DEPARTMENT OF HEALTH AND HUMAN SERVICES

Centers for Disease Control and Prevention Model Performance Evaluation Program

$CD4^+$ T-Cell Determinations

Report of Results for the Performance Evaluation Survey Conducted in April 2005

CENTERS FOR DISEASE™
CONTROL AND PREVENTION

Coordinating Center for Health Information and Service
Division of Laboratory Systems
Atlanta, Georgia

Analysis of the Testing Results Submitted by Laboratories Participating in the Centers for Disease Control and Prevention (CDC) Model Performance Evaluation Program (MPEP) for $CD4^+$ T-Cell Determinations Conducted in April 2005

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Overview of April 2005 $CD4^+$ T-cell Determinations Performance Evaluation

Introduction

This report analyzes testing results reported by laboratories participating in the Centers for Disease Control and Prevention (CDC) Model Performance Evaluation Program (MPEP) for $CD4^+$ T-cell determination ($CD4^+$ T-cell) performance evaluation specimens sent on April 12 and April 19, 2005. This program was discontinued in May 2005. This is the final report for this activity.

Laboratory Response

Of the 256 laboratories receiving specimen panels, 248 (96.9%) reported testing results.

- Of the 8 nonreporting laboratories, one laboratory indicated they no longer were performing CD4 T-cell determinations. Seven laboratories provided no explanation for nonparticipation.
- The majority of the laboratories (77.8%) reported their testing results using the online data entry system.

Significant Findings

The majority of the results (93.9%) returned by the laboratories participating in the April 2005 performance evaluation panel shipment were within the established 95% confidence limits.

- In particular, 92.9% of the absolute $CD4^+$ and 91.9% of the $CD8^+$ T-cell counts were within the established 95% confidence limits.
- As has been seen in previous surveys, the range of results reported for absolute $CD4^+$ and $CD8^+$ T-cell counts differed depending on the method used to obtain the result, i.e., single-platform or dual-platform.
- The ranges of dual-platform absolute $CD4^+$ and $CD8^+$ T-cell counts were significantly wider due to the large ranges of hematology instrument-derived absolute lymphocyte count results.
- According to the CDC guidelines for $CD4^+$ T-cell testing (*MMWR*: 1997; 46, RR-2), specimens should be processed for hematologic testing and immunophenotyping within 30 hours after collection. A total of 60 laboratories reported specimen preparation delays (3 laboratories reported both late deliveries and delays in processing). These specimen preparation delays may have affected the testing results from these laboratories (see Discussion, page 27).

Materials and Methods

Specimen panels

Each laboratory received a total of five whole blood specimens collected in K_3EDTA, three HIV-1 antibody-positive and two HIV-1 antibody-negative specimens. One of the HIV-1 antibody-positive whole blood specimens was sent to the participant laboratories in duplicate. Not all laboratories received the same panel of specimens.

Specimen numbers and donor information

Table 1 contains the specimen numbers and donor information for each performance evaluation specimen.

Table 1. Donor Identification for April 2005 Shipment Specimens

Panel Letter	Participant Laboratory Vial Label	CDC Donor Number	Donor Information (HIV-1* status)
A	A1, A4	02	HIV-1 Antibody-Positive
	A2	01	HIV-1 Antibody-Negative
	A3	05	HIV-1 Antibody-Positive
	A5	03	HIV-1 Antibody-Negative
B	B1	03	HIV-1 Antibody-Negative
	B2, B3	04	HIV-1 Antibody-Positive
	B4	01	HIV-1 Antibody-Negative
	B5	05	HIV-1 Antibody-Positive
C	C1	08	HIV-1 Antibody-Negative
	C2, C4	07	HIV-1 Antibody-Positive
	C3	10	HIV-1 Antibody-Positive
	C5	09	HIV-1 Antibody-Negative
D	D1	09	HIV-1 Antibody-Negative
	D2, D5	06	HIV-1 Antibody-Positive
	D3	10	HIV-1 Antibody-Positive
	D4	08	HIV-1 Antibody-Negative

* Human immunodeficiency virus type 1

Continued on next page

Materials and Methods, Continued

Preshipment notification

To facilitate and prevent delays in specimen receipt and processing, laboratories were notified a month in advance of the date of the shipment.

- An air-bill tracking number was included in these notifications, enabling the laboratories to locate the specimens in the event the shipment was not received by noon on the scheduled date of their receipt.
- Participant laboratories were instructed to process and test the MPEP $CD4^+$ T-cell specimens as they would patient specimens routinely received by their laboratory.

$CD4^+$ T-cell testing guidelines

Participant laboratories were encouraged to use the CDC guidelines for $CD4^+$ T-cell testing (MMWR, vol. 46, no. RR-2, January 10, 1997, MMWR, vol. 52, no. RR-2:1-13, January 31, 2003) in performing $CD4^+$ T-cell determinations on patient specimens.

- The result reporting booklet used for the April 2005 specimen shipment was designed to be consistent with these guidelines.
- According to these guidelines, specimens should be processed for hematologic testing and flow cytometric immunophenotyping within 30 hours of collection.

Absolute cell count methods

Methods used to derive the cell marker-specific absolute cell count were classified as either dual-platform or single-platform.

- Dual-platform methods are those which use the results from the flow cytometer (cell marker percentages) combined with the results from a hematology analyzer (white blood cell count, percent lymphocytes, and absolute lymphocyte count) to calculate the specific absolute cell count.
- Single-platform methods are those whereby the absolute cell count is derived using a single instrument (e.g., FACSCount, TruCount, or Flow-Count).

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Materials and Methods, Continued

Grouping of test results for analysis

Participant laboratories used various methods of determining cell marker percentage and absolute cell counts. For establishing 95% confidence limits, we combined the results from the various methods.

- All cell marker percentage results reported by the laboratories were grouped according to the cell marker of interest, regardless of the flow cytometer or monoclonal antibody combination used to derive the specific result, e.g., $CD4^+$ results were grouped from laboratories using CD3/CD4, CD3/CD4/CD8, CD45/CD3/CD4, and CD45/CD3/CD4/CD8.
- Similarly, regardless of the method used to obtain the absolute cell count (single-platform or dual-platform), we also grouped all results for $CD4^+$ and $CD8^+$ absolute cell counts.

Calculations of 95% confidence limits

Results submitted by participant laboratories were used to calculate 95% confidence limits for each donor and cell marker using the SAS procedure PROC GLM (general linear model).

- Before calculation, data were analyzed for possible outliers. If the absolute value of the jack-knife residual was greater than 3.0, then the data point was considered to be an outlier for calculating the 95% confidence limits.
- Only 204 (2.0%) of 10,046 results were considered to be outliers. These outlier results were removed before the 95% confidence limits shown in Table 3 were calculated.
- No data from any laboratory were removed from the aggregate results table comparing values obtained by the laboratories against the 95% confidence limits.
- Because of insufficient data, 95% confidence limits could not be calculated for $CD3^-/CD16^+$. Table 3 shows the entire range of laboratory results (maximum and minimum) reported for this cell marker.

Overall Summary of Results Submitted

Introduction

The majority of the results (93.9%) returned by the laboratories participating in the April 2005 performance evaluation panel shipment were within the established 95% confidence limits.

Summary of participant results

The percentages of participating laboratory results within the 95% confidence limits established for the cell-marker percentage results, the marker-specific absolute cell counts, white blood cell (WBC) count, lymphocyte percentage, and absolute lymphocyte count are shown in the table below.

