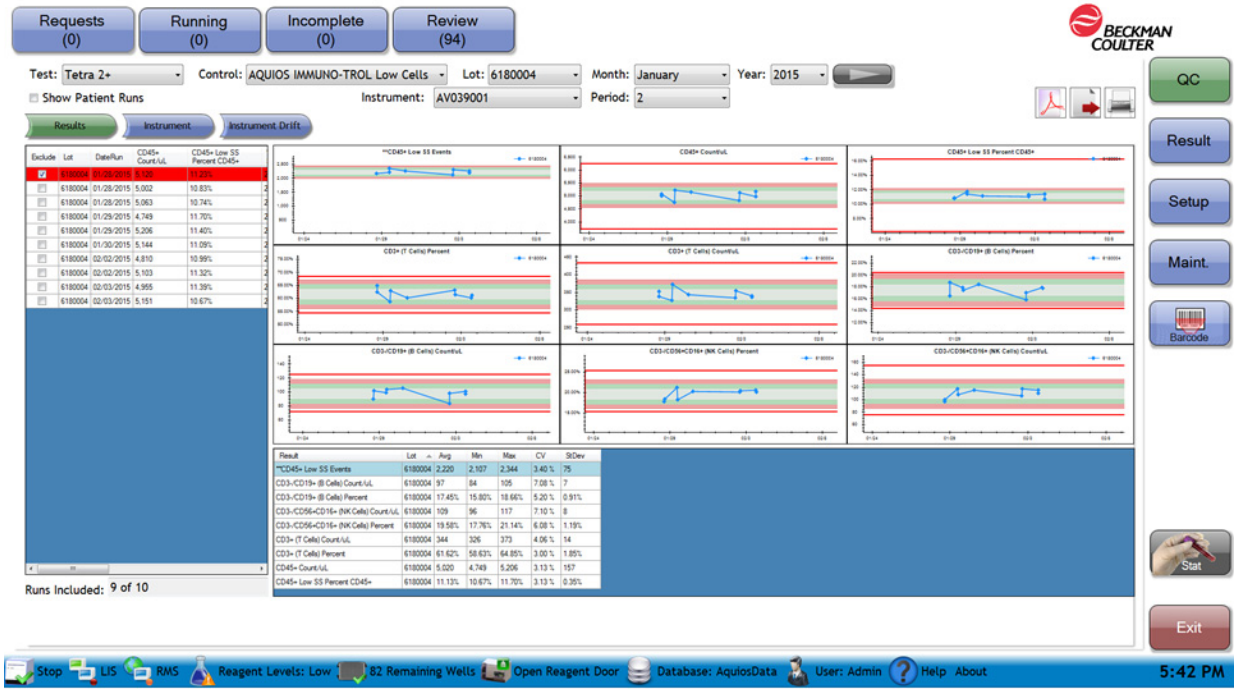

Figure A.33 QC Report: AQUIOS IMMUNO-TROL Low, AQUIOS Tetra-2+ (Page 1)

Instrument SN: All | Test: Tetra 2+ | Control: AQUIOS IMMUNO-TROL Low Cells | Lot: 6180004 | Date: January 2015

Results:

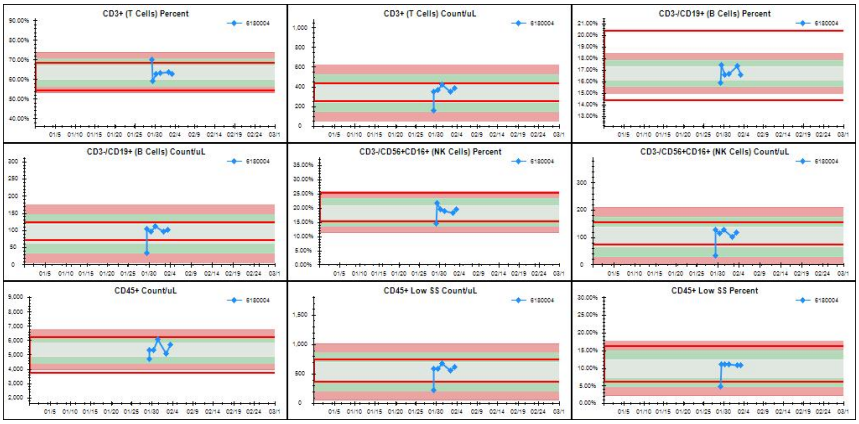

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Figure A.34 QC Report: AQUIOS IMMUNO-TROL Low, AQUIOS Tetra-2+ (Page 2)

Instrument SN: All | Test: Tetra 2+ | Control: AQUIOS IMMUNO-TROL Low Cells | Lot: 6180004 | Date: January 2015

Instrument:

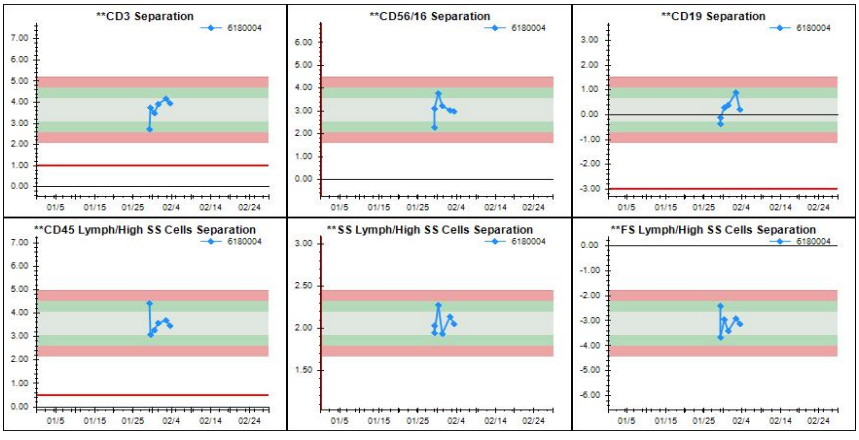

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Figure A.35 QC Report: AQUIOS IMMUNO-TROL Low, AQUIOS Tetra-2+ (Page 3)

