CPAL
Central Pennsylvania Alliance Laboratory

Technical Bulletin
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Flow Cytometry
Lymphocyte Subset Analysis Testing Platform Change

Change Effective Date: February 6, 2017

Methods and Clinical Use: CPAL currently uses a combination of Beckman Coulter's tetraCHROME IVD kit, in conjunction with a Beckman Coulter Navios flow cytometer, to perform lymphocyte subset testing. Acceptable performance characterization and validation studies have been performed on the Beckman Coulter Aquios CL flow cytometer using the Aquios Tetra IVD assay. Immunophenotypic analysis of lymphocytes may assist in the evaluation of cellular immunocompetency in suspected cases of primary or secondary immunodeficiency states. It may also help to evaluate helper/suppressor cell immune status in diseases such as AIDS.

> **Note:** *Absolute count determinations for the subsets may yield slightly different results when compared from one method/platform to another. This is typical and expected in this clinical laboratory discipline.*

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Testing Schedule: Mon-Fri, morning and afternoon.
Saturday, morning and afternoon (specimens must be received by 1 PM)

Specimen:

- 4 ml whole blood (2 mL minimum) collected in EDTA (lavender top)
- Do not freeze or refrigerate
- ***Specimens must be tested within 48 hours of collection***! (Call CPAL to arrange for special courier pick-up if necessary).
- **STORE AND TRANSPORT AT ROOM TEMPERATURE!**

CPAL Lymphocyte Subset Panels

Effective February 6th, the lymphocyte subset panels available at CPAL are as follows:

CPAL Test Name	CPAL Test Mnemonic	CPAL Test Code	Result Paramters	LOINC Code(s)	CPT Code(s)
B-cell, Total Count	BCC	4000500	CD3-CD19+ Percent	15196-9	86355
			CD3-CD19+ Absolute Count	15195-1	
T-cell, Absolute CD4 Count	CD4C	4000600	CD3+ Percent	8124-0	86359
			CD3+ Absolute Count	8122-4	
			CD3+CD4+ Percent	8123-2	86361
			CD3+CD4+ Absolute Count	24467-3	
T-cell Count with Ratio	TCC	4000550	CD3+ Percent	8124-0	86359
			CD3+ Absolute Count	8122-4	
			CD3+CD4+ Percent	8123-2	86360
			CD3+CD4+ Absolute Count	24467-3	
			CD3+CD8+ Percent	8101-8	
			CD3+CD8+ Absolute Count	14135-8	
			CD3+CD4+/CD3+CD8+ Ratio	54218-3	
T and B-cell Panel	TBCC	4000700	CD3+ Percent	8124-0	86359
			CD3+ Absolute Count	8122-4	
			CD3+CD4+ Percent	8123-2	86360
			CD3+CD4+ Absolute Count	24467-3	
			CD3+CD8+ Percent	8101-8	
			CD3+CD8+ Absolute Count	14135-8	
			CD3+CD4+/CD3+CD8+ Ratio	54218-3	
			CD3-CD19+ Percent	15196-9	86355
			CD3-CD19+ Absolute Count	15195-1	
Total Lymphocyte Count	TLC	4000410	CD3+ Percent	8124-0	86359
			CD3+ Absolute Count	8122-4	
			CD3+CD4+ Percent	8123-2	86360
			CD3+CD4+ Absolute Count	24467-3	
			CD3+CD8+ Percent	8101-8	
			CD3+CD8+ Absolute Count	14135-8	
			CD3+CD4+/CD3+CD8+ Ratio	54218-3	
			CD3-CD19+ Percent	15196-9	86355
			CD3-CD19+ Absolute Count	15195-1	
			CD3-CD16+CD56+ Percent	8112-5	86357
			CD3-CD16+CD56+ Absolute Count	9728-7	

New Method Description:

The Aquios flow cytometer is an automated analyzer that combines automated sample processing and cellular analysis of whole blood samples. The system performs flow cytometric applications that utilize a no-wash sample preparation to prevent cellular loss due to specimen processing.

The Aquios Tetra assay, used on the Beckman Coulter Aquios, is considered a single platform method of lymphocyte subset testing. The assay is designed to enable determinations of both absolute count and percent lymphocyte subset values on the same instrument from a single sample. In the assay, specific leukocyte staining is accomplished by incubating whole blood with one and/or two monoclonal antibody reagent cocktails. Each antibody cocktail is a combination of four or five murine monoclonal antibodies conjugated to a fluorochrome. Each monoclonal antibody-fluorochrome conjugate is specific for a different lymphocyte cell surface antigen. The red blood cells (RBCs) are removed by lysis and the leukocytes, which are unaffected by lysis, are analyzed by flow cytometry. A syringe mechanism is used to obtain absolute counts. The syringe pump regulates the flow cell aspiration volume and provides metered sample delivery for accurate cell counting and is based on the Coulter principle of electronic impendence.