Table 2. Total Percentage of Participant Laboratory Results Within or Outside the Established 95% Confidence Limits

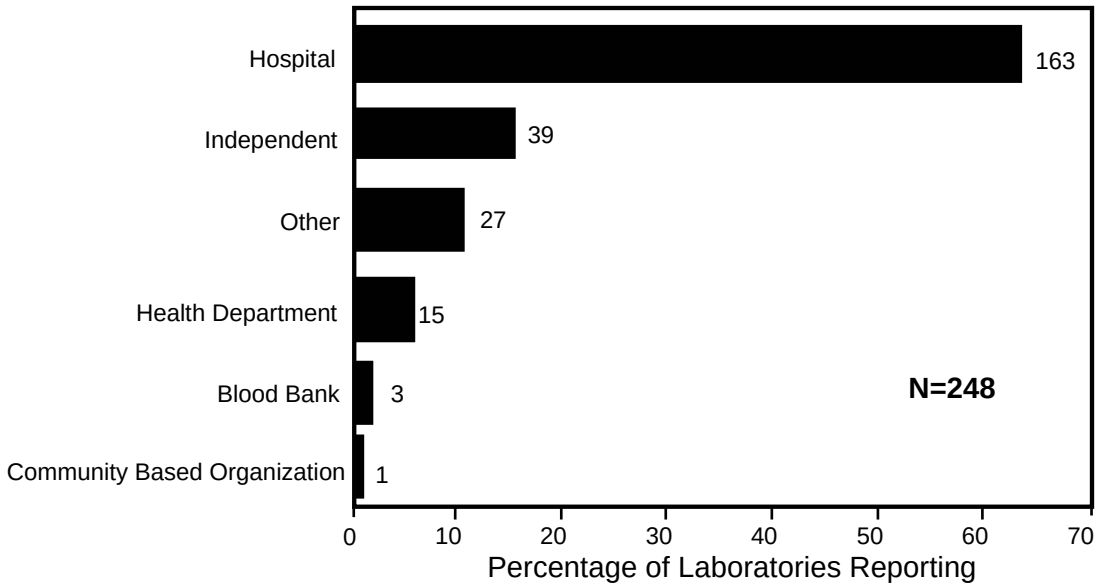
	Cell-Marker Percentage		Absolute Cell Counts			Hematology Results		
Cell Marker	Within 95% Confidence Limits	Outside 95% Confidence Limits	Within 95% Confidence Limits	Outside 95% Confidence Limits			Within 95% Confidence Limits	Outside 95% Confidence Limits
$CD3^+$	94.2%	5.8%				White Blood Cell Count	92.7%	7.3%
$CD4^+$	94.5%	5.5%	92.9%	7.1%		Lymphocyte Percentage	92.5%	7.5%
$CD8^+$	95.2%	4.8%	91.9%	8.1%		Absolute Lymphocyte Count	92.7%	7.3%
$CD14^+$	97.1%	2.9%						
$CD19^+$	96.0%	4.0%						
$CD45^+$	96.5%	3.5%						
$CD3^-/CD56^+$	93.9%	6.1%						
$CD3^-/CD(56+16)^+$	94.9%	5.1%						

Description of Laboratories, Methods, and Instruments

Types of laboratories

The primary classifications of laboratories participating in the April 2005 $CD4^+$ T-cell determinations shipment are shown in Figure 1.

Figure 1. Types of Participant Laboratories



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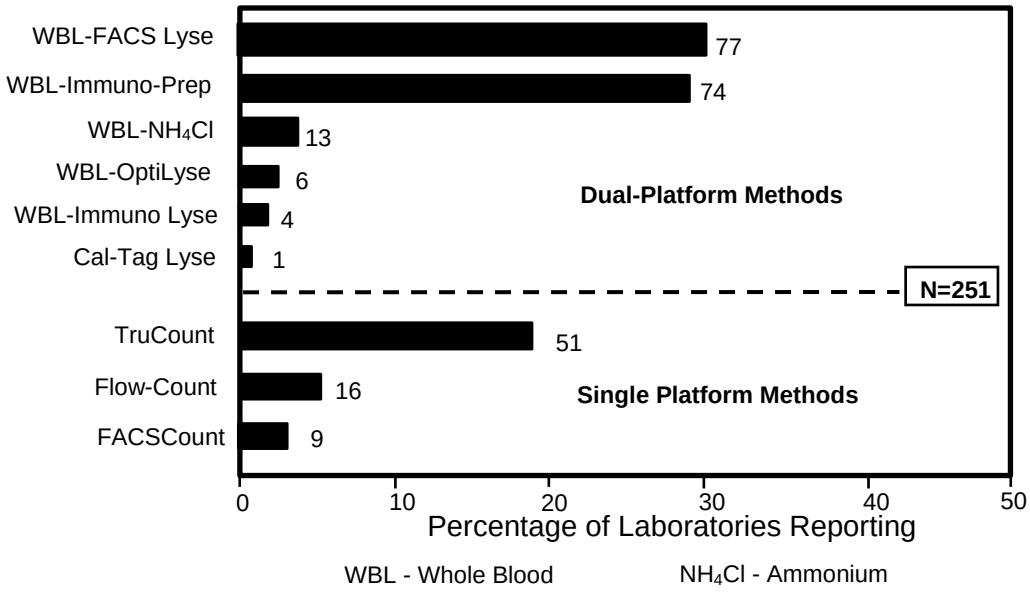
Description of Laboratories, Methods, and Instruments,
Continued

Specimen preparation methods

Figure 2 shows the methods used by the laboratories to prepare specimens for $CD4^+$ T-cell determinations. All of the laboratories performing dual-platform methods reported using a method of whole blood lysis to prepare specimens for $CD4^+$ T-cell (including 1 method described as "Other"). The frequency of preparation methods specific for single-platform methods is also reflected in Figure 2.

Figure 2. Specimen Preparation Methods Used



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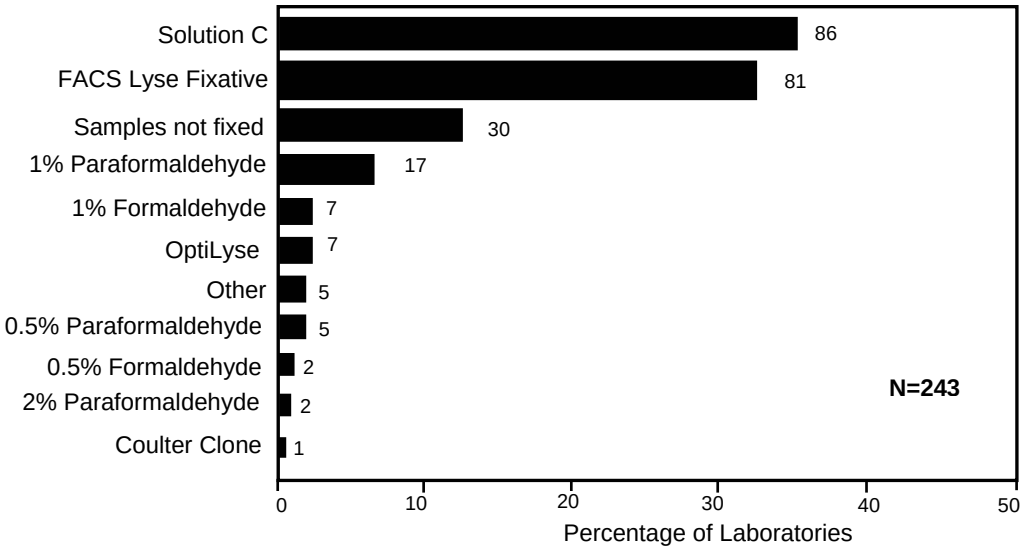
Description of Laboratories, Methods, and Instruments,
Continued

Specimen fixation methods

Figure 3 shows the methods used by the laboratories to fix their $CD4^+$ T-cell specimens before flow cytometric analysis.

