WHITE PAPER

EVALUATION OF THE ANALYTICAL PERFORMANCE OF THE AQUIOS CL FLOW CYTOMETER IN A MULTI-CENTER STUDY

Diana Careaga[1], Robert Magari[1], Karen Lo1, Michael Keeney[2], Ben Hedley[2], Dominika Benjamins[2], Dr. You-Wen Qian[3], Larry Hinson[3], Cristina Ceja[3], Alexandra Amador[4], Casiana Fernandez-Bango[4], Rosa Colon[5], Oilda Rubio[5], Eileen Landrum[5], Justin Rohrbach[1], Robert Ortega[1], Ana Vieira[1], Dawn Holmes[1], Raquel Cabana[6], Pedro Diaz[6], and Lilliana Tejidor[1]

Beckman Coulter Diagnostics, Inc., [1]Clinical Research, Miami, Florida, USA

[2]London Health Sciences Centre, London, Ontario, Canada

[3]The University of Texas Medical Branch, Galveston, Texas, USA

[4]University of Miami Miller School of Medicine, Miami, Florida, USA

Beckman Coulter, Inc., [5]Clinical Quality Assurance, Research and Development, Miami, Florida, USA

Beckman Coulter Life Science, Inc., [6]Research and Development, Miami, Florida, USA

Introduction

A multi-center study was conducted to establish the performance of the AQUIOS CL Flow Cytometer with AQUIOS Tetra Panel reagents for lymphocyte subset analysis in patients suspected of immunodeficiency, post-transplant patients and apparently healthy individuals. Performance equivalency was evaluated compared to the BD FACSCalibur™ Flow Cytometer with Multitest™ IMK kit and TruCount tubes.

Objectives and Methods

Precision performance of the AQUIOS Tetra system was evaluated based on CLSI EP5-A2 using control materials as samples.

To assess substantial equivalency, the AQUIOS CL Flow Cytometer with AQUIOS Tetra reagents was compared against the BD FACSCalibur™ Flow Cytometer with BD Multitest™ reagents using a single platform approach for absolute counts. A multi-center study was conducted to target the intended use population following CLSI EP09-A3. The AQUIOS Tetra gating method was compared to the FACSCalibur™ system with Multiset™ software in ≥400 samples covering the CD4+ medical decision levels. Fully automated sample preparation was performed by the AQUIOS CL Flow Cytometer and manually for analysis on the FACSCalibur™ Flow Cytometer.

Hematologically normal adult donors between 18 – 65 years old with a targeted ratio of 50:50 males to females from three (3) geographical locations were combined to establish the reference interval for AQUIOS Tetra-1 Panel and Tetra-2+ Panel. The adult reference interval was determined following CLSI EP28-A2.

Results

The AQUIOS CL Flow Cytometer showed excellent repeatability and reproducibility. Tables 1 and 2 summarize representative results for AQUIOS Tetra-1 and AQUIOS Tetra-2+ reagents for all sites combined. Two levels of control material were evaluated at three (3) sites. Tables 1 and 2 provide results for AQUIOS IMMUNO-TROL Cells and AQUIOS IMMUNO-TROL Low Cells, respectively. The repeatability and reproducibility for very low CD4 levels (123 cells/µL) was <5%.

Substantial equivalency of the AQUIOS CL system with AQUIOS Tetra reagents was demonstrated against the BD FACSCalibur system with BD Multitest™ reagents [CD3-FITC/CD8-PE/CD45-PerCP/CD4-APC and CD3-FITC/CD16+CD56-PE/CD45-PerCP/CD19-APC] using a single platform approach for absolute counts [TruCount]. A total

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of 443 samples combined from four (4) clinical sites covering the CD4 analytical measuring range with emphasis on the medical decision ranges were tested on both platforms.

Percent marker recovery comparisons had clinically insignificant to no bias. A negative bias for the comparison of the AQUIOS system to the BD FACSCalibur™ system was observed in the absolute count marker recovery for all four (4) sites combined. This negative trend observed for all markers was not clinically significant. Smaller differences were observed for CD3+/CD4+ counts compared to other markers as summarized in Tables 3 and 4. The bias was clinically insignificant at the medical decision points for CD3+/CD4+ as noted in Table 5. The additional parameters that AQUIOS provides (CD45+ and CD45+ Low SS) are not provided.

Figures 1 and 2 illustrate regression and Bland-Altman plots, respectively for CD3+/CD4+ count confirming comparability of AQUIOS Tetra application to the BD FACSCalibur™ Flow Cytometer.

