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 October 2004

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 October 2004

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Overview of October 2004 $CD4^+$ 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 October 12 and October 19, 2004.

Laboratory Response

Of the 259 laboratories receiving specimen panels, 247 (95.4%) reported testing results.

- Of the 12 nonreporting laboratories, one laboratory indicated they had mistakenly held the specimens for longer than 48 hours before processing and, therefore, did not consider their testing results reliable. Eleven laboratories provided no explanation for nonparticipation.
- The majority of the laboratories (78.1%) 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 October 2004 performance evaluation panel shipment were within the established 95% confidence limits.

- In particular, 92.4% of the absolute $CD4^+$ and 92.0% 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 October 2004 Shipment Specimens

Panel Letter	Participant Laboratory	CDC Donor Number	Donor Information (HIV-1* status)
A	A1	03	HIV-1 Antibody-Negative
	A2, A5	02	HIV-1 Antibody-Positive
	A3	01	HIV-1 Antibody-Negative
	A4	05	HIV-1 Antibody-Positive
B	B1	05	HIV-1 Antibody-Positive
	B2	03	HIV-1 Antibody-Negative
	B3, B4	04	HIV-1 Antibody-Positive
	B5	01	HIV-1 Antibody-Negative
C	C1	09	HIV-1 Antibody-Negative
	C2	08	HIV-1 Antibody-Negative
	C3, C5	07	HIV-1 Antibody-Positive
	C4	10	HIV-1 Antibody-Positive
D	D1, D3	06	HIV-1 Antibody-Positive
	D2	09	HIV-1 Antibody-Negative
	D4	10	HIV-1 Antibody-Positive
	D5	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) in performing $CD4^+$ T-cell determinations on patient specimens.

- The result reporting booklet used for the October 2004 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).

Continued on next page

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 191 (1.9%) of 9,914 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 October 2004 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

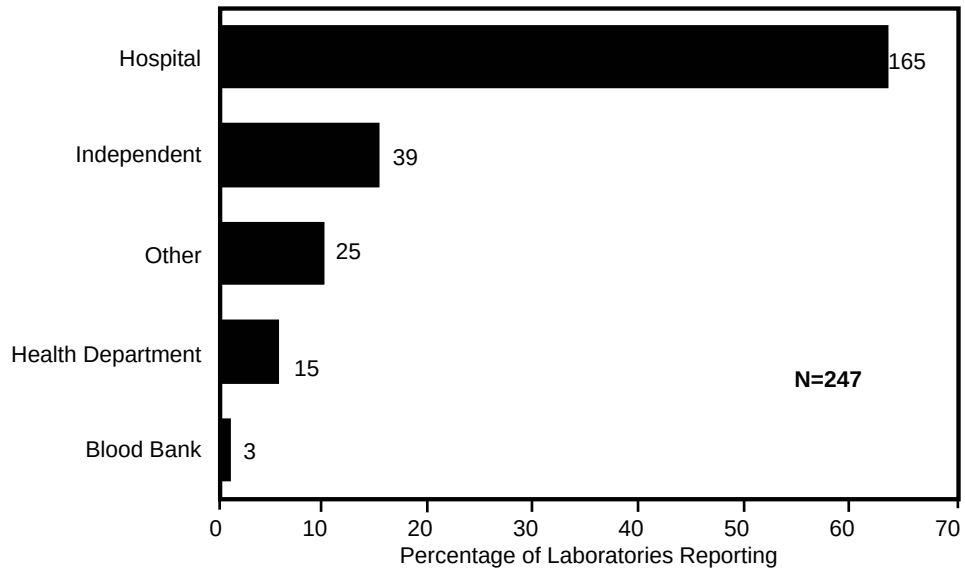
	Cell-Marker Percentage		Absolute Cell Counts			Hematology Results		
Cell Marker	Within 95% Confidence Limits	Outside 95% Confidence Limits	Within 95% Confidence Limits	Outside 95% Confidence Limits			Within 95% Confidence Limits	Outside 95% Confidence Limits
$CD3^+$	94.8%	5.2%				White Blood Cell Count	92.8%	7.2%
$CD4^+$	94.8%	5.2%	92.4%	7.6%		Lymphocyte Percentage	92.2%	7.8%
$CD8^+$	94.8%	5.2%	92.0%	8.0%		Absolute Lymphocyte Count	91.6%	8.4%
$CD14^+$	96.8%	3.2%						
$CD19^+$	96.2%	3.8%						
$CD45^+$	96.9%	3.1%						
$CD3^-/CD56^+$	96.1%	3.9%						
$CD3^-/CD(56+16)^+$	94.7%	5.3%						

Description of Laboratories, Methods, and Instruments

Types of laboratories

The primary classifications of laboratories participating in the October 2004 $CD4^+$ T-cell determinations shipment are shown in Figure 1.

Figure 1. Types of Participant Laboratories



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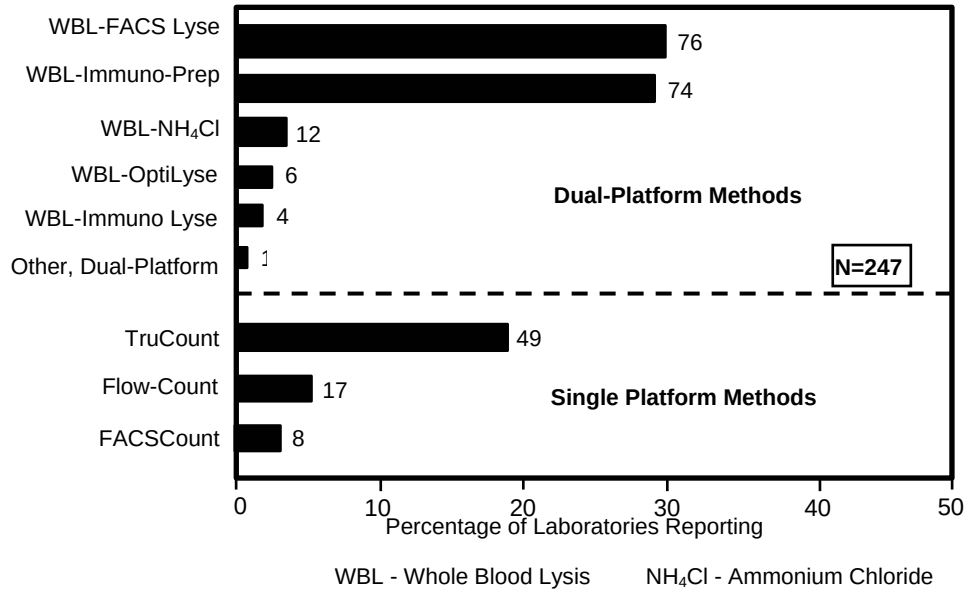
Description of Laboratories, Methods, and Instruments,
Continued