Instrument SN: All | Test: Tetra 2+ | Control: AQUIOS IMMUNO-TROL Low Cells | Lot: 6180004 | Date: January 2015

Instrument Drift:

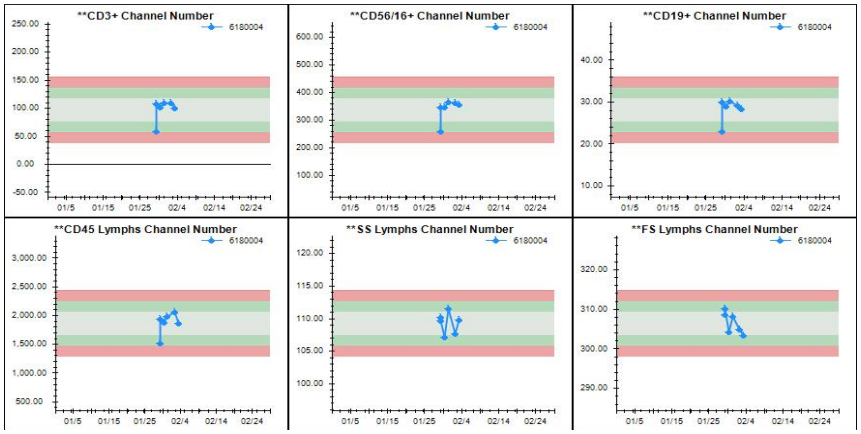

Figure A.36 QC Report: AQUIOS IMMUNO-TROL Low, AQUIOS Tetra-2+ (Page 4)

Instrument SN: All | Test: Tetra 2+ | Control: AQUIOS IMMUNO-TROL Low Cells | Lot: 6180004 | Date: January 2015

Result	Lot	Avg	Min	Max	CV	StDev
CD3+ (T Cells) Percent	6180004	63.56%	59.24%	69.79%	5.40 %	3.43%
CD3+ (T Cells) Count/uL	6180004	337	153	421	27.88 %	94
CD3-/CD19+ (B Cells) Percent	6180004	16.71%	15.83%	17.40%	3.42 %	0.57%
CD3-/CD19+ (B Cells) Count/uL	6180004	90	35	111	30.76 %	28
CD3-/CD56+CD16+ (NK Cells) Percent	6180004	18.58%	14.27%	21.57%	12.93 %	2.40%
CD3-/CD56+CD16+ (NK Cells) Count/uL	6180004	102	31	127	35.37 %	36
CD45+ Count/uL	6180004	5,349	4,688	6,038	8.75 %	468
CD45+ Low SS Count/uL	6180004	537	219	667	29.85 %	160
CD45+ Low SS Percent	6180004	9.91%	4.68%	11.10%	25.87 %	2.56%

Exclude (Yes/No)	Lot	DateRun	CD3+ (T Cells) Percent	Pass/Fail	CD3+ (T Cells) Count/uL	CD3 -/CD19+ (B Cells) Percent	CD3 -/CD19+ (B Cells) Count/uL	CD3 -/CD56+CD16+ (NK Cells) Percent	CD3 -/CD56+CD16+ (NK Cells) Count/uL	CD45+ Count/uL	CD45+ Low SS Count/uL	CD45+ Low SS Percent	**CD3 Separation	**CD56/16 Separation	**CD19 Separation	**CD45 Lymph/High SS Cells Separation	**SS Lymph/High SS Cells Separation	**FS Lymph/High SS Cells Separation	**CD3+ Channel Number	**CD56/16+ Channel Number	**CD19+ Channel Number
No	6180004	01/29/2015	69.79%	Fail	153	15.83%	35	14.27%	31	4,688	219	4.68%	2.70	2.27	-0.39	4.40	2.02	-2.43	58.33	258.28	22.98
No	6180004	01/29/2015	59.24%	Pass	349	17.40%	102	21.57%	127	5,309	589	11.10%	3.73	3.10	-0.13	3.05	1.94	-3.68	106.55	344.07	29.82
No	6180004	01/30/2015	62.80%	Pass	366	16.58%	97	19.32%	113	5,325	583	10.95%	3.47	3.76	0.27	3.24	2.27	-2.97	100.31	343.49	28.79
No	6180004	01/31/2015	63.03%	Fail	421	16.64%	111	18.88%	126	6,038	667	11.06%	3.90	3.20	0.37	3.57	1.93	-3.44	109.36	365.27	30.00
No	6180004	02/02/2015	63.64%	Pass	351	17.29%	95	18.13%	100	5,065	551	10.88%	4.16	3.00	0.89	3.66	2.13	-2.94	109.27	360.52	29.03
No	6180004	02/03/2015	62.83%	Pass	385	16.55%	101	19.29%	118	5,668	612	10.80%	3.92	2.97	0.18	3.46	2.05	-3.16	99.69	353.10	28.30

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AQUIOS Tetra System Guide
PN B26364
- Use and Function
- Operation Principles
- Sample Analysis
- Data Review
- Performance Characteristics
- Application Specifications
- Appendix
- References

AQUIOS CL Flow Cytometer Instructions for Use
PN B21896
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- Messages
- Messages from ASTM Engine

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