A total of 161 hematological normal adult donors were combined to establish reference Interval. Normal reference intervals for lymphocyte subsets (data not shown) were consistent with published values.

AQUIOS Tetra Precision Performance: 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]

Table 1. Precision results from the combined sites for a normal CD4 level (AQUIOS IMMUNO-TROL as sample)

COMBINED SITES		IMMUNO TROL		REPEATABILITY	WITHIN SITE	REPRODUCIBILITY
Name	**Unit**	**N**	**Mean**	**CV%**	**CV%**	**CV%**
CD3+	%	311	72.40	1.23	1.38	1.39
	cells/µL	311	831	2.66	2.96	3.27
CD3+/CD4+	%	311	48.37	1.76	1.86	1.86
	cells/µL	311	555	2.94	3.32	3.58
CD3+/CD8+	%	311	22.16	3.11	3.15	3.17
	cells/µL	311	254	3.93	3.99	4.36
CD3+	%	311	72.00	1.37	1.49	1.55
	cells/µL	311	833	2.81	3.10	3.79
CD3-/CD19+	%	311	13.65	3.39	3.45	3.86
	cells/µL	311	158	4.38	4.56	5.51
CD3-/ CD56+16+	%	311	12.30	5.28	5.48	5.52
	cells/µL	311	142	6.69	6.87	7.34

Table 2. Precision results from the combined sites for a low CD4 level (AQUIOS IMMUNO-TROL Low as sample)

COMBINED SITES		IMMUNO TROL LOW		REPEATABILITY	WITHIN SITE	REPRODUCIBILITY
Name	**Unit**	**N**	**Mean**	**CV%**	**CV%**	**CV%**
CD3+	%	307	58.00	2.07	2.21	2.22
	cells/µL	307	388	3.09	3.40	3.70
CD3+/CD4+	%	307	18.37	3.84	4.23	4.23
	cells/µL	307	123	4.52	4.92	4.96
CD3+/CD8+	%	307	35.60	3.06	3.14	3.24
	cells/µL	307	238	3.92	4.23	4.69
CD3+	%	307	57.45	2.06	2.17	2.22
	cells/µL	307	387	2.89	3.32	3.68
CD3-/CD19+	%	307	17.33	4.46	4.46	4.46
	cells/µL	307	117	5.10	5.10	5.24
CD3-/ CD56+16+	%	307	23.65	4.25	4.29	4.29
	cells/µL	307	159	5.78	5.90	6.01

AQUIOS Tetra (Test) vs. BD FACSCalibur (Reference): 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]

Table 3. Bias and Its Confidence Intervals at three percentiles

CONDUCTED AT 4 SITES IN NORTH AMERICA					95% CONFIDENCE LIMITS	
Analyte	**Unit**	**Percentile**	**Level**	**Bias**	**Lower**	**Upper**
CD3+ (Tetra-1)	%	25	66.10	0.31	0.09	0.53
	%	50	73.77	0.03	-0.16	0.21
	%	75	79.56	-0.19	-0.41	0.03
	cells/µL	25	643	-3.88	-10.56	2.80
	cells/µL	50	1094	-13.88	-25.56	-2.20
	cells/µL	75	1545	-23.88	-40.96	-6.79
CD3+/CD4+	%	25	18.66	0.18	0.04	0.32
	%	50	31.38	0.15	0.02	0.28
	%	75	44.04	0.12	-0.07	0.30
	cells/µL	25	184	-0.41	-2.69	1.88
	cells/µL	50	428	-4.39	-9.67	0.89
	cells/µL	75	770	-9.97	-20.43	0.49
CD3+/CD8+	%	25	24.93	-0.21	-0.38	-0.03
	%	50	36.34	-0.40	-0.55	-0.24
	%	75	50.94	-0.64	-0.86	-0.42
	cells/µL	25	300	-5.73	-9.06	-2.39
	cells/µL	50	508	-12.94	-18.71	-7.17
	cells/µL	75	805	-23.24	-32.88	-13.60