Reference Ranges

All Adult reference ranges were obtained from the Beckman Coulter *Aquios Tetra System Guide, April 2015* and verified at CPAL. CPAL has *not* independently established Pediatric lymphocyte subset reference values. The pediatric reference values will be attached to the clinical report as appropriate. Pediatric reference ranges were adapted from *Tosato F, Bucciol G, Putti MC, et al: Lymphocytes Subsets Reference Values in Childhood: Cytometry Part A 2015; 87A:81-85*. All results should be interpreted with consideration of the entire clinical context.

*CPAL Lymphocyte Subset Reference Ranges (**Adult**)*

	CD3		CD3/CD4		CD3/CD8		CD19		CD16/CD56		CD45+	CD45+ Low SSC	
	%	cells/µl	%	cells/µl	%	cells/µl	%	cells/µl	%	cells/µl	cells/µL	%	cells/µL
Adult	58-84	857-2245	34-65	518-1472	13-38	205-924	6-25	87-507	4-27	74-562	3897-9997	18-43	1198-2856

*CPAL Lymphocyte Subset Reference Ranges (**Pediatric**)*

	CD3		CD3/CD4		CD3/CD8		CD19		CD16/CD56	
Age	%	cells/µl	%	cells/µl	%	cells/µl	%	cells/µl	%	cells/µl
0-2 years	58-77	2668-5036	37-57	1809-3346	15-27	631-1459	14-28	608-1653	4-14	205-798
2-6 years	60-78	1578-3707	31-47	870-2144	16-27	472-1107	13-29	434-1274	5-16	155-565
6-18 Years	63-79	1097-2471	32-49	628-1480	18-30	323-847	12-22	225-663	5-17	103-493

Clinical Information:

Lymphocyte subset analysis by flow cytometry identifies and enumerates total CD3+, dual CD3+CD4+, dual CD3+CD8+, CD3-CD19+, and CD3-(CD16+CD56+) lymphocyte percentages and absolute counts in whole blood, as well as the CD3+CD4+/CD3+CD8+ ratio.

The lymphocyte population of human peripheral blood is composed of three cell types: T (thymus-derived), B (bone marrow-derived), and NK (Natural Killer) cells. T, B, and NK lymphocytes play central roles in immune system function. CD3+, CD3+CD4+, CD3+CD8+, and/or CD3-CD19+ lymphocyte percentages and absolute counts may be used as aids to evaluate immune competency underlying known or unknown disease states and to monitor lymphocyte levels following organ transplantation. NK lymphocyte populations identified as CD3-, CD16+, and CD56+ have been functionally defined as a lymphocyte population capable of mediating non-MHC restricted cytotoxicity against targets such as certain tumor and virus-infected cells. Identification of abnormal levels of CD3+CD4+ lymphocytes, and corresponding CD3+CD4+/CD3+CD8+ ratios, might also aid in the diagnosis and/or prognosis of immunodeficiency diseases. For example, infection with human immunodeficiency virus (HIV), the etiologic agent of acquired immunodeficiency syndrome (AIDS), results in profound immunosuppression due predominantly to a selective depletion of the CD3+CD4+ lymphocytes that express the receptor for the virus. Disease-related changes in CD3+CD4+ and/or CD3+CD8+ lymphocyte levels might alter CD3+CD4+/CD3+CD8+ helper/suppressor cell ratios. As a result, CD3+CD4+/CD3+CD8+ ratios might be useful as diagnostic and/or prognostic indicators of immune competence. CD4/CD8 ratios in conjunction with CD4+ lymphocyte cell numbers have been the most widely used laboratory parameters for evaluation/monitoring of AIDS-related complex and AIDS treatment protocols.

Validation Summary

Reference Range Verification: CPAL tested 42 samples from normal, healthy adult males and females to verify Beckman Coulter's stated normal range. For all parameters of the assay, no more than 10% of the 42 samples were outside the manufacturer's reference range.

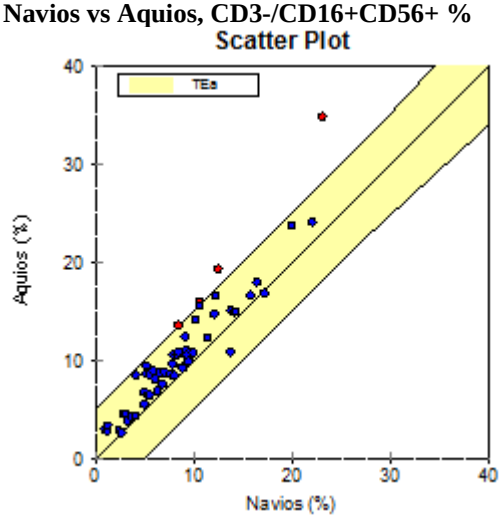
Linearity and Accuracy: Studies were performed and found to be acceptable.