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



The "Other" dual-platform method was described as Cal-Lyse (CalTag).

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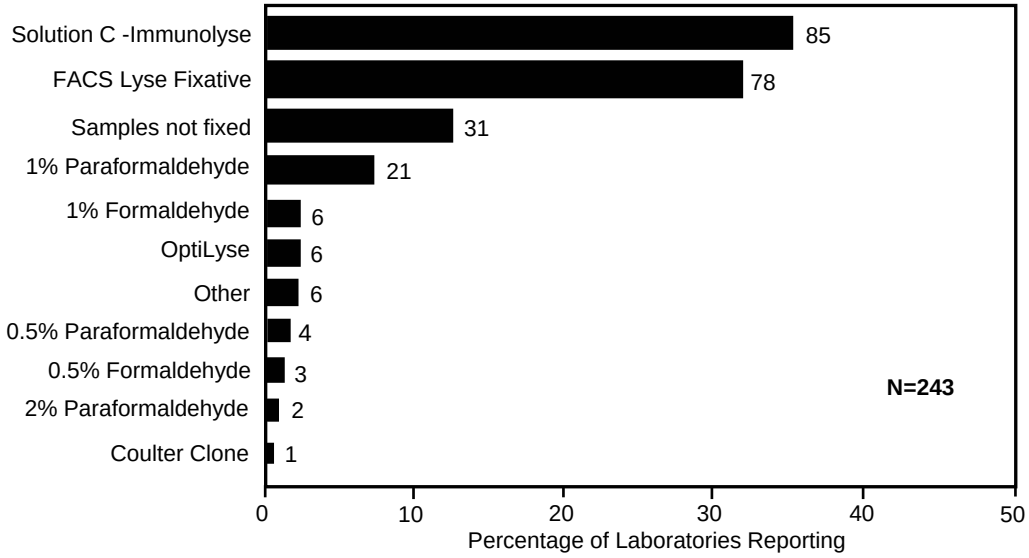
Description of Laboratories, Methods, and Instruments,
Continued

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 (3), 2.5% formaline (1), 0.5-2.0% formaldehyde in Cal-Lys (1), and 0.1% paraformaldehyde (1).

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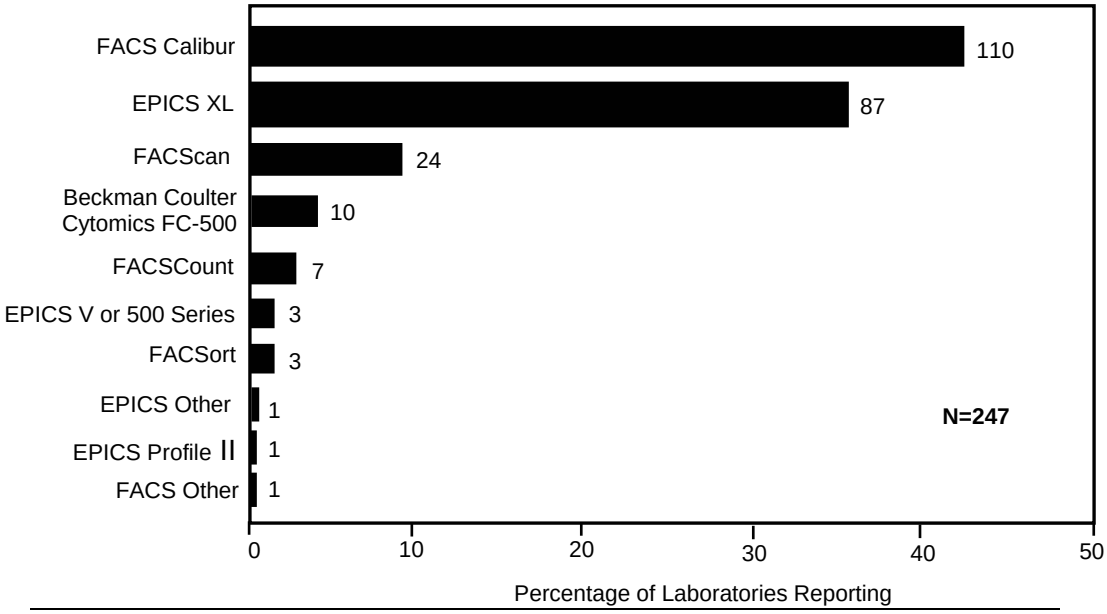
Description of Laboratories, Methods, and Instruments,
Continued

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 247 laboratories reporting results, 210 reported absolute cell counts.

- Of these, 136 (64.8%) of 210 used a dual-platform method to derive marker-specific absolute cell counts.
- Seventy-four (35.2%) of 210 laboratories used a single-platform method.