- Of laboratories reporting testing results, 31 (12.8%) of 243 specifically stated that they did not fix their $CD4^+$ T-cell specimens before analyzing them, even though the panel sent to the laboratories contained known HIV antibody-positive specimens.
- This practice may be a potential biohazard for flow cytometry personnel.

Figure 3. Methods of Specimen Fixation



"Other" types of fixative used were described as: 5% formaldehyde (2), 2.5% formaline (1), 0.5-2.0% formaldehyde in Cal-Lyse (1), and FACSCount solution (1).

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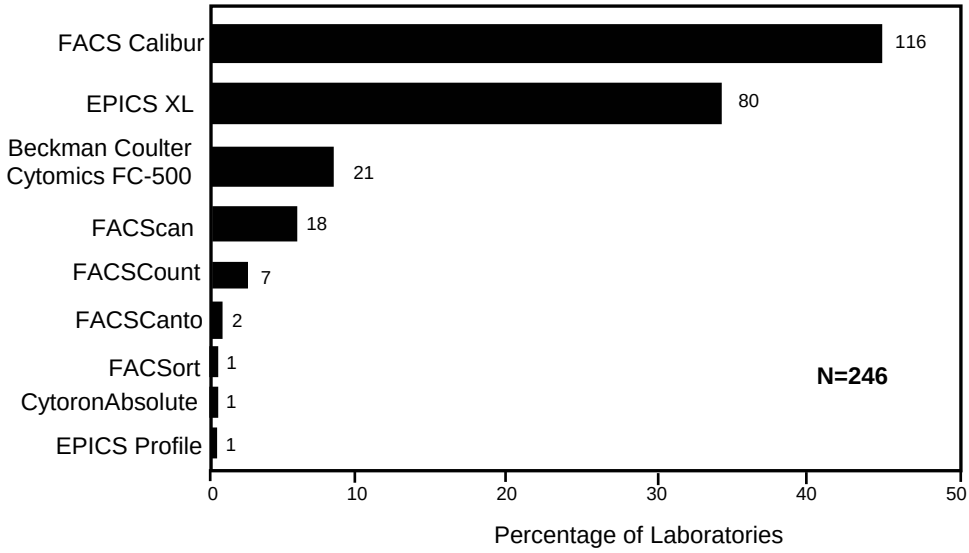
Description of Laboratories, Methods, and Instruments,
Continued

Types of flow cytometers used

Figure 4 shows the types of flow cytometers used by the participant laboratories.

Figure 4. Types of Flow Cytometers Used



Number of laboratories using single- vs. dual-platform methods

Among the 248 laboratories reporting results, 212 reported absolute cell counts.

- Of these, 136 (64.2%) of 212 used a dual-platform method to derive marker-specific absolute cell counts.
- Seventy-two (34.4%) of 212 laboratories used a single-platform method.
- Three (1.4%) of 212 laboratories reported both single-platform and dual-platform derived results.

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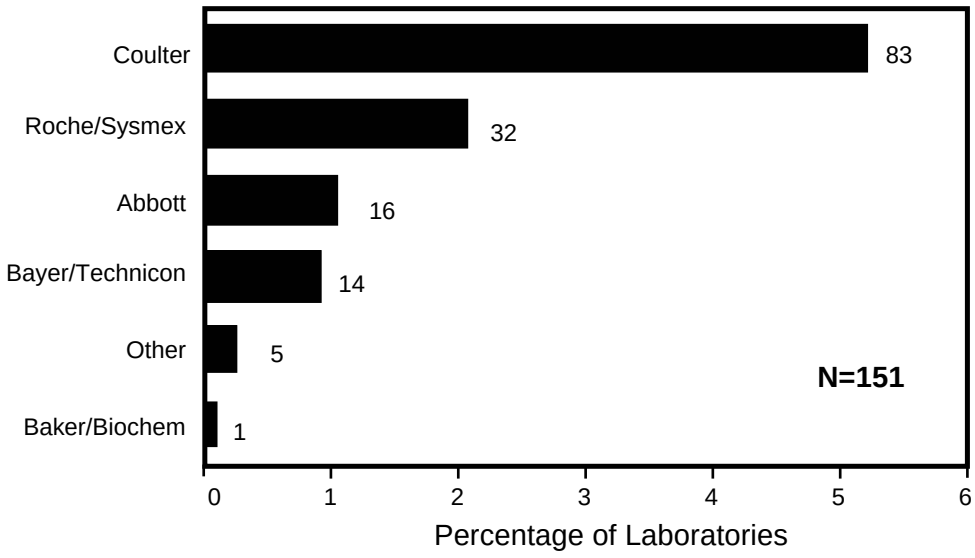
Description of Laboratories, Methods, and Instruments,
Continued

Hematology instruments used

Of the 248 participant laboratories, 151 (60.9%) identified the manufacturer of the hematology instrument being used in their laboratory. These manufacturers are shown in Figure 5.

Figure 5. Types of Hematology Instruments Used



Cell Marker Results and Distributions

Introduction

This section describes the aggregate cell marker percentage and absolute counts results submitted by the participant laboratories.

- Table 3 on the following pages shows the frequency of participant laboratory lymphocyte immunophenotyping percentage results by donor and cell marker, within, above, or below the 95% confidence limits established using results from all laboratories, regardless of the monoclonal antibody combination or manufacturer of flow cytometry instrument used to obtain these percentage results.
- Table 3 also shows the frequency of participant laboratory hematology results (white blood cell count, percentage of lymphocytes and absolute lymphocyte count) and absolute cell count results for $CD4^+$ and $CD8^+$, within, above, or below the statistically established 95% confidence limits.
- Distributions of the $CD4^+$ T-cell absolute counts obtained by single-platform methods are compared with those same results obtained by dual-platform methods in Figure 6.
- The significance of difference in the mean values of these $CD4^+$ T-cell distributions is shown in Table 4.
- The effect of hematology values (absolute lymphocyte count) on the distribution of dual-platform results is shown in Figure 7.

Continued on next page

Table 3. Participant Laboratory Results for the April 2005 Shipment

Donor Number 1 - Donor Status: HIV-antibody Negative

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	97 - 100	15		
	< 97	0		
CD14	> 1	0		
	0 - 1	15		
	< 0	0		
CD4	> 68	4	> 2,426	4
	60 - 68	115	1,359 - 2,426	100
	< 60	2	< 1,359	5
CD8	> 25	0	> 839	3
	21 - 25	119	520 - 839	100
	< 21	2	< 520	6
CD19	> 8	0		
	4 - 8	98		
	< 4	3		
CD56	> 6	1		
	2 - 6	32		
	< 2	0		
CD56+16	> 6	3		
	4 - 6	60		
	< 4	3		
CD3 Average	> 93	4		
	85 - 93	91		
	< 85	0		
CD16	Not Applicable	0		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 8,811	1
	6,934 - 8,811	67
	< 6,934	5
% Lymphs	> 44	4
	34 - 44	68
	< 34	1
Absolute Lymphs	> 3,734	1
	2,395 - 3,734	69
	< 2,395	3

Legend:
95% Confidence limits highlighted
"No." represents number of laboratories reporting in these ranges.
No confidence limits established for CD16 - maximum and minimum values reported

