Comparison of AQUIOS tetra and Navios tetra system performance

Violetta Headley[1], Michael Keeney[2], Dominika Benjamins[2], Justin Rohrbach[3], Robert Ortega[3], Karen Lo[3], Liliana Tejidor[3] and Elena Afonina[1]

[1]Clinical Application Development, Life Sciences, Beckman Coulter, Inc [2]London Health Sciences Centre, London, Ontario, Canada [3]Clinical Affairs, Diagnostics, Beckman Coulter, Inc

BECKMAN COULTER Life Sciences

Background

The CD4+ T-cell count is a critical parameter in monitoring HIV disease. Flow cytometry remains the gold standard technology for enumeration of CD4+ T-cells, because of its accuracy, precision and reproducibility[1]. The AQUIOS™ CL is a fully automated flow cytometer with integrated sample loading, preparation and analysis. In this study, we demonstrate that the AQUIOS Tetra algorithm provides accurate results for enumeration of lymphocyte subsets in samples tested up to 24 hours post venipuncture. The recovery of the T, B and NK cell lymphocyte subsets using AQUIOS Tetra method was compared to the Navios Tetra system.

Methods

Systems

The AQUIOS CL instrument is a load-and-go IVD flow cytometry system that was recently cleared by the US FDA for testing in clinical labs. The system incorporates on-board sample preparation and automated analysis with LIS capabilities. The instrument employs a volumetric approach for enumerating specific cell populations. In this study, the AQUIOS CL system performance for immunophenotyping lymphocyte cell populations was compared to the Navios tetra system (tetraCHROME application run on Navios instrument with Flow-Count Fluorospheres), a currently used flow cytometry method for measuring the T, B and NK-cells.

Table 1 Detailed components of each system used in the study

	AQUIOS Tetra System	Navios Tetra System
Flow Cytometry Analyzer	AQUIOS CL	Navios
Sample Preparation	On-board of AQUIOS CL instrument	Manual
Lysing preparation	On-board of AQUIOS CL instrument	TQ-prep
Antibody reagents	AQUIOS Tetra 1 (CD45-FITC/CD4-PE/CD8-ECD/CD3-PC5) and AQUIOS Tetra-2+ (CD45-FITC/CD56-PE/**CD16-PE**/CD19-ECD/CD3-PC5)	tetraCHROME Tube 1 (CD45-FITC/CD4-PE/CD8-ECD/CD3-PC5) and tetraCHROME Tube 2 (CD45-FITC/CD56-PE/CD19-ECD/CD3-PC5)
Lysing Reagents	AQUIOS Lysing Reagent Kit	ImmunoPrep reagent
Algorithm	AQUIOS-Tetra	Navios-Tetra
Analysis principle	Volumetric	Flow Count based

Specimens

Sixty seven (67) specimens, including HIV+ clinical patients (61) were analyzed in the study. All testing was performed on spent blood after clinical testing had been performed. Specimens were targeted for normal and clinical range on the CD4+ T-cells. The distribution included CD4 expression levels at 32 cells/µL – 1500 cells/uL; with the majority (63%) of samples representing the clinical decision points under 500 cells/uL.

Table 2: Number of specimens analyzed per CD4 count range

CD4 Count Range	Number of Specimens
20 – 50	2
51 – 500	40
≥501	25
Total:	67

The whole blood samples were prepared within 24±2 hours of collection. The same specimens were prepared and analyzed by both systems in duplicates. For analysis on Navios instrument, samples were prepared manually and the red blood cells were lysed using the ImmunoPrep reagents and a TQ-prep instrument from Beckman Coulter, Inc. The statistical analysis included data from the first replicate only.

Data analysis

The recovery of the absolute count and percent positive parameters for CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/CD19+ and CD3-/CD56+16+ (or CD3-/CD56+ for Navios-tetra system) lymphocyte subsets were obtained and compared between the methods. Data was inspected for outliers prior to statistical analysis and no outliers were observed. Basic summary statistics and Bland-Altman plots were calculated for each marker. Deming approach was used to estimate regression parameters for percent positive measurements of each marker while weighted Deming approach was used for cell counts because the variability (scatter) of the data depended on the range of measurements. Regression analysis was performed. Bias between methods was calculated from the regression line at the 25th, 50th and 75th percentile of the range of the comparator and different medical decision points of CD4+ T-cell counts. Confidence limits of bias estimates were calculated based on standard errors of bias and 95% confidence. %Bias along with their confidence limits were also calculated at medical decision levels and 50th percentile at the median level based on the regression model.