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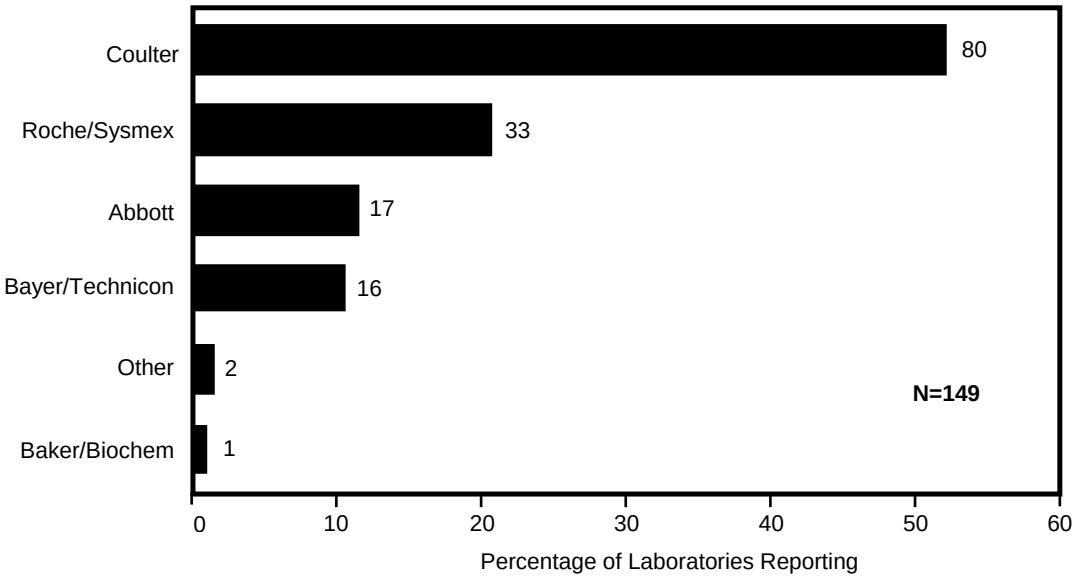
Description of Laboratories, Methods, and Instruments,
Continued

Hematology instruments used

Of the 247 participant laboratories, 149 (60.3%) 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.

Table 3. Participant Laboratory Results for the October 2004 Shipment

Donor Number 1 - Donor Status: HIV-antibody Negative

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	96 - 100	14		
	< 96	2		
CD14	> 1	1		
	0 - 1	14		
	< 0	0		
CD4	> 53	3	> 1,266	6
	47 - 53	113	812 - 1,266	95
	< 47	1	< 812	1
CD8	> 32	3	> 705	4
	25 - 32	113	467 - 705	94
	< 25	1	< 467	3
CD19	> 13	1		
	9 - 13	96		
	< 9	2		
CD56	> 9	0		
	4 - 9	27		
	< 4	1		
CD56+16	> 10	2		
	6 - 10	63		
	< 6	2		
CD3 Average	> 84	2		
	77 - 84	91		
	< 77	1		
CD16	7 - 7	2		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 4,901	1
	4,197 - 4,901	60
	< 4,197	3
% Lymphs	> 54	5
	40 - 54	58
	< 40	1
Absolute Lymphs	> 2,486	5
	1,760 - 2,486	59
	< 1,760	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 2 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	95 - 100	12		
	< 95	0		
CD14	> 1	1		
	0 - 1	11		
	< 0	0		
CD4	> 24	4	> 834	2
	19 - 24	111	376 - 834	98
	< 19	3	< 376	6
CD8	> 66	7	> 2,242	2
	59 - 66	109	1,256 - 2,242	95
	< 59	2	< 1,256	9
CD19	> 12	0		
	7 - 12	92		
	< 7	4		
CD56	> 2	0		
	0 - 2	30		
	< 0	0		
CD56+16	> 6	0		
	1 - 6	62		
	< 1	0		
CD3 Average	> 89	3		
	82 - 89	88		
	< 82	3		
CD16	0 - 0	0		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 6,914	0
	4,418 - 6,914	60
	< 4,418	6
% Lymphs	> 56	5
	45 - 56	59
	< 45	2
Absolute Lymphs	> 3,480	0
	2,205 - 3,480	61
	< 2,205	5

Table 3. Participant Laboratory Results for the October 2004 Shipment

Donor Number 3 - Donor Status: HIV-antibody Negative

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	95 - 100	15		
	< 95	1		
CD14	> 3	0		
	0 - 3	15		
	< 0	0		
CD4	> 57	5	> 2,029	6
	50 - 57	110	1,329 - 2,029	95
	< 50	3	< 1,329	2
CD8	> 18	2	> 650	4
	14 - 18	113	364 - 650	97
	< 14	3	< 364	1
CD19	> 23	1		
	17 - 23	95		
	< 17	4		
CD56	> 7	0		
	3 - 7	29		
	< 3	0		
CD56+16	> 9	3		
	5 - 9	62		
	< 5	2		
CD3 Average	> 76	3		
	67 - 76	90		
	< 67	3		
CD16	5 - 6	2		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 11,893	1
	9,575 - 11,893	61
	< 9,575	3
% Lymphs	> 36	5
	24 - 36	60
	< 24	0
Absolute Lymphs	> 4,120	6
	2,445 - 4,120	59
	< 2,445	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 4 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	94 - 100	20		
	< 94	0		
CD14	> 2	0		
	0 - 2	18		
	< 0	0		
CD4	> 43	6	> 1,350	4
	36 - 43	108	883 - 1,350	93
	< 36	4	< 883	3
CD8	> 52	5	> 1,561	2
	41 - 52	113	1,020 - 1,561	93
	< 41	0	< 1,020	3
CD19	> 15	2		
	6 - 15	98		
	< 6	4		
CD56	> 3	0		
	1 - 3	28		
	< 1	0		
CD56+16	> 5	4		
	1 - 5	68		
	< 1	0		
CD3 Average	> 91	3		
	82 - 91	93		
	< 82	1		
CD16	2 - 2	4		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 8,509	0
	5,674 - 8,509	60
	< 5,674	4
% Lymphs	> 51	3
	31 - 51	61
	< 31	0
Absolute Lymphs	> 3,353	4
	2,371 - 3,353	59
	< 2,371	1

