



CPAL

Central Pennsylvania Alliance Laboratory

Technical Bulletin

No. 78

January 5, 2010

Flow Cytometry Lymphocyte Subset Analysis

Starting Date: January 11, 2010

Clinical use: Immunophenotypic analysis may assist in evaluating cellular immunocompetency in suspected cases of primary and secondary immunodeficiency states. Helps 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 laboratory to another. This is typical and expected in this clinical laboratory discipline.* However, based on studies presented below, correlation between CPAL and Quest Diagnostics subset analysis results is expected to be high. (See correlation study results presented below).

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Testing Schedule: Mon-Fri, morning and afternoon;
Saturday, morning (specimen must be here by 7am)

Specimen: 2 ml whole blood (1ml minimum); EDTA (lavender top)
Do not freeze or refrigerate. Specimen must be tested within 24 hrs of collection

Instructions: Do not freeze or refrigerate. Specimen must be tested within 24 hrs of collection. **TRANSPORT AT ROOM TEMPERATURE!**

CPAL Lymphocyte Subset Panels

Effective January 11, 2010 several lymphocyte subset panels will be available at CPAL. The available panels are as follows:

<u>Markers</u>		<u>CPT</u>	<u>Corresponds to Quest</u>
T-cells, absolute CD4 count (CD4C)	CD3% and count	86359	#8360
	CD4% and count	86361	
T-cells, CD4/CD8 w/ratio (TCC)	CD3% /count	86359	#7195
	CD4% /count and CD8% /count with ratio	86360	
B-cells, total count (BCC)	CD19% and count	86355	no equivalent
Total lymphocyte counts (TLC)	CD3% and count	86359	#7197
	CD4% /count and CD8% /count with ratio	86360	
	CD19% and count	86355	
	CD16+56% and count	86357	
T&B cells, total counts w/ratio (TBCC)	CD3% and count	86359	#36420
	CD4% /count and CD8% /count with ratio	86360	
	CD19% and count	86355	

Reference Ranges

A reference range verification study was performed in order to verify the reference ranges that the manufacturer reports in the package insert for the assay. The manufacturer's reference ranges require laboratory specific verification. Sixty CBC samples were obtained from York Hospital. These were initially characterized by that institution as having "normal" CBC results, based on the failure to note "flags" during analysis on Hematology cell counters. Ten of these specimens were rejected for the verification study - prior to statistical analysis - based on one of two parameters that were measured during flow cytometry testing. The first was an absolute lymphocyte count less than 1000 per cubic mm; the second was a CD4:CD8 ratio less than 1.0.

Verification criteria used the 10% Rule in which ranges are verified if <10% of study group results are outside of proposed reference range (10% Rule). Results of verification analyses are presented below. All reference ranges were verified (Absolute and Percentage). Parametric and Non-parametric analysis was performed for comparison but limited sample size limits the utility of these analyses for establishment of independent reference ranges. Quest and Lab Corp reference ranges were also examined for comparison.

CPAL Lymphocyte Subset Reference Ranges* (Adult).

Adult lymphocyte subset reference values

	CD3		CD3/CD4		CD3/CD8		CD19		CD16+56	
	%	cells/µl	%	cells/µl	%	cells/µl	%	cells/µl	%	cells/µl
Adult	49-84	603-2990	28-63	441-2156	10-40	125-1312	7-27	107-698	4-25	95-640

*Verified in-house and adopted from the Becton Dickenson (*BD Multitest 6-color TBNK Reagent, July 2009*) package insert.

CPAL Lymphocyte Subset Reference Ranges (Pediatric).

CPAL has not established independent Pediatric lymphocytes subset reference values. The following set of reference values will be attached to the clinical report as appropriate. All results should be interpreted with consideration of the entire clinical context.