Donor Number 2 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	98 - 100	14		
	< 98	0		
CD14	> 1	0		
	0 - 1	14		
	< 0	0		
CD4	> 42	5	> 1,402	5
	36 - 42	119	968 - 1,402	104
	< 36	2	< 968	3
CD8	> 51	4	> 1,689	3
	45 - 51	118	1,277 - 1,689	103
	< 45	4	< 1,277	6
CD19	> 8	0		
	4 - 8	102		
	< 4	2		
CD56	> 5	2		
	0 - 5	30		
	< 0	0		
CD56+16	> 6	2		
	3 - 6	63		
	< 3	1		
CD3 Average	> 92	2		
	86 - 92	96		
	< 86	2		
CD16	Not Applicable	0		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 6,929	1
	6,148 - 6,929	73
	< 6,148	2
% Lymphs	> 53	6
	42 - 53	69
	< 42	1
Absolute Lymphs	> 3,483	4
	2,715 - 3,483	69
	< 2,715	3

Table 3. Participant Laboratory Results for the April 2005 Shipment

Donor Number 3 - Donor Status: HIV-antibody Negative

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	97 - 100	13		
	< 97	2		
CD14	> 2	0		
	0 - 2	15		
	< 0	0		
CD4	> 53	4	> 1,527	7
	48 - 53	114	845 - 1,527	100
	< 48	3	< 845	2
CD8	> 20	3	> 554	8
	16 - 20	117	295 - 554	100
	< 16	1	< 295	1
CD19	> 21	0		
	17 - 21	95		
	< 17	6		
CD56	> 13	2		
	5 - 13	30		
	< 5	1		
CD56+16	> 13	2		
	8 - 13	63		
	< 8	1		
CD3 Average	> 73	6		
	67 - 73	85		
	< 67	4		
CD16	Not Applicable	0		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 9,901	2
	8,542 - 9,901	68
	< 8,542	3
% Lymphs	> 34	7
	19 - 34	66
	< 19	0
Absolute Lymphs	> 3,081	5
	1,776 - 3,081	67
	< 1,776	1

Legend:
95% Confidence limits highlighted
"No." represents number of laboratories reporting in these ranges.
No confidence limits established for CD16 - maximum and minimum values reported

Donor Number 4 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	96 - 100	16		
	< 96	0		
CD14	> 2	0		
	0 - 2	16		
	< 0	0		
CD4	> 40	1	> 1,086	4
	35 - 40	109	771 - 1,086	100
	< 35	6	< 771	2
CD8	> 52	4	> 1,446	4
	47 - 52	108	1,050 - 1,446	98
	< 47	4	< 1,050	4
CD19	> 11	1		
	7 - 11	96		
	< 7	1		
CD56	> 3	2		
	1 - 3	32		
	< 1	0		
CD56+16	> 5	1		
	1 - 5	65		
	< 1	0		
CD3 Average	> 90	0		
	85 - 90	89		
	< 85	1		
CD16	Not Applicable	0		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 6,149	3
	5,253 - 6,149	64
	< 5,253	3
% Lymphs	> 49	1
	42 - 49	67
	< 42	2
Absolute Lymphs	> 2,847	2
	2,313 - 2,847	65
	< 2,313	3

Table 3. Participant Laboratory Results for the April 2005 Shipment

Donor Number 5 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	97 - 100	13		
	< 97	2		
CD14	> 1	1		
	0 - 1	14		
	< 0	0		
CD4	> 5	7	> 102	5
	3 - 5	114	25 - 102	104
	< 3	0	< 25	0
CD8	> 74	1	> 1,362	8
	67 - 74	118	749 - 1,362	100
	< 67	2	< 749	1
CD19	> 12	0		
	6 - 12	99		
	< 6	2		
CD56	> 9	2		
	2 - 9	31		
	< 2	0		
CD56+16	> 10	1		
	5 - 10	62		
	< 5	3		
CD3 Average	> 87	2		
	79 - 87	93		
	< 79	0		
CD16	Not Applicable	0		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 4,959	2
	4,055 - 4,959	70
	< 4,055	1
% Lymphs	> 44	5
	26 - 44	68
	< 26	0
Absolute Lymphs	> 2,073	5
	1,107 - 2,073	67
	< 1,107	1

Legend:
95% Confidence limits highlighted
"No." represents number of laboratories reporting in these ranges.
No confidence limits established for CD16 - maximum and minimum values reported

Donor Number 6 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	95 - 100	20		
	< 95	0		
CD14	> 3	0		
	0 - 3	20		
	< 0	0		
CD4	> 50	2	> 1,056	7
	41 - 50	117	578 - 1,056	95
	< 41	2	< 578	1
CD8	> 33	2	> 766	8
	26 - 33	115	271 - 766	93
	< 26	4	< 271	2
CD19	> 17	3		
	12 - 17	100		
	< 12	4		
CD56	> 9	1		
	2 - 9	19		
	< 2	0		
CD56+16	> 11	3		
	6 - 11	72		
	< 6	2		
CD3 Average	> 80	5		
	72 - 80	106		
	< 72	0		
CD16	8 - 11	4		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 9,278	6
	5,774 - 9,278	64
	< 5,774	2
% Lymphs	> 35	6
	15 - 35	66
	< 15	0
Absolute Lymphs	> 2,535	7
	1,176 - 2,535	65
	< 1,176	0

Table 3. Participant Laboratory Results for the April 2005 Shipment

Donor Number 7 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	96 - 100	18		
	< 96	0		
CD14	> 0	2		
	0 - 0	16		
	< 0	0		
CD4	> 47	6	> 1,040	7
	38 - 47	115	578 - 1,040	101
	< 38	3	< 578	0
CD8	> 35	7	> 850	5
	29 - 35	116	408 - 850	102
	< 29	1	< 408	1
CD19	> 9	3		
	4 - 9	95		
	< 4	2		
CD56	> 18	1		
	8 - 18	29		
	< 8	2		
CD56+16	> 18	0		
	12 - 18	52		
	< 12	4		
CD3 Average	> 81	6		
	75 - 81	100		
	< 75	4		
CD16	Not Applicable	0		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 4,518	4
	2,907 - 4,518	61
	< 2,907	1
% Lymphs	> 58	4
	46 - 58	62
	< 46	0
Absolute Lymphs	> 2,334	3
	1,519 - 2,334	62
	< 1,519	1

Legend:
95% Confidence limits highlighted
"No." represents number of laboratories reporting in these ranges.
No confidence limits established for CD16 - maximum and minimum values reported

Donor Number 8 - Donor Status: HIV-antibody Negative

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	96 - 100	18		
	< 96	1		
CD14	> 1	1		
	0 - 1	18		
	< 0	0		
CD4	> 55	5	> 1,411	10
	47 - 55	115	737 - 1,411	94
	< 47	2	< 737	1
CD8	> 30	5	> 787	9
	25 - 30	114	397 - 787	94
	< 25	3	< 397	2
CD19	> 14	1		
	9 - 14	98		
	< 9	4		
CD56	> 10	1		
	4 - 10	24		
	< 4	1		
CD56+16	> 11	0		
	6 - 11	64		
	< 6	2		
CD3 Average	> 82	7		
	76 - 82	102		
	< 76	1		
CD16	7 - 9	2		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 6,153	2
	5,311 - 6,153	64
	< 5,311	3
% Lymphs	> 50	6
	27 - 50	63
	< 27	0
Absolute Lymphs	> 2,924	6
	1,520 - 2,924	63
	< 1,520	0