Table 3. Participant Laboratory Results for the October 2004 Shipment

Donor Number 5 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	92 - 100	15		
	< 92	1		
CD14	> 2	1		
	0 - 2	14		
	< 0	0		
CD4	> 8	5	> 172	6
	4 - 8	113	49 - 172	97
	< 4	0	< 49	0
CD8	> 70	1	> 1,732	6
	61 - 70	114	692 - 1,732	94
	< 61	3	< 692	2
CD19	> 17	1		
	9 - 17	95		
	< 9	4		
CD56	> 8	1		
	1 - 8	28		
	< 1	0		
CD56+16	> 11	4		
	4 - 11	62		
	< 4	1		
CD3 Average	> 84	2		
	74 - 84	92		
	< 74	2		
CD16	5 - 5	2		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 10,492	0
	7,891 - 10,492	62
	< 7,891	3
% Lymphs	> 32	6
	12 - 32	59
	< 12	0
Absolute Lymphs	> 3,020	5
	1,042 - 3,020	60
	< 1,042	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 6 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	91 - 100	24		
	< 91	0		
CD14	> 1	0		
	0 - 1	24		
	< 0	0		
CD4	> 36	3	> 900	6
	28 - 36	115	338 - 900	96
	< 28	4	< 338	3
CD8	> 75	2	> 1,570	4
	49 - 75	113	763 - 1,570	95
	< 49	7	< 763	6
CD19	> 7	0		
	1 - 7	102		
	< 1	0		
CD56	> 6	2		
	1 - 6	30		
	< 1	0		
CD56+16	> 11	3		
	2 - 11	55		
	< 2	0		
CD3 Average	> 94	2		
	82 - 94	107		
	< 82	3		
CD16	5 - 6	2		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 7,049	3
	3,035 - 7,049	65
	< 3,035	3
% Lymphs	> 52	4
	28 - 52	66
	< 28	1
Absolute Lymphs	> 2,749	3
	1,273 - 2,749	66
	< 1,273	2

Table 3. Participant Laboratory Results for the October 2004 Shipment

Donor Number 7 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	96 - 100	22		
	< 96	0		
CD14	> 1	0		
	0 - 1	22		
	< 0	0		
CD4	> 37	2	> 1,113	6
	32 - 37	112	513 - 1,113	102
	< 32	2	< 513	4
CD8	> 54	4	> 1,604	7
	47 - 54	111	782 - 1,604	101
	< 47	1	< 782	4
CD19	> 9	0		
	4 - 9	92		
	< 4	1		
CD56	> 6	0		
	0 - 6	22		
	< 0	0		
CD56+16	> 7	0		
	4 - 7	67		
	< 4	3		
CD3 Average	> 90	2		
	84 - 90	94		
	< 84	0		
CD16	0 - 0	0		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 7,521	1
	5,040 - 7,521	73
	< 5,040	6
% Lymphs	> 48	6
	29 - 48	74
	< 29	0
Absolute Lymphs	> 3,270	7
	1,556 - 3,270	70
	< 1,556	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 8 - Donor Status: HIV-antibody Negative

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	95 - 100	23		
	< 95	0		
CD14	> 1	1		
	0 - 1	22		
	< 0	0		
CD4	> 62	5	> 1,826	5
	52 - 62	112	1,156 - 1,826	100
	< 52	2	< 1,156	4
CD8	> 23	5	> 712	7
	19 - 23	113	420 - 712	101
	< 19	1	< 420	1
CD19	> 16	1		
	10 - 16	92		
	< 10	4		
CD56	> 8	0		
	2 - 8	25		
	< 2	2		
CD56+16	> 9	2		
	5 - 9	58		
	< 5	4		
CD3 Average	> 85	5		
	74 - 85	97		
	< 74	2		
CD16	9 - 9	1		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 9,558	1
	7,265 - 9,558	68
	< 7,265	7
% Lymphs	> 39	5
	24 - 39	70
	< 24	1
Absolute Lymphs	> 3,354	5
	1,943 - 3,354	69
	< 1,943	2

Table 3. Participant Laboratory Results for the October 2004 Shipment

Donor Number 9 - Donor Status: HIV-antibody Negative

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	96 - 100	22		
	< 96	1		
CD14	> 1	2		
	0 - 1	21		
	< 0	0		
CD4	> 52	3	> 2,022	7
	46 - 52	115	1,363 - 2,022	100
	< 46	2	< 1,363	2
CD8	> 31	6	> 1,233	7
	27 - 31	113	806 - 1,233	99
	< 27	1	< 806	3
CD19	> 17	2		
	13 - 17	95		
	< 13	1		
CD56	> 5	0		
	2 - 5	25		
	< 2	2		
CD56+16	> 5	0		
	3 - 5	62		
	< 3	3		
CD3 Average	> 83	3		
	77 - 83	99		
	< 77	3		
CD16	6 - 6	1		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 10,359	1
	8,361 - 10,359	73
	< 8,361	2
% Lymphs	> 44	6
	31 - 44	70
	< 31	0
Absolute Lymphs	> 4,168	5
	2,849 - 4,168	70
	< 2,849	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 10 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	92 - 100	22		
	< 92	1		
CD14	> 2	0		
	0 - 2	23		
	< 0	0		
CD4	> 21	5	> 271	5
	15 - 21	115	154 - 271	102
	< 15	0	< 154	2
CD8	> 61	7	> 813	6
	43 - 61	112	421 - 813	100
	< 43	1	< 421	3
CD19	> 29	0		
	8 - 29	92		
	< 8	6		
CD56	> 4	3		
	0 - 4	24		
	< 0	0		
CD56+16	> 10	2		
	2 - 10	63		
	< 2	0		
CD3 Average	> 82	8		
	62 - 82	96		
	< 62	1		
CD16	12 - 12	1		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 3,777	3
	2,769 - 3,777	70
	< 2,769	3
% Lymphs	> 46	4
	30 - 46	71
	< 30	1
Absolute Lymphs	> 1,501	3
	960 - 1,501	71
	< 960	2