Pediatric lymphocyte subset reference values

	CD3		CD3/CD4		CD3/CD8		CD19		CD16+56	
	%	cells/µl	%	cells/µl	%	cells/µl	%	cells/µl	%	cells/µl
Neonatal	28-76	600-5000	17-52	400-3500	10-41	200-1900	5-22	40-1100	6-58	100-1900
1 wk-2 mo	60-85	1300-7000	41-68	1700-5300	9-23	400-1700	4-26	600-1900	3-23	200-1400
2-5 mo	48-75	2300-6500	33-58	1500-5000	11-25	500-1600	14-39	600-3000	2-14	100-1300
5-9 mo	50-77	2400-6900	33-58	1400-5100	13-26	600-2200	13-35	700-2500	2-13	100-1000
9-15 mo	54-76	1600-6700	31-54	1000-4600	12-28	400-2100	15-39	600-2700	3-17	200-1200
15-24 mo	39-73	1400-8000	25-50	900-5500	11-32	400-2300	17-41	600-3100	3-16	100-1400
2-5 yr	43-76	900-4500	23-48	500-2400	14-33	300-1600	14-44	200-2100	4-23	100-1000
5-10 yr	55-78	700-4200	27-53	300-2000	19-34	300-1800	10-31	200-1600	4-26	90-900
10-16 yr	52-78	800-3500	25-48	400-2100	9-35	200-1200	8-24	200-600	6-27	70-1200

Source: Comans-Bitter WM, et al. Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations.

[J Pediatr 1997 Mar;130\(3\):388-393.](#)

Summary:

Lymphocyte subset analysis is performed at CPAL using the BD Multitest™ test kit (a six-color direct immunofluorescence reagent for use with BD FACSCanto™ II flow cytometers) to identify and determine the percentages and absolute counts of T, B, and natural killer (NK) cells as well as the CD4 and CD8 subpopulations of T cells in peripheral blood.

Human lymphocytes can be divided into three major subset populations based on their biologic function and cell-surface antigen expression: T lymphocytes (CD3+), B lymphocytes (CD19+), and NK lymphocytes (CD16+CD56+). The BD Multitest 6-color TBNK system can be used to characterize these lymphocyte subset populations. When the reagent is used with BD Trucount tubes, BD FACSCanto clinical software calculates lymphocyte subset percentages and absolute counts using flow data from the BD Trucount beads and the sample.

Determining percentages or counts of CD3+CD4+ lymphocytes can be useful in monitoring human immunodeficiency virus (HIV)-infected individuals, who typically exhibit a steady decrease of CD3+CD4+ lymphocyte counts as the infection progresses. CD3+CD4+ percentages or counts and total T and B lymphocytes are used to characterize and monitor some forms of immunodeficiency and autoimmune diseases. NK lymphocytes identified as CD3- and CD16+ and/or CD56+ have been shown to mediate cytotoxicity against certain tumors and virus-infected cells.