Results

Figure 1 shows side by side comparison of cytometry analysis results between the methods. The SS vs CD45-FITC scatter plot shows lymphocyte gating done by each system algorithm; the CD3-PC5 histogram is used for separation of CD3+ and CD3- cells for further gating of CD3+/CD4+ and CD3/CD8+ T-cells, CD3-/CD19+ B-cells and CD3-/CD56+CD16+ NK-cells. The cell recovery for percent positive and absolute counts for each lymphocyte subset is shown under each corresponding plot. The AQUIOS-Tetra system provides absolute counts for an additional IVD marker CD45+ Low SS.

The statistical analysis demonstrated a negative bias (lower 95% limit of bias for CD3+/CD4+ count at 100, 200 and 500 cell/µL was -17, -20 and -37 cells, respectively) between the two systems for all parameters and all markers except for the CD3-/CD56+CD16+ marker. A positive bias (upper 95% limit of bias for CD3-/CD56+16+ count at 25th, 50th and 75th percentile cell/µL was 29, 38 and 57 cells, respectively) was observed between the methods for CD3-/CD56+CD16+ marker. A larger bias for NK-cells population recovery may be explained by inclusion of the CD16 monoclonal conjugated with the same fluorochrome as the CD56 antibody in the AQUIOS Tetra-2+ cocktail.

Conclusions

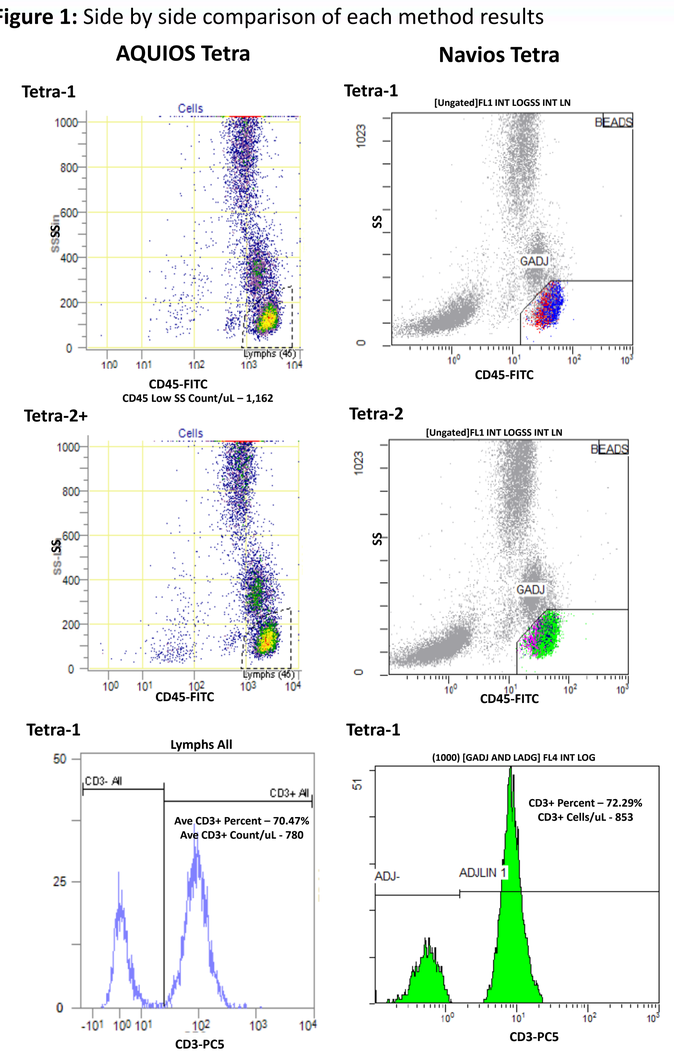
The new AQUOIS Tetra method demonstrated comparable results to the Navios Tetra application for measuring recovery of the T, B and NK cell lymphocyte subsets that is performed without the requirement of a fluorescent bead. All markers had < 8% Bias at 50th percentile except for NK-cells. Overall the Aquios has the advantage of a full walkaway system with integrated quality control and reagent accountability offering a standardized approach to lymphocyte subset analysis, while reducing hands-on time and operator variability.