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

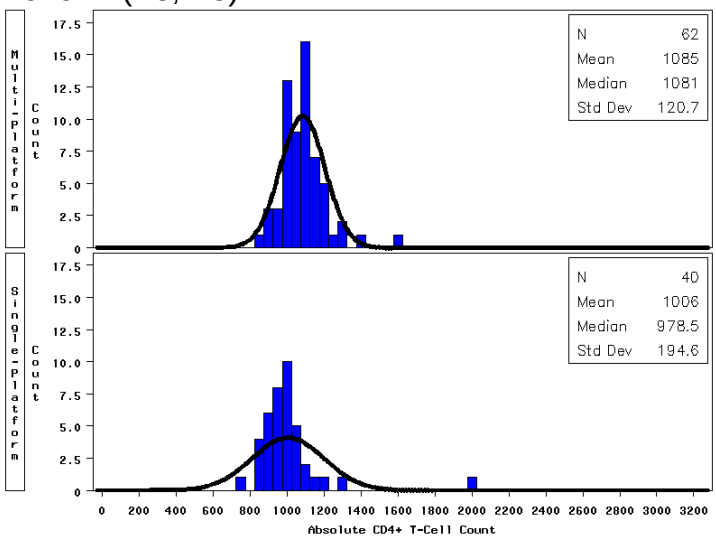
Figure 6. Absolute CD4$^+$ T-cell counts, by donor, by method

Description of graphs depicted below:

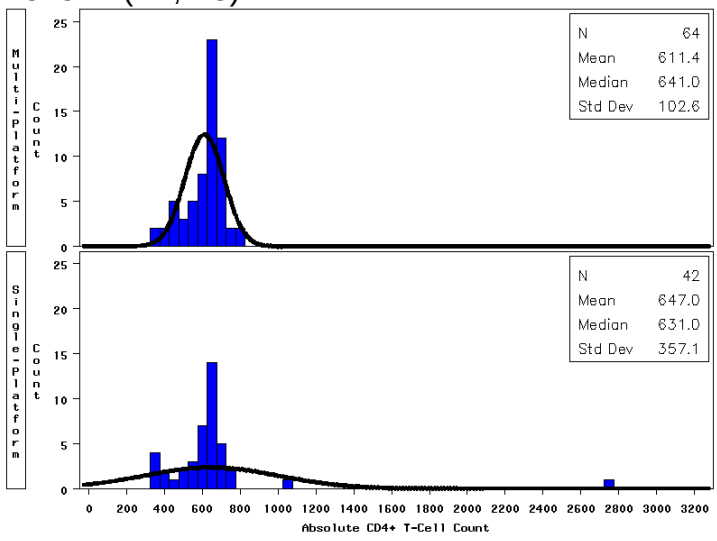
- Upper plot -- absolute CD4$^+$ T-cell count derived using dual-platform methods.
- Lower plot -- absolute CD4$^+$ T-cell count derived using single-platform methods.
- X-axis -- range of absolute CD4$^+$ T-cell counts.
- Y-axis --number of laboratories obtaining a particular CD4$^+$ T-cell count.

Absolute CD4$^+$ T-Cell Count

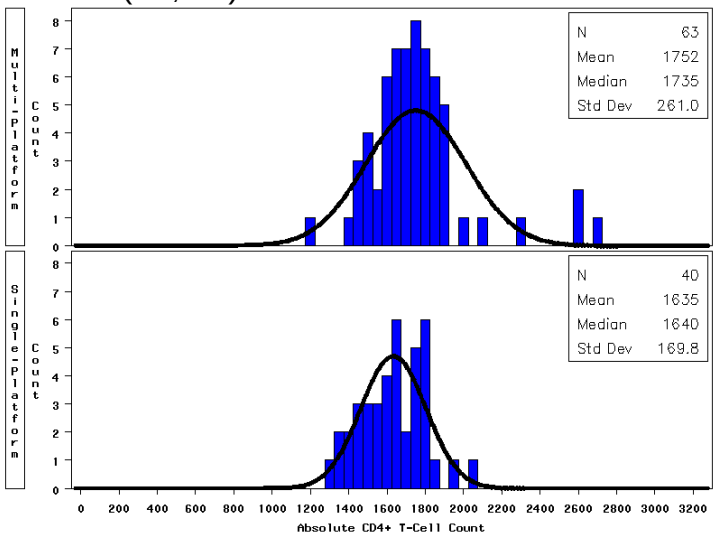
Donor 1 (A3, B5)



Donor 2 (A2, A5)



Donor 3 (A1, B2)



Donor 4 (B3, B4)