Table 3. Participant Laboratory Results for the April 2005 Shipment

Donor Number 9 - Donor Status: HIV-antibody Negative

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	95 - 100	18		
	< 95	1		
CD14	> 2	0		
	0 - 2	19		
	< 0	0		
CD4	> 54	6	> 1,838	7
	45 - 54	116	893 - 1,838	98
	< 45	0	< 893	0
CD8	> 26	4	> 832	6
	19 - 26	117	417 - 832	97
	< 19	1	< 417	1
CD19	> 26	1		
	18 - 26	99		
	< 18	3		
CD56	> 3	0		
	1 - 3	26		
	< 1	0		
CD56+16	> 5	2		
	1 - 5	64		
	< 1	0		
CD3 Average	> 78	6		
	70 - 78	101		
	< 70	3		
CD16	2 - 4	2		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 7,656	7
	4,342 - 7,656	62
	< 4,342	0
% Lymphs	> 56	5
	36 - 56	64
	< 36	0
Absolute Lymphs	> 3,689	3
	1,873 - 3,689	66
	< 1,873	0

Legend:
95% Confidence limits highlighted
"No." represents number of laboratories reporting in these ranges.
No confidence limits established for CD16 - maximum and minimum values reported

Donor Number 10 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	92 - 100	19		
	< 92	0		
CD14	> 3	1		
	0 - 3	18		
	< 0	0		
CD4	> 13	5	> 203	6
	7 - 13	114	66 - 203	98
	< 7	2	< 66	0
CD8	> 53	1	> 770	7
	33 - 53	115	356 - 770	95
	< 33	5	< 356	2
CD19	> 15	5		
	5 - 15	97		
	< 5	0		
CD56	> 19	2		
	0 - 19	23		
	< 0	0		
CD56+16	> 40	1		
	16 - 40	62		
	< 16	3		
CD3 Average	> 65	1		
	43 - 65	102		
	< 43	6		
CD16	28 - 32	2		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 5,488	4
	2,118 - 5,488	64
	< 2,118	0
% Lymphs	> 49	5
	24 - 49	63
	< 24	0
Absolute Lymphs	> 2,086	4
	712 - 2,086	64
	< 712	0

Cell Marker Results and Distributions, Continued

Effect of cell analysis method on the range of results

As shown in Figure 6 on the following pages, the range of results reported for absolute $CD4^+$ and $CD8^+$ T-cell counts was different depending on the method used to obtain the result, i.e., single-platform vs. dual-platform.

- These are inclusive ranges (lowest value to highest value) and are not 95% confidence limits as presented in the results in Table 3.
- The bars in the graphs represent the data submitted by the participant laboratories. The lines in the graphs represent the normalized plot of the results.
- The mean and standard deviation in each of the graphs is based on the normalized distribution of the results.
- As demonstrated by the difference in the standard deviations for the normalized distribution of results, the dual-platform ranges were larger than the corresponding single-platform ranges for both $CD4^+$ and $CD8^+$ absolute T-cell counts (for most donor specimens).

Continued on next page

Cell Marker Results and Distributions, Continued

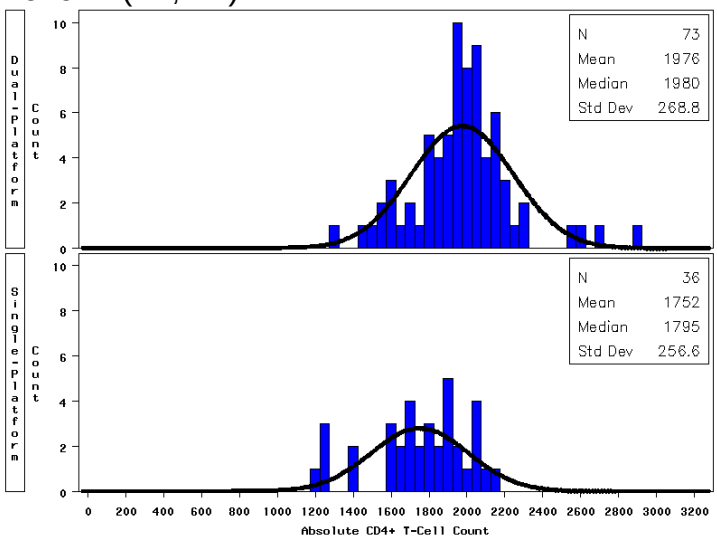
Figure 6. Absolute CD4$^+$ T-cell counts, by donor, by method

Description of graphs depicted below:

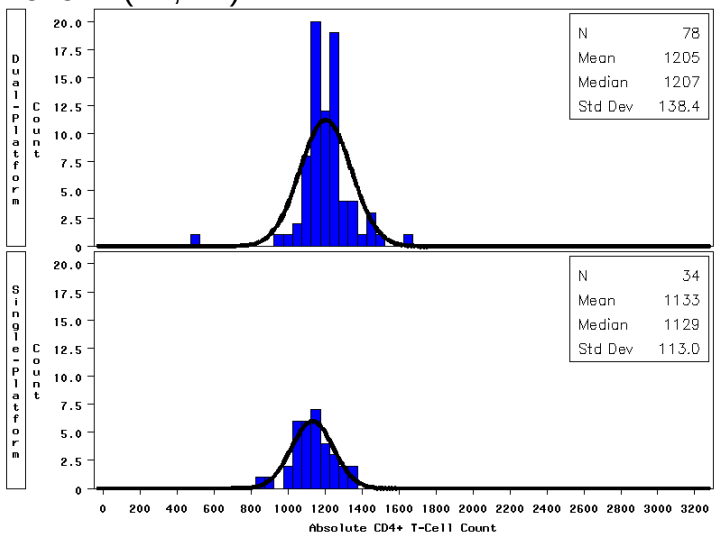
- Upper plot -- absolute CD4$^+$ T-cell count derived using dual-platform methods.
- Lower plot -- absolute CD4$^+$ T-cell count derived using single-platform methods.
- X-axis -- range of absolute CD4$^+$ T-cell counts.
- Y-axis --number of laboratories obtaining a particular CD4$^+$ T-cell count.

Absolute CD4$^+$ T-Cell Count

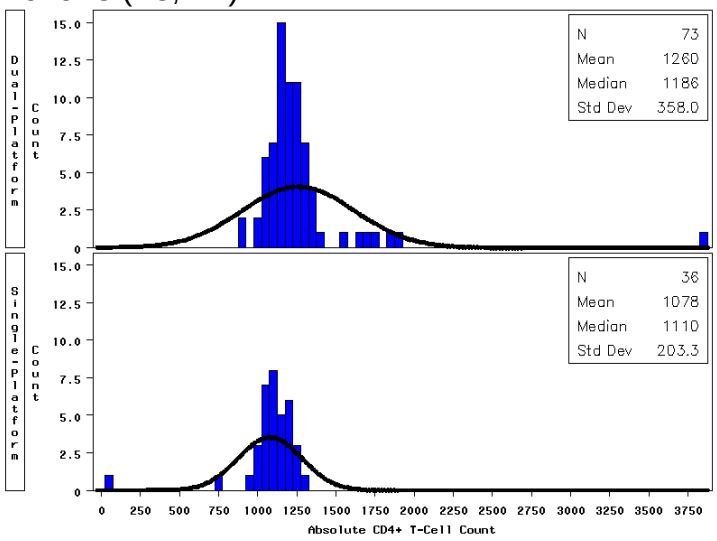
Donor 1 (A2, B4)



Donor 2 (A1, A4)



Donor 3 (A5, B1)



Donor 4 (B2, B3)