NOTE: The additional parameters that AQUIOS provides (CD45+ and CD45+ Low SS) in comparison to Navios are not presented

References:

1. CD4+ T- CELL ENUMERATION TECHNOLOGIES TECHNICAL INFORMATION by World Health Organization at http://www.who.int/diagnostics_laboratory/faq/cd4/en/

Figure 1: Side by side comparison of each method results



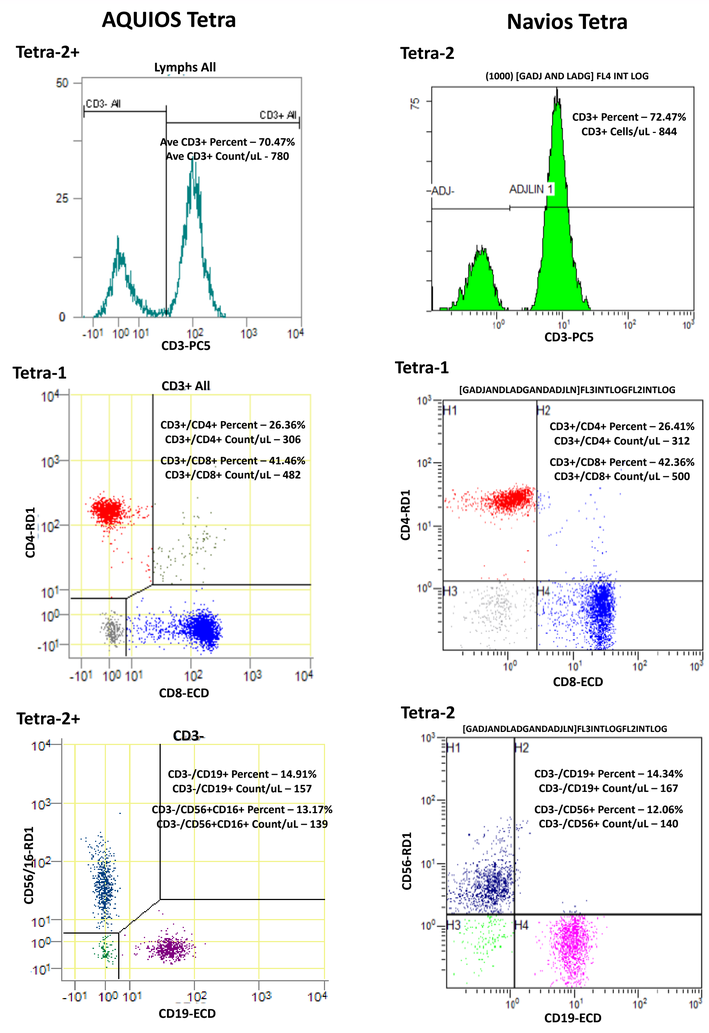


Table 3 shows general statistics, including number of tests (N), mean values, total bias difference in recovery between methods and 95% confidence limits of the difference.

			Means			95% Confidence Limits	
Analyte	**Unit**	**N**	**Reference**	**Test**	**Difference**	**Lower**	**Upper**
CD3+ (Tetra-1)	%	67	76.60	74.68	-1.92	-2.33	-1.50
	cells/µL	67	1338	1299	-39	-59	-20
CD3+/CD4+	%	67	29.91	28.43	-1.48	-1.81	-1.15
	cells/µL	67	535	504	-31	-41	-21
CD3+/CD8+	%	67	45.00	44.79	-0.20	-0.63	0.22
	cells/µL	67	775	770	-5	-18	7
CD3+ (Tetra-2+)	%	67	76.80	74.97	-1.83	-2.18	-1.49
	cells/µL	67	1371	1304	-67	-86	-48
CD3-/CD56+CD16+	%	67	8.51	10.79	2.27	1.91	2.63
	cells/µL	67	144	178	35	27	43
CD3-/CD19+	%	67	12.79	12.64	-0.15	-0.46	0.16
	cells/µL	67	227	216	-11	-18	-3