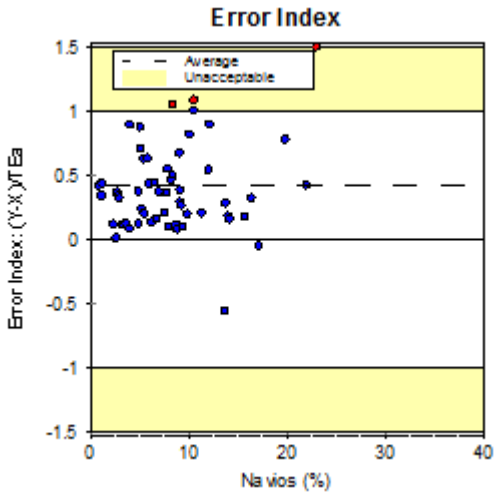
Analytical Measurement Range: Concentration and dilution studies were performed to verify Beckman Coulter's stated analytical measurement ranges for the parameters of this assay:

Parameter	Units	Aquios Tetra AMR
CD3+	Cells/uL	55-4700
CD3+/CD4+	Cells/uL	35-3000
CD3+/CD8+	Cells/uL	45-1600
CD3-/CD19+	Cells/uL	25-1000
CD3-/CD56+ CD16+	Cells/uL	20-1000
CD45+	Cells/uL	350-26500
CD45+ low SSC	Cells/uL	80-6500

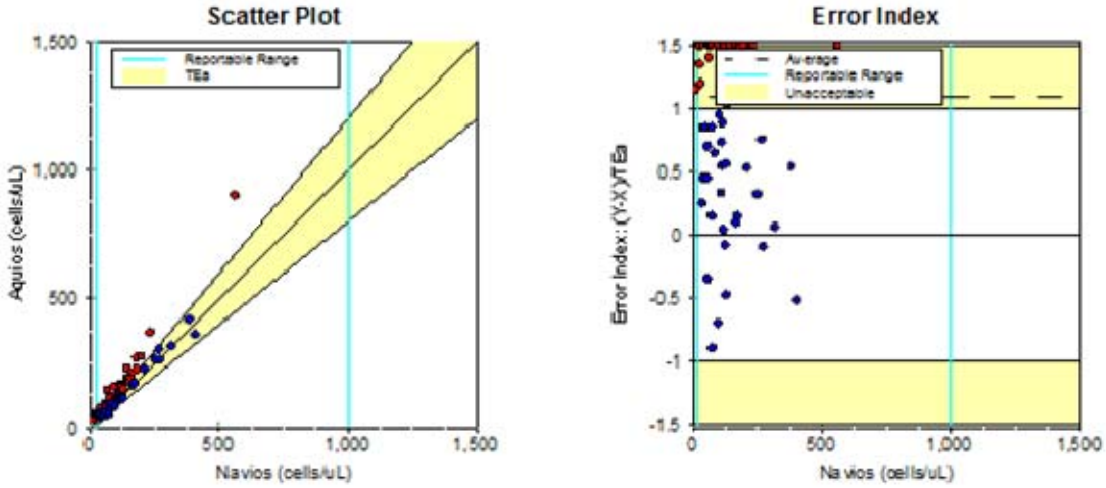
Method Comparison: Fifty-seven samples submitted for routine testing on the current platform, the Beckman Coulter Navios/ Navios Tetra assay, were tested concurrently on the Beckman Coulter Aquios. All parameters (percent and absolute counts) were found to be within the goals set for total allowable error except for the NK cell (CD3-/CD16+CD56+) subset percent and absolute counts.

Navios vs Aquios, CD3-/CD16+CD56+ %





Navios vs Aquios, CD3-/CD16+CD56+ Count



A high bias is seen in the Aquios for the NK cell subset (CD3-/CD16+CD56+) compared to the Navios NK cell subset (CD3-/CD56+) for both percent and absolute count parameters. It is well-documented in the literature that laboratories using CD3-/CD56+ alone to enumerate NK-cells produce significantly lower values for both percent and absolute count values when compared to either CD3-/CD16+ alone or CD3-/CD16+CD56+. The results obtained with analysis on the Beckman Coulter Aquios for the NK cell subset are in line with the expected results due to the difference in antibody cocktail reagents.

Specimen Stability Study: CPAL performed a study to extend the manufacturer's claims of specimen stability. Beckman Coulter claims that specimens are stable within 24 hours of collection for testing. CPAL was able to extend this claim to 48 hours post-collection and show acceptable comparable results within defined error limits.

Precision Studies: Both Within Run and Between Run Precision studies were within defined error limits and were acceptable.

References

1. Aquios Instructions for Use, 03/2016

2. Aquios Tetra System Guide, 04/2015

3. Aquios Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 (PN B23533) and Aquios Tetra-2+ Panel CD45-FITC/(CD56+CD16)-RD1/CD19-ECD/CD3-PC5 (PN B23534) Technical Data Sheet, 08/2015

4. Clinical and Laboratory Standards Institute. Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; *Approved Guideline-Second Edition*. CLSI document H42-A2 [ISBN 1-56238-640-9]. Clinical and Laboratory Standards Institute. 940 West Valley Road, Suite 1400, Wayne PA 19087-1898 USA 2007.

5. Tosato F, Bucciol G, Putti MC, et al: Lymphocytes Subsets Reference Values in Childhood: Cytometry Part A 2015; 87A:81-85.