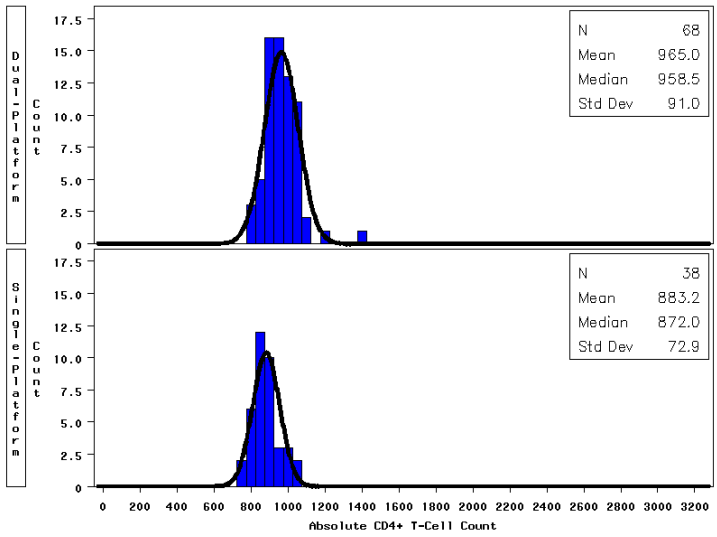


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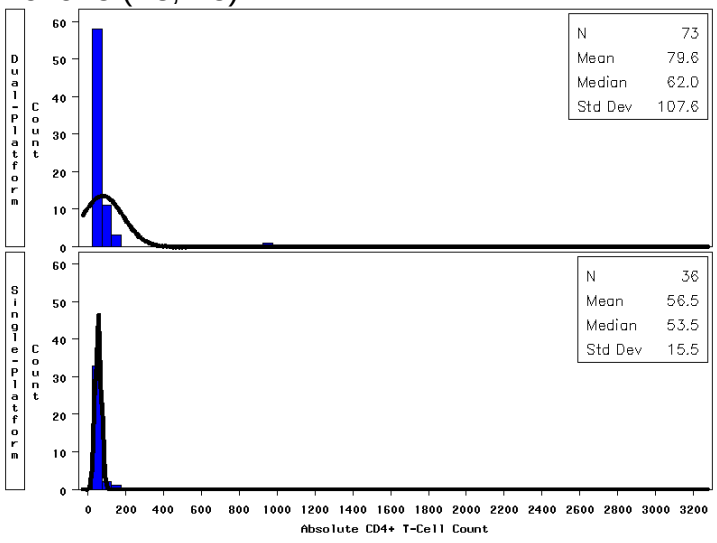
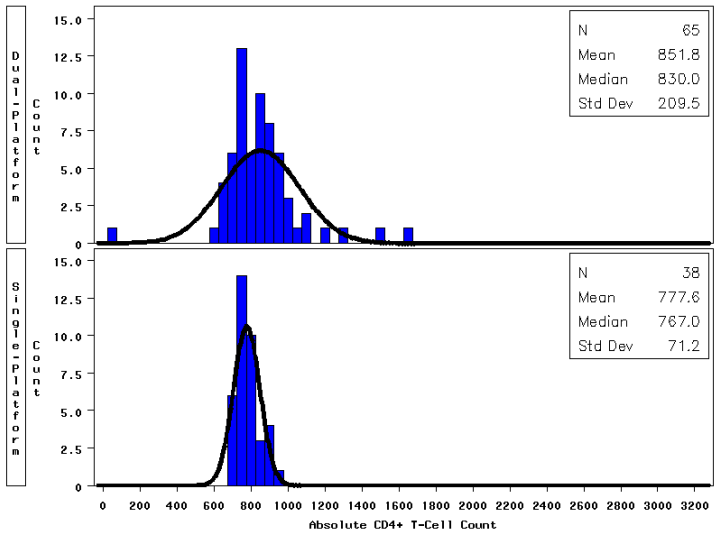
Figure 6, continued. **Absolute CD4$^+$ T-cell counts, by donor, by method**

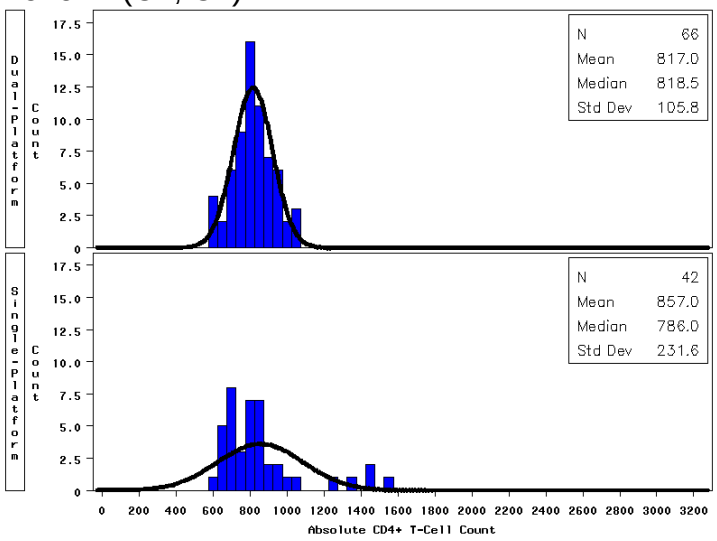
Donor 5 (A3, B5)



Donor 6 (D2, D5)



Donor 7 (C2, C4)



Donor 8 (C1, D4)