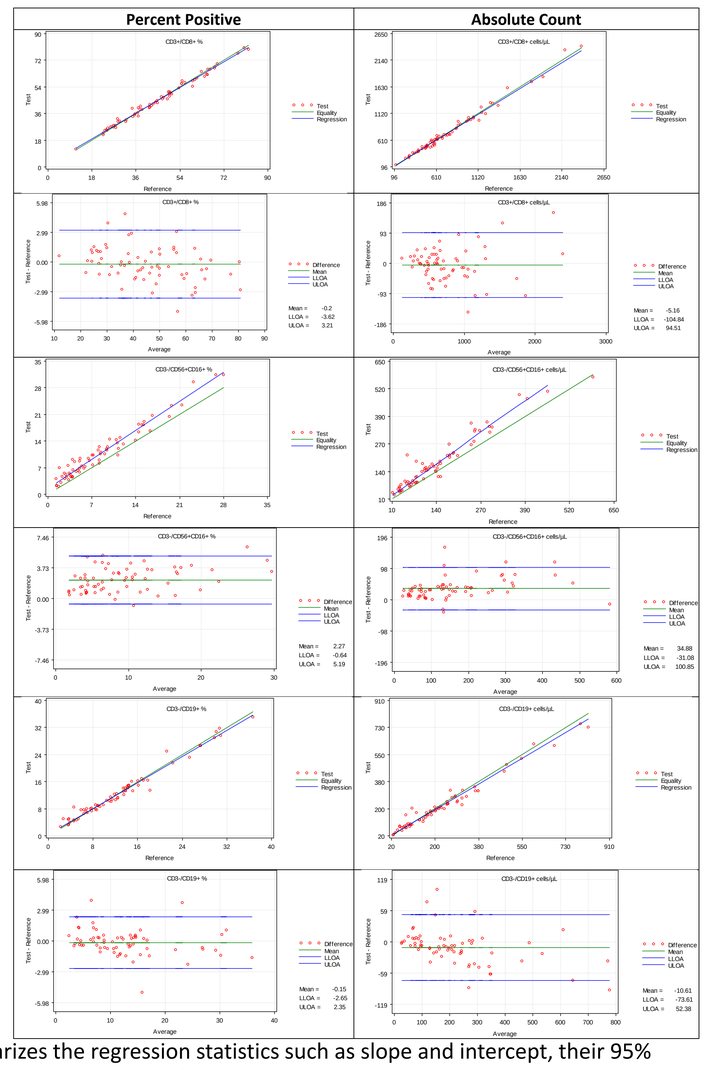


Figure 2 displays the regression graphs followed by the Bland-Altman plots for each individual marker for both AQUIOS Tetra-1 and AQUIOS Tetra-2+ Panel reagents. For the regression graphs, the green line represents the equality line and the blue lines represent the regression line. For the Bland-Altman plots, the green line represents the mean bias and the blue lines represent the mean ⍰2SD also known as the 95% limits of agreement (LOA). LOA are limits calculated from the data based on the variability of the collected sample data. They are based on the confidence level (95% confidence) which implies that a certain percent of the data could be outside of those limits.