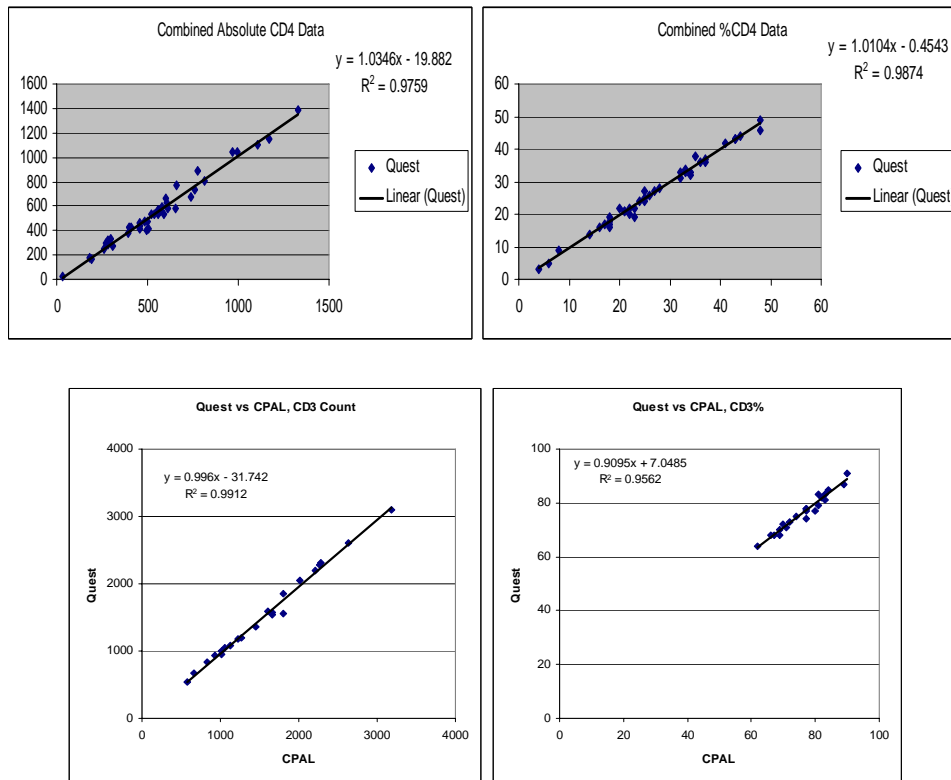
Validation Studies

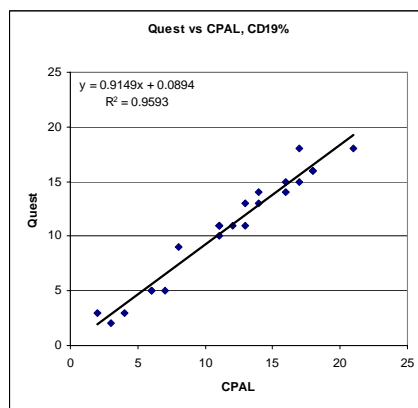
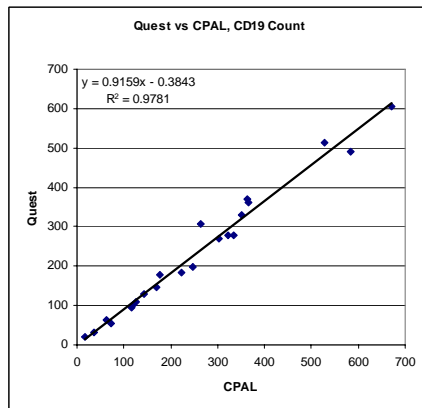
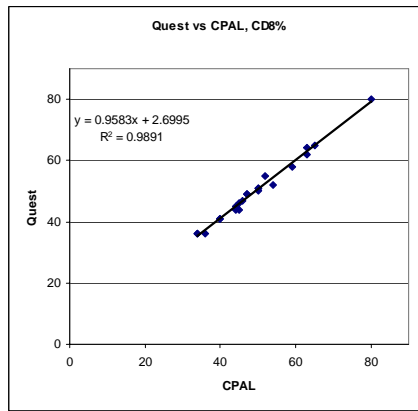
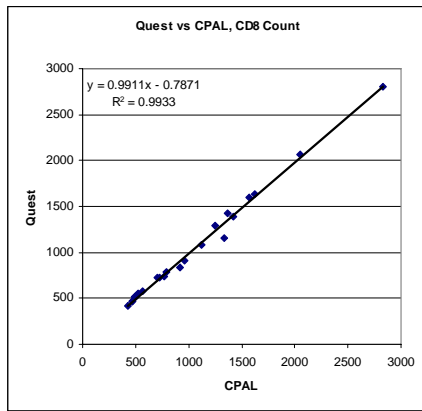
Correlation, precision, linearity and reference range verification studies were performed at CPAL in November and December 2009 in order to determine the performance characteristics of lymphocyte subset analysis by using the BD Multitest™ test kit with the BD FACSCanto™ II flow cytometer. In summary, the assays performed as expected. Specimens from 20 patients presenting at Pinnacle with a request for lymphocyte subset enumeration were used for correlation studies. (Forty specimens were used for the CD4 correlation study). The specimens were sent to CPAL, where an aliquot was removed for testing at that site; the remaining specimen was forwarded to Quest Diagnostics such that it was subject to the same handling in the same time-frame as would have occurred without the comparison testing performed in this instance. CPAL performed their TLC panel, which corresponds to Quest's Panel 1 (#7197). Both of these assays measure percentages and absolute counts for CD3, CD4, CD8, CD19 and CD16+56. Results from each site on each specimen were compared and the results are presented. **Table 1** lists a summary of the result analysis of the correlation studies. Graphical representations of the data are also presented below.

Table 1. Subset Correlations

Analyte	Correlation Coefficient (R ²)	Slope
CD4 Absolute Count	0.9759	1.0346
CD4 Percent	0.9874	1.1014
CD3 Absolute Count	0.9912	0.996
CD3 Percent	0.9562	0.9095
CD8 Absolute Count	0.9933	0.9911
CD8 Percent	0.9891	0.9583
CD19 Absolute Count	0.9781	0.9159
CD19 Percent	0.9593	0.9149
CD16+56 Absolute Count	0.9292	0.9315
CD16+56 Percent	0.9474	0.9571

Correlation studies demonstrated good comparison to Quest Diagnostics method. No inherent bias was noted for any parameter. Presented below are the correlation plots for each analyte.





For detailed analysis of the verification studies, or to discuss any aspect of lymphocyte subset analysis testing performed at CPAL, please call the laboratory (see contact information listed above).