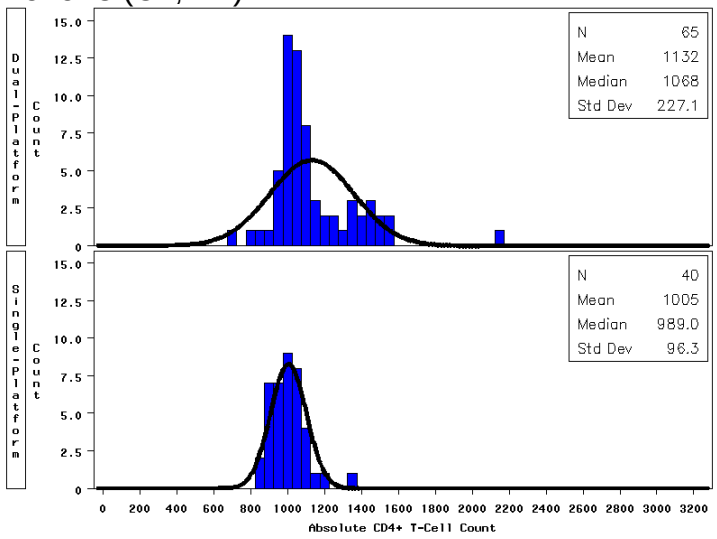


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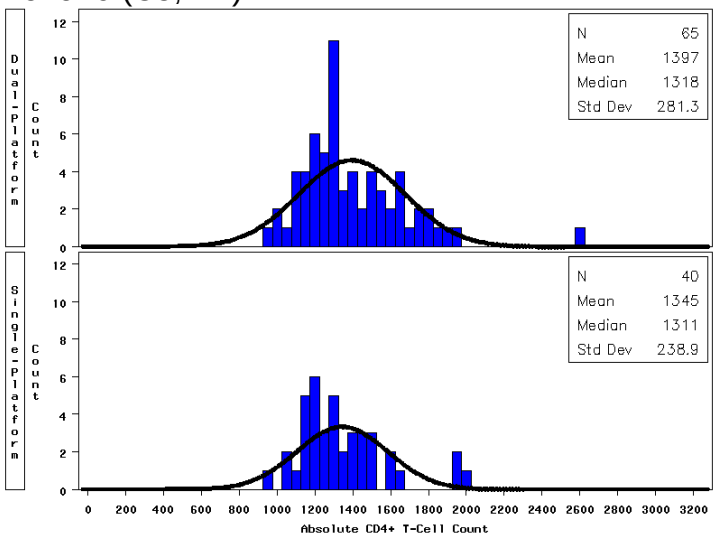
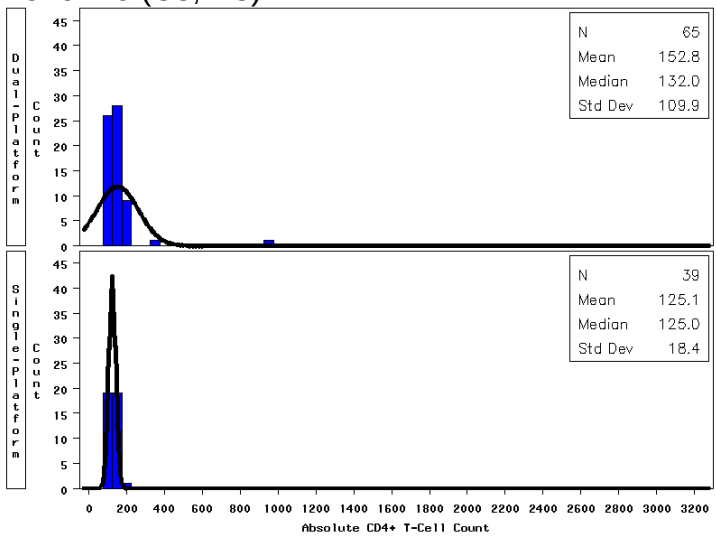
Figure 6, continued. **Absolute CD4$^+$ T-cell counts, by donor, by method**

Donor 9 (C5, D1)



Donor 10 (C3, D3)



Reporting errors

The magnitude of the ranges shown in Figure 6 may be partially due to reporting errors on the part of the laboratories.

- One laboratory for one of the specimens they tested reported a lymphocyte count result that was in error by nearly a factor of 2 (e.g., the laboratory reported a WBC of 4720 and a lymphocyte percent of 36, which should have yielded a lymphocyte count of 1699; however, the laboratory reported a lymphocyte count of 3620).
- One laboratory reported the same values for lymphocyte percent and lymphocyte count for all five specimens.
- In total, four laboratories reported lymphocyte counts that differed by more than 5% from the true calculated lymphocyte count (WBC X Lymphocyte percent) on at least one specimen.

Continued on next page

Significance of method of analysis on mean CD4 value

- In general, the mean CD4 value of the normalized curve for the dual-platform results was larger than the mean CD4 value of the normalized curve for the single-platform results.
- As can be seen in Table 4 below, for some donors this shift in the mean CD4 values was statistically significant.
- If the shift in CD4 value occurs around a medical treatment or AIDS case defining decision point (e.g., 500 or 200 absolute CD4 counts), the shift may have clinical significance.

Table 4. Mean CD4 values, Dual-Platform vs. Single-Platform Methods

Donor	Dual-Platform mean CD4+ Value	Single-Platform mean CD4+ Value	p value	Significance
1	1976	1752	p=<.0001	Significant*
2	1205	1133	p=0.0091	Significant
3	1260	1079	p=0.0011	Significant
4	965	883	p=<.0001	Significant
5	80	57	p=0.0761	Not Significant
6	852	778	p=0.0107	Significant
7	817	857	p=0.2969	Not Significant
8	1133	1005	p=0.0001	Significant
9	1397	1345	p=0.3309	Not Significant
10	153	125	p=0.0508	Not Significant
	* Significant if p-value is <0.05			

Effect of hematology results on dual-platform methods, Figure 7

The ranges of dual-platform results were affected by the extent of variability in the absolute lymphocyte count results derived from hematology instruments. As shown in Figure 7 on the following pages, often the range of results was quite large.

Continued on next page

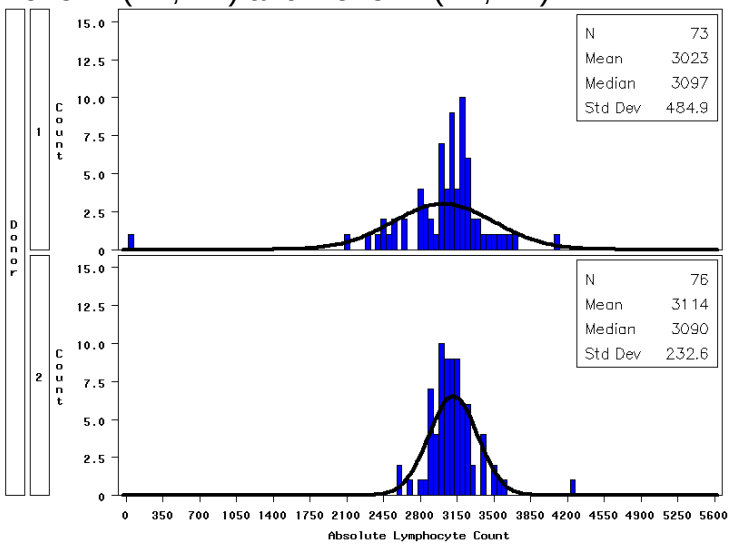
Figure 7. Absolute Lymphocyte Counts, by Donor

Description of graphs depicted below:

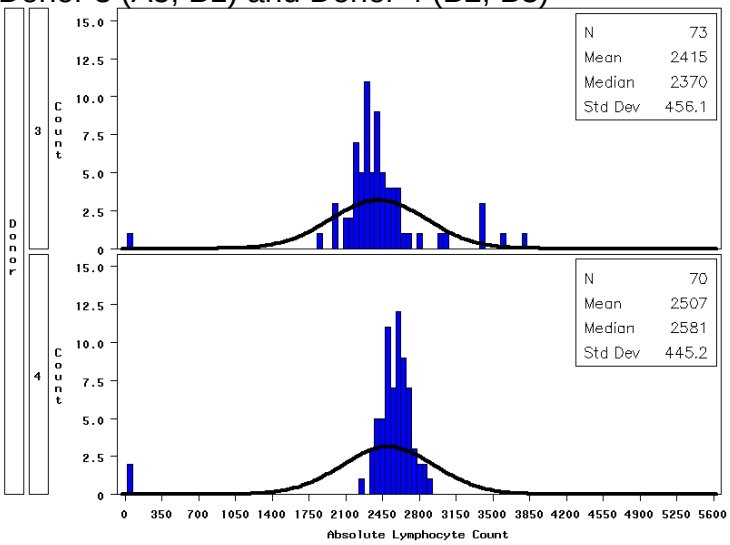
- Upper plot -- absolute lymphocyte count for one donor.
- Lower plot -- absolute lymphocyte count for another donor.
- The identity of the donors is noted in the bars on the left hand side of the plot.
- X-axis -- range of absolute lymphocyte counts.
- Y-axis -- number of laboratories obtaining a particular absolute lymphocyte count.