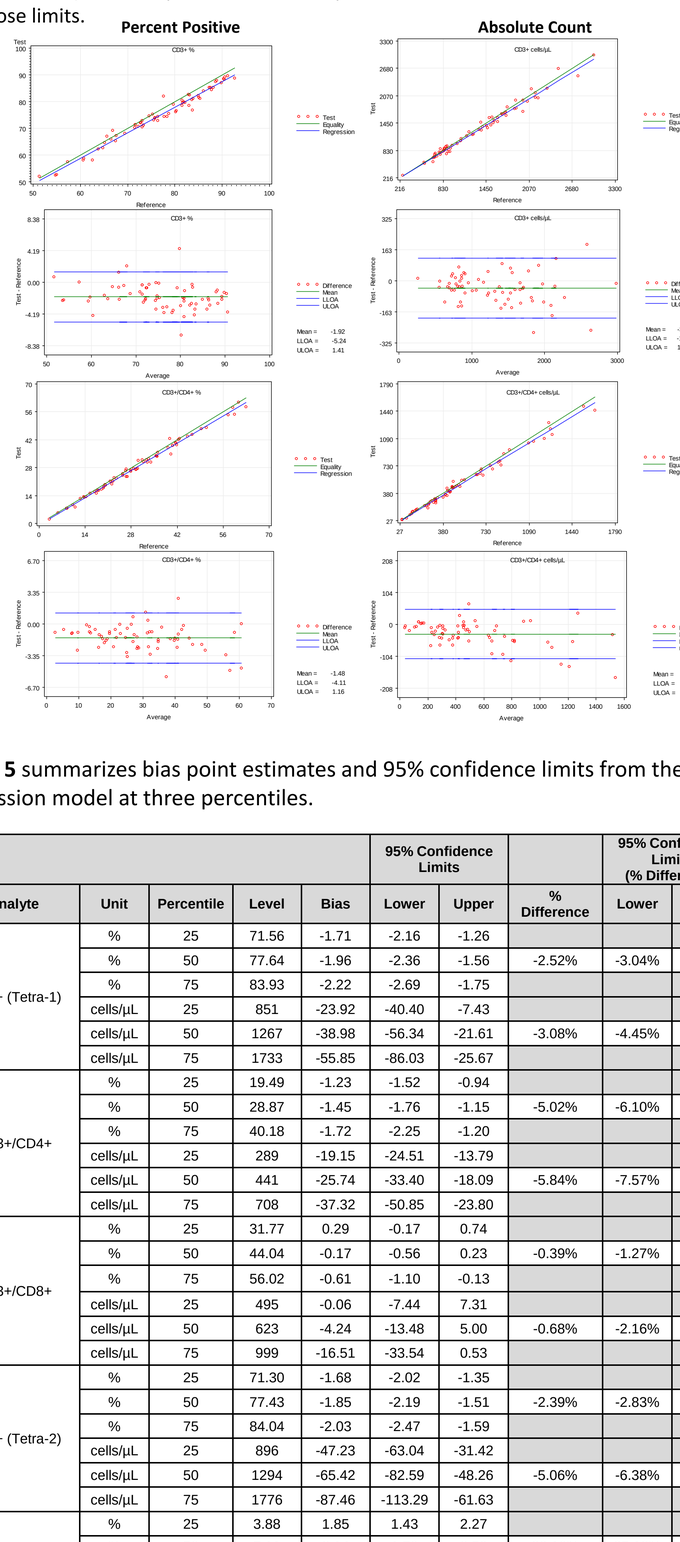


Table 4 summarizes the regression statistics such as slope and intercept, their 95% confidence intervals, and correlation between methods

			95% Confidence Limits			95% Confidence Limits		
Analyte	**Unit**	**Slope**	**Lower**	**Upper**	**Intercept**	**Lower**	**Upper**	**Correlation**
CD3+ (Tetra-1)	%	0.959	0.922	0.995	1.242	-1.606	4.091	0.986
	cells/µL	0.964	0.926	1.001	7.020	-34.484	48.524	0.991
CD3+/CD4+	%	0.976	0.949	1.003	-0.763	-1.447	-0.079	0.996
	cells/µL	0.957	0.931	0.982	-6.618	-13.936	0.701	0.995
CD3+/CD8+	%	0.963	0.941	0.984	1.472	0.483	2.461	0.995
	cells/µL	0.967	0.943	0.992	16.114	5.186	27.043	0.994
CD3+ (Tetra-2)	%	0.973	0.944	1.002	0.232	-1.938	2.402	0.990
	cells/µL	0.954	0.927	0.982	-6.194	-38.148	25.759	0.991
CD3-/CD56+CD16+	%	1.092	1.033	1.150	1.491	0.915	2.068	0.978
	cells/µL	1.148	1.036	1.260	14.239	5.297	23.180	0.964
CD3-/CD19+	%	0.962	0.920	1.003	0.338	-0.191	0.867	0.987
	cells/µL	0.950	0.890	1.009	4.083	-3.269	11.436	0.985

Table 5 summarizes bias point estimates and 95% confidence limits from the regression model at three percentiles.