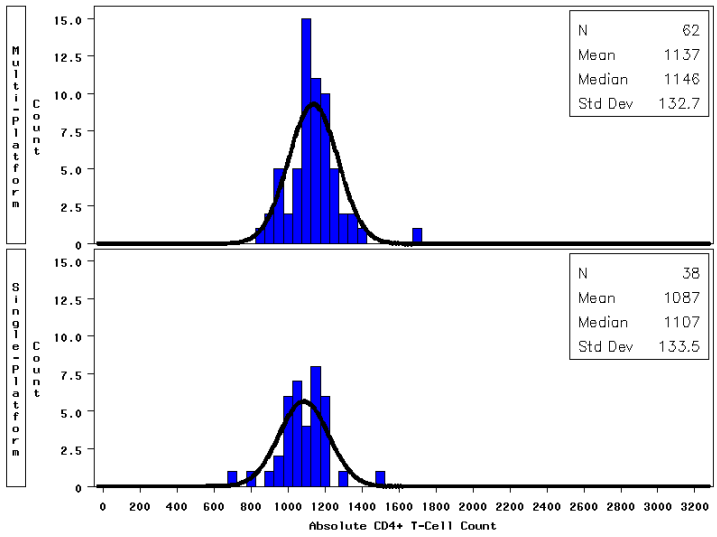
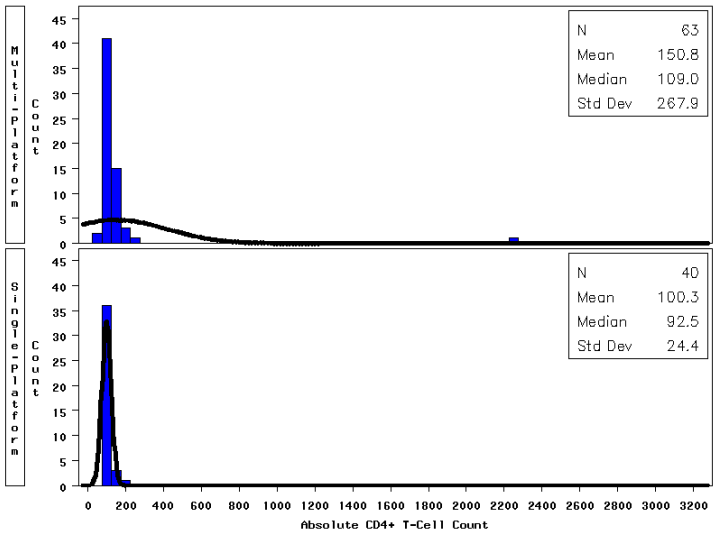
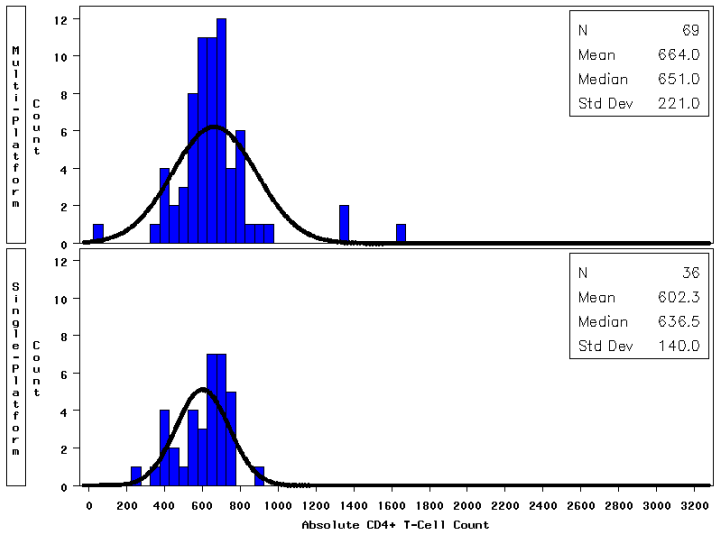


Figure 6, continued. **Absolute CD4$^+$ T-cell counts, by donor, by method**

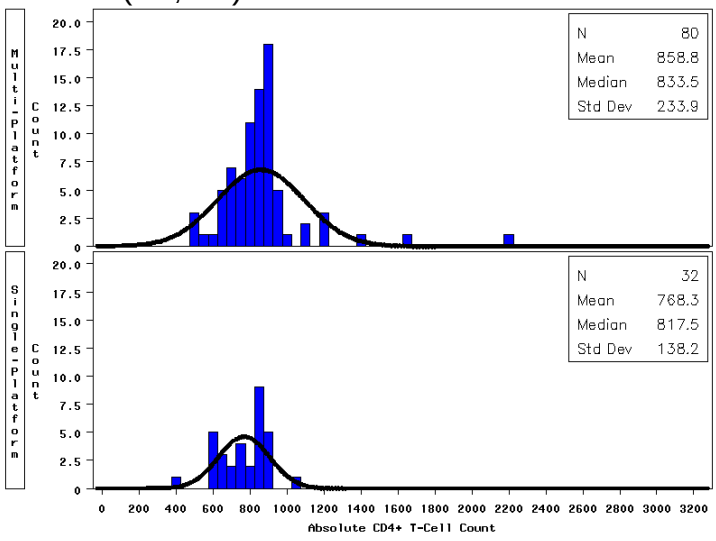
Donor 5 (A4, B1)



Donor 6 (D1, D3)



Donor 7 (C3, C5)



Donor 8 (C2, D5)