Absolute Lymphocyte Count (Hematology Instrument)

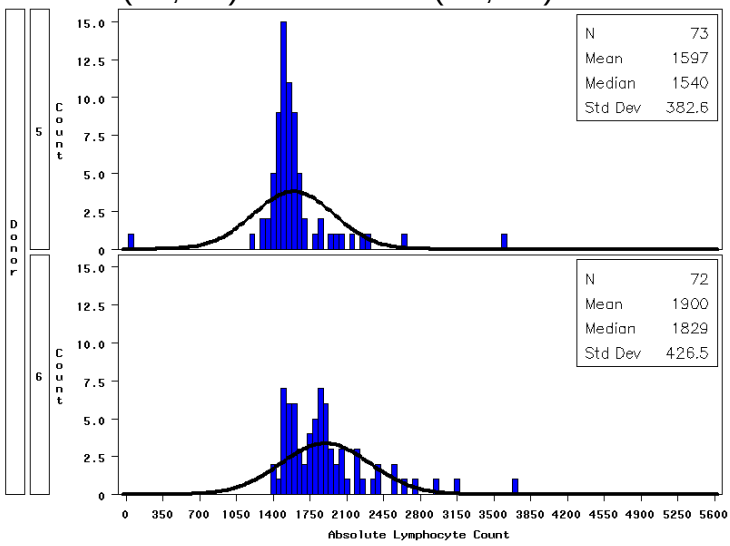
Donor 1 (A2, B4) and Donor 2 (A1, A4)



Donor 3 (A5, B1) and Donor 4 (B2, B3)



Donor 5 (A3, B5) and Donor 6 (D2, D5)



Donor 7 (C2, C4) and Donor 8 (C1, D4)

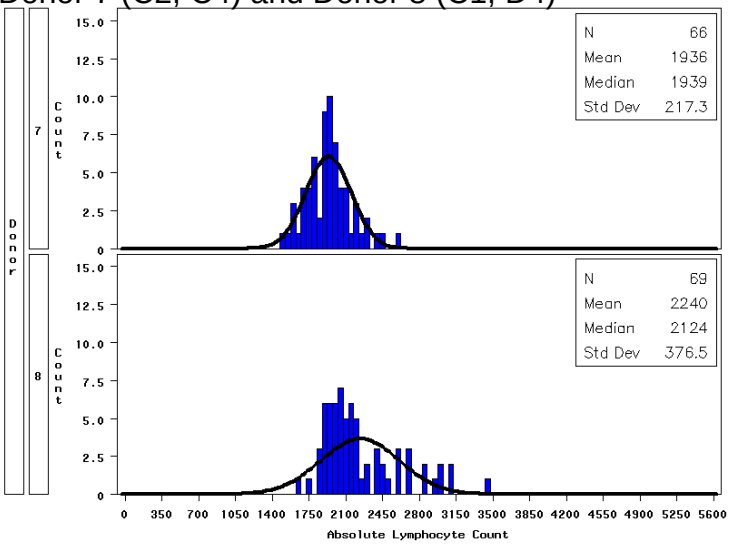


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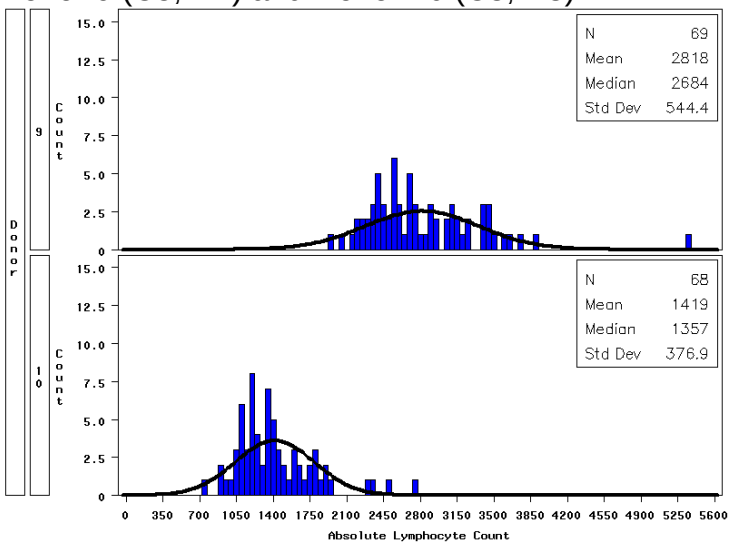
Figure 7, continued**. Absolute Lymphocyte Counts, by Donor**

Donor 9 (C5, D1) and Donor 10 (C3, D3)



Discussion

Effect of delayed specimen preparation

Several laboratories reported delays in preparing specimens for analysis. These delays were related to delay in receipt due to problems with the overnight courier, delivery problems within the receiving institution, and delay in processing the specimens after receipt in the laboratory.

A total of 60 laboratories reported specimen preparation delays (3 laboratories reported both late deliveries and delays in processing).

These specimen preparation delays may have affected the testing results from these laboratories.

- Of the 60 laboratories reporting specimen preparation delays, 34 laboratories (56.7%) reported one or more results outside the established 95% confidence ranges.
- One laboratory reported 18 of 50 results (36.0%) submitted and another laboratory reported 17 of 50 results (34.0%) submitted outside the 95% confidence ranges.

Possible reasons for differences in laboratory performance

Differences in laboratory performance of cell marker analysis may be related to:

- the use of the CDC $CD4^+$ T-cell testing guidelines
- the use of dual-platform versus single-platform procedures
- the use of different flow cytometer, hematology instrument, and reagent manufacturer combinations
- factors associated with specimen preparation (including specimen fixation before analysis and delay in preparing specimens for analysis), and
- reporting errors on the part of the laboratories.

Ensuring accurate calculated results

Laboratories should have a mechanism in place to ensure accurate and reliable calculated results. Laboratories are reminded that this is a requirement in the regulations implementing the Clinical Laboratory Improvement Amendments (CLIA) [Sec. 493.1291 (a) (1)]. This standard is as follows:

> " (a) The laboratory must have adequate manual or electronic systems in place to ensure test results and other patient-specific data are accurately and reliably sent from the point of data entry (whether interfaced or entered manually) to final report destination, in a timely manner. This includes the following:
>
> (1) Results reported from calculated data."

Continued on next page

Discussion, Continued

Discontinuation of CDC MPEP for CD4+ T-cell Determinations

This document represents the final report for this program.

The CDC MPEP for CD4+ T-cell determinations is being discontinued for the following reasons:

- it has met the goals set out for it when it was initiated 15 years ago;
- the quality of the performance of the enrolled laboratories has been consistently high;
- technology has evolved to the point where this testing has become simpler and not subject to as much variability as seen early on; and
- other external quality assessment programs for $CD4^+$ T-cell testing are available for U.S. laboratories.

Summary of Program Outcomes

During its 15-year life span, this program has provided useful information on the quality of CD4+ testing and quality assurance and quality control practices through the dedicated participation of several hundred of the Nation's laboratories. In addition, data collected through this program has contributed to the publication of four sets of CDC $CD4^+$ T-cell testing guidelines:

- "Guidelines for the Performance of $CD4^+$ T-cell Determinations in Persons with Human Immunodeficiency Virus Infection" *MMWR* 1992; 41(RR-8).
- "1994 Revised Guidelines for the Performance of $CD4^+$ T-cell Determinations in Persons with Human Immunodeficiency Virus (HIV) Infection" *MMWR* 1994; 43(No.RR-3).
- "1997 Revised Guidelines for the Performance of $CD4^+$ T-cell Determinations in Persons Infected with Human Immunodeficiency Virus (HIV)" *MMWR* 1997; 46(No.RR-2).
- "Guidelines for Performing Single-Platform Absolute $CD4^+$ T-cell Determinations with CD45 Gating for Persons Infected with Human Immunodeficiency Virus" *MMWR* 2003; 52(No.RR-2):1-13.

Thank you

We thank the laboratories that participated in this program. Their willingness to share performance and practice data has contributed to the evaluation of and collective knowledge about the state of $CD4^+$ T-cell testing in the United States.