Table 4. Bias and Its Confidence Intervals at three percentiles

CONDUCTED AT 4 SITES IN NORTH AMERICA					95% CONFIDENCE LIMITS	
Analyte	**Unit**	**Percentile**	**Level**	**Bias**	**Lower**	**Upper**
CD3+ (Tetra-2+)	%	25	66.09	0.45	0.23	0.67
	%	50	73.76	0.22	0.04	0.40
	%	75	79.38	0.05	-0.16	0.26
	cells/µL	25	657	-23.40	-30.40	-16.40
	cells/µL	50	1125	-46.88	-59.34	-34.43
	cells/µL	75	1565	-68.97	-86.88	-51.05
CD3-/ CD56+CD16+	%	25	6.94	0.26	0.10	0.43
	%	50	10.46	0.25	0.13	0.37
	%	75	16.38	0.24	0.02	0.46
	cells/µL	25	87	-0.30	-1.89	1.30
	cells/µL	50	155	-2.59	-5.64	0.47
	cells/µL	75	240	-5.45	-11.05	0.14
CD3-/CD19+	%	25	8.69	-0.34	-0.48	-0.21
	%	50	13.05	-0.38	-0.51	-0.26
	%	75	17.68	-0.43	-0.59	-0.27
	cells/µL	25	95	-2.42	-10.41	5.56
	cells/µL	50	202	-16.04	-18.70	-13.38
	cells/µL	75	315	-30.42	-39.25	-21.58

Table 5. Bias and Its Confidence Intervals at Medical Decision Points

CONDUCTED AT 4 SITES IN NORTH AMERICA				95% CONFIDENCE LIMITS	
Analyte	**Unit**	**Level**	**Bias**	**Lower**	**Upper**
CD3+/CD4+	cells/µL	50	1.78	-0.54	4.09
		100	0.96	-1.10	3.03
		200	-0.67	-3.08	1.74
		500	-5.56	-11.90	0.78

Figure 1. CD3+CD4+ Absolute Count - Regression Plot

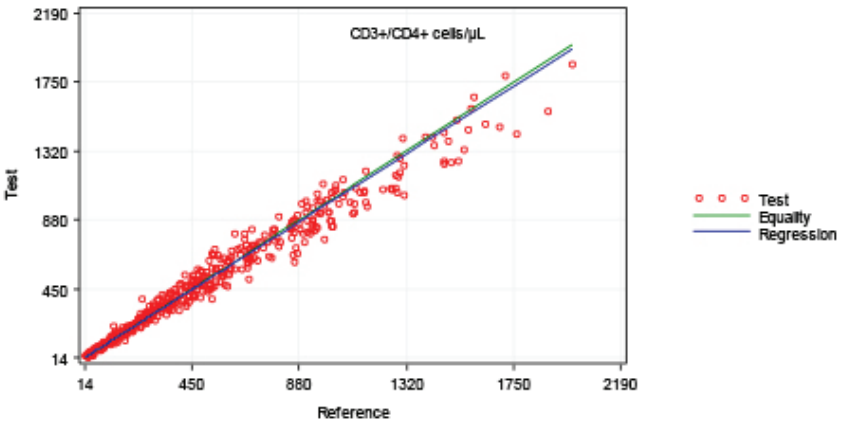
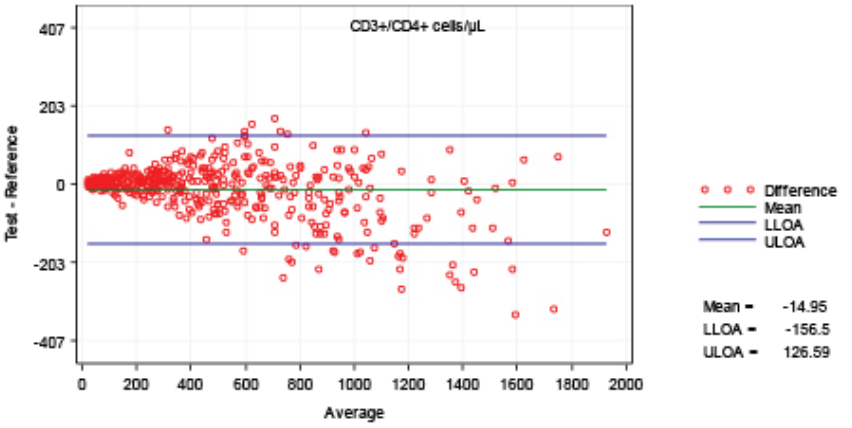


Figure 2. CD3+CD4+ Absolute Count - Bland-Altman Plot



Conclusions

The AQUIOS CL flow cytometer demonstrated excellent performance in lymphocyte subset analysis with AQUIOS Tetra Panel Reagents.

AQUIOS CL is a Class I Laser Product.

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