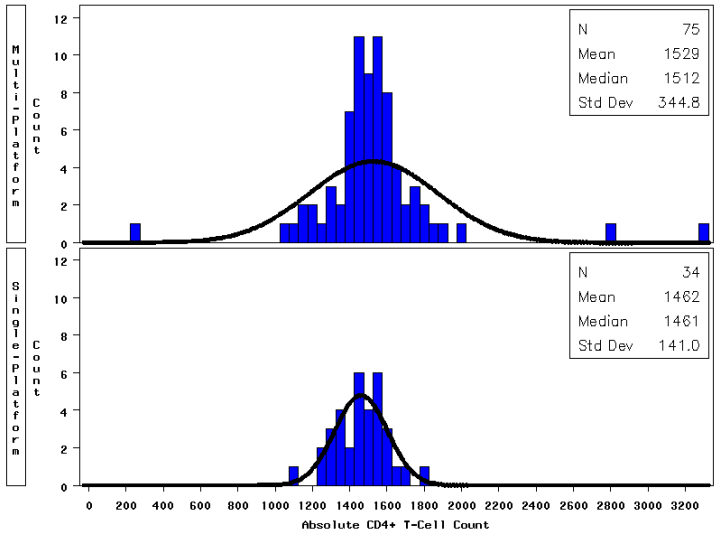
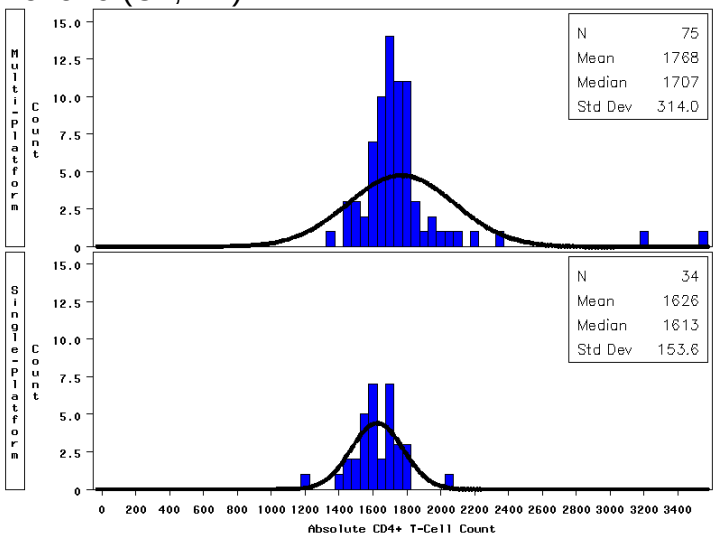
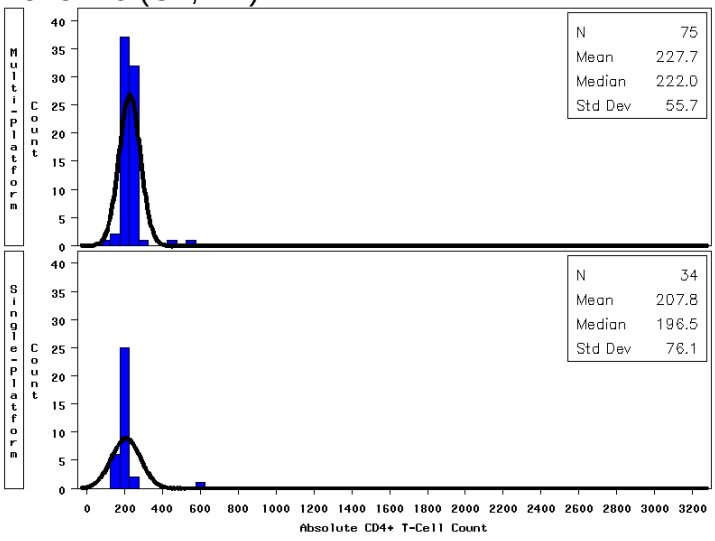


Figure 6, continued. **Absolute CD4$^+$ T-cell counts, by donor, by method**

Donor 9 (C1, D2)



Donor 10 (C4, D4)



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 10 (e.g., the laboratory reported a WBC of 1110 and a lymphocyte percent of 29, which should have yielded a lymphocyte count of 322; however, the laboratory reported a lymphocyte count of 3185).
- Two other laboratories reported lymphocyte counts on one of the specimens they tested that were in error by a factor of 2.
- In total, six 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.

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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	1085	1006	p=0.025	Significant*
2	611	647	p=0.533	Not Significant
3	1752	1635	p=0.007	Significant
4	1137	1087	p=0.066	Not Significant
5	151	100	p=0.142	Not Significant
6	664	602	p=0.084	Not Significant
7	859	768	p=0.013	Significant
8	1529	1462	p=0.150	Not Significant
9	1768	1626	p=0.002	Significant
10	228	208	p=0.021	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

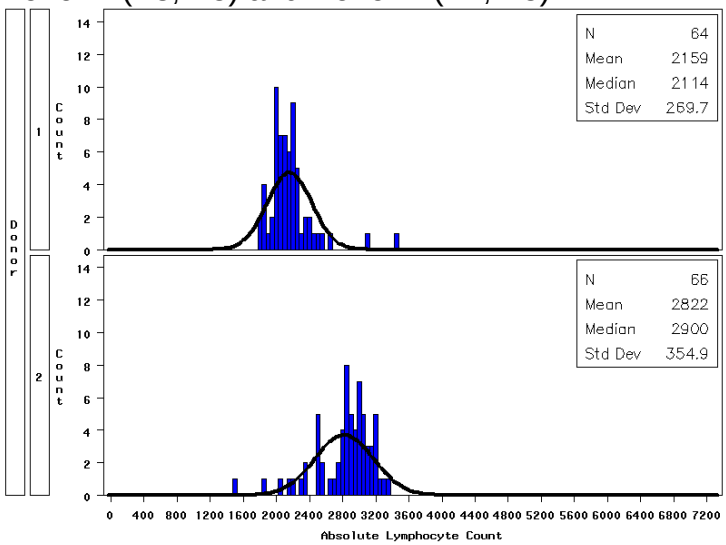
Figure 7. Absolute Lymphocyte Counts, by Donor

Description of graphs depicted below:

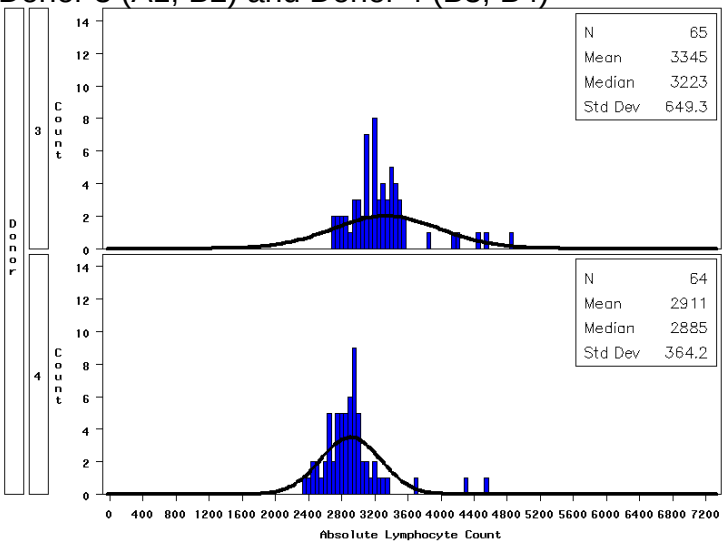
- Upper plot -- absolute lymphocyte count for one donor.
- Lower plot -- absolute lymphocyte count for another donor.
- The identity of the donors can be read in the bars on the left hand side of the plot.
- X-axis -- range of absolute lymphocyte counts.
- Y-axis -- number of laboratories obtaining a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

Absolute Lymphocyte Count (Hematology Instrument)

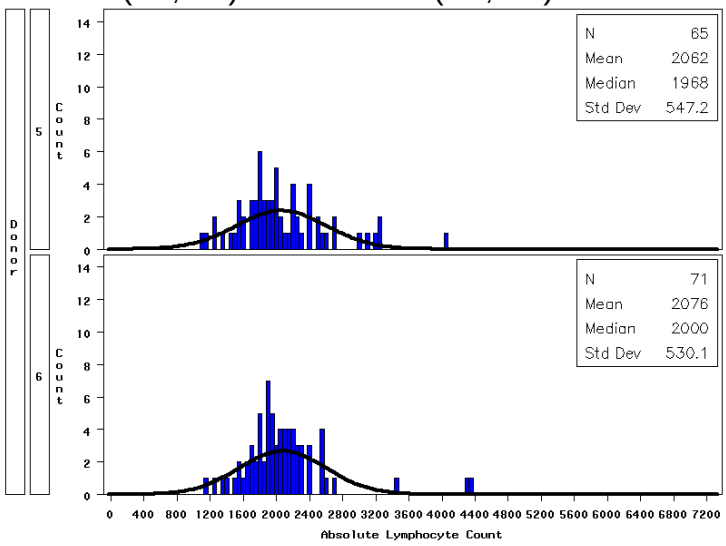
Donor 1 (A3, B5) and Donor 2 (A2, A5)



Donor 3 (A1, B2) and Donor 4 (B3, B4)



Donor 5 (A4, B1) and Donor 6 (D1, D3)



Donor 7 (C3, C5) and Donor 8 (C2, D5)

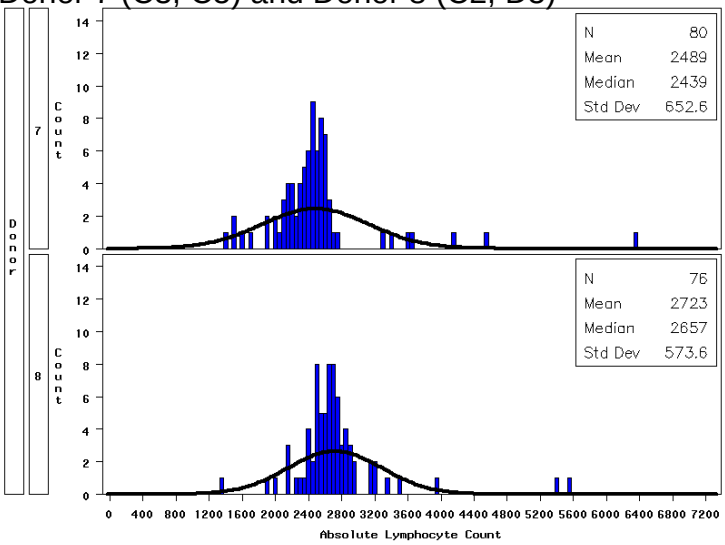


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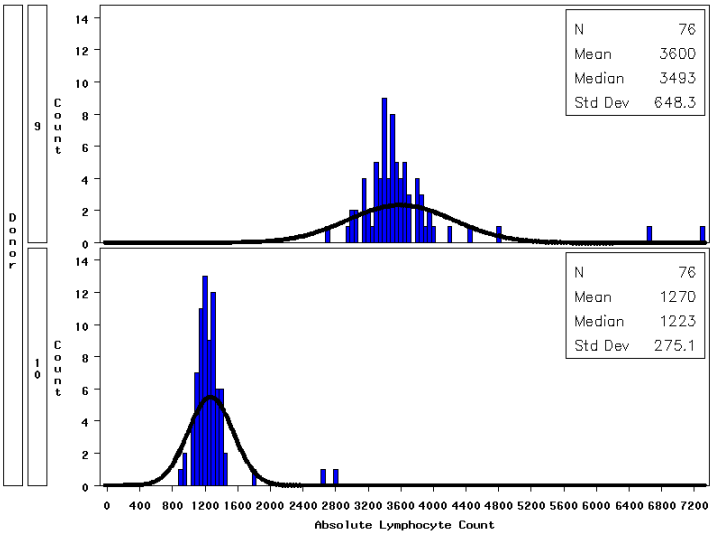
Figure 7, continued**. Absolute Lymphocyte Counts, by Donor**

Donor 9 (C1, D2) and Donor 10 (C4, D4)



Discussion

Effect of delayed shipments

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, 39 laboratories (65.0%) reported one or more results outside the established 95% confidence ranges.
- One laboratory reported 13 of 35 results (37.1%) submitted and another laboratory reported 19 of 50 results (38.0%) 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."

CDC Guidelines

Those laboratories performing $CD4^+$ T-cell determinations using a single-platform method are encouraged to follow the recently published CDC *Guidelines for Performing Single-Platform Absolute CD4+ T-Cell Determinations with CD45 Gating for Persons Infected with Human Immunodeficiency Virus* [MMWR 2003; 52(RR-2):1-13].