					95% Confidence Limits			95% Confidence Limits (% Difference)	
Analyte	**Unit**	**Percentile**	**Level**	**Bias**	**Lower**	**Upper**	**% Difference**	**Lower**	**Upper**
CD3+ (Tetra-1)	%	25	71.56	-1.71	-2.16	-1.26			
	%	50	77.64	-1.96	-2.36	-1.56	-2.52%	-3.04%	-2.01%
	%	75	83.93	-2.22	-2.69	-1.75			
	cells/µL	25	851	-23.92	-40.40	-7.43			
	cells/µL	50	1267	-38.98	-56.34	-21.61	-3.08%	-4.45%	-1.71%
	cells/µL	75	1733	-55.85	-86.03	-25.67			
CD3+/CD4+	%	25	19.49	-1.23	-1.52	-0.94			
	%	50	28.87	-1.45	-1.76	-1.15	-5.02%	-6.10%	-3.98%
	%	75	40.18	-1.72	-2.25	-1.20			
	cells/µL	25	289	-19.15	-24.51	-13.79			
	cells/µL	50	441	-25.74	-33.40	-18.09	-5.84%	-7.57%	-4.10%
	cells/µL	75	708	-37.32	-50.85	-23.80			
CD3+/CD8+	%	25	31.77	0.29	-0.17	0.74			
	%	50	44.04	-0.17	-0.56	0.23	-0.39%	-1.27%	0.52%
	%	75	56.02	-0.61	-1.10	-0.13			
	cells/µL	25	495	-0.06	-7.44	7.31			
	cells/µL	50	623	-4.24	-13.48	5.00	-0.68%	-2.16%	0.80%
	cells/µL	75	999	-16.51	-33.54	0.53			
CD3+ (Tetra-2)	%	25	71.30	-1.68	-2.02	-1.35			
	%	50	77.43	-1.85	-2.19	-1.51	-2.39%	-2.83%	-1.95%
	%	75	84.04	-2.03	-2.47	-1.59			
	cells/µL	25	896	-47.23	-63.04	-31.42			
	cells/µL	50	1294	-65.42	-82.59	-48.26	-5.06%	-6.38%	-3.73%
	cells/µL	75	1776	-87.46	-113.29	-61.63			
CD3-/CD56+CD16+	%	25	3.88	1.85	1.43	2.27			
	%	50	7.11	2.14	1.79	2.50	30.10%	25.18%	35.16%
	%	75	11.26	2.52	2.11	2.93			
	cells/µL	25	62	23.32	17.88	28.75			
	cells/µL	50	112	30.66	22.96	38.35	27.38%	20.50%	34.24%
	cells/µL	75	189	41.96	26.87	57.05			
CD3-/CD19+	%	25	6.93	0.07	-0.25	0.40			
	%	50	11.89	-0.12	-0.41	0.18	-1.01%	-3.45%	1.51%
	%	75	15.21	-0.24	-0.59	0.10			
	cells/µL	25	97	-0.87	-5.82	4.08			
	cells/µL	50	193	-5.74	-13.58	2.09	-2.97%	-7.04%	1.08%
	cells/µL	75	292	-10.77	-23.75	2.20			

Table 6 summarizes the bias point estimates from the regression model for CD4 at medical decision levels (50, 100, 200 & 500 cells/ul) and their upper and lower 95% confidence limits.

				95% Confidence Limits			95% Confidence Limits (% Difference)	
Analyte	**Unit**	**Level**	**Bias**	**Lower**	**Upper**	**% Bias**	**Lower**	**Upper**
CD3+/CD4+	cells/µL	50	-8.78	-15.23	-2.33	-17.56%	-30.46%	-4.66%
CD3+/CD4+	cells/µL	100	-10.95	-16.68	-5.22	-10.95%	-16.68%	-5.22%
CD3+/CD4+	cells/µL	200	-15.29	-20.28	-10.29	-7.65%	-10.14%	-5.15%
CD3+/CD4+	cells/µL	500	-28.3	-37.14	-19.46	-5.66%	-7.43%	-3.89%