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 2004

CENTERS FOR DISEASE™
CONTROL AND PREVENTION

PUBLIC HEALTH PRACTICE PROGRAM OFFICE
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 2004

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Overview of April 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 April 13 and April 20, 2004.

Laboratory Response

Of the 274 laboratories receiving specimen panels, 256 (93.4%) reported testing results.

- Of the 18 nonreporting laboratories, one laboratory indicated they were no longer performing $CD4^+$ T-cell testing, and 17 provided no explanation.
- The majority of the laboratories (67.2%) reported their testing results using the online data entry system.

Significant Findings

The majority of the results (93.8%) returned by the laboratories participating in the April 2004 performance evaluation panel shipment were within the established 95% confidence limits.

- In particular, 92.1% of the absolute $CD4^+$ and 91.3% 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 multi-platform.

- The ranges of multi-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 58 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.

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 2004 Shipment Specimens

Panel Letter	Participant Laboratory	CDC Donor Number	Donor Information (HIV-1* status)
A	A1, A5	02	HIV-1 Antibody-Positive
	A2	01	HIV-1 Antibody-Negative
	A3	05	HIV-1 Antibody-Positive
	A4	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, C3	07	HIV-1 Antibody-Positive
	C2	10	HIV-1 Antibody-Positive
	C4	09	HIV-1 Antibody-Negative
	C5	08	HIV-1 Antibody-Negative
D	D1, D4	06	HIV-1 Antibody-Positive
	D2	10	HIV-1 Antibody-Positive
	D3	08	HIV-1 Antibody-Negative
	D5	09	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 April 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 multi-platform or single-platform.

- Multi-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 multi-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 232 (2.2%) of 10,360 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 (93.8%) returned by the laboratories participating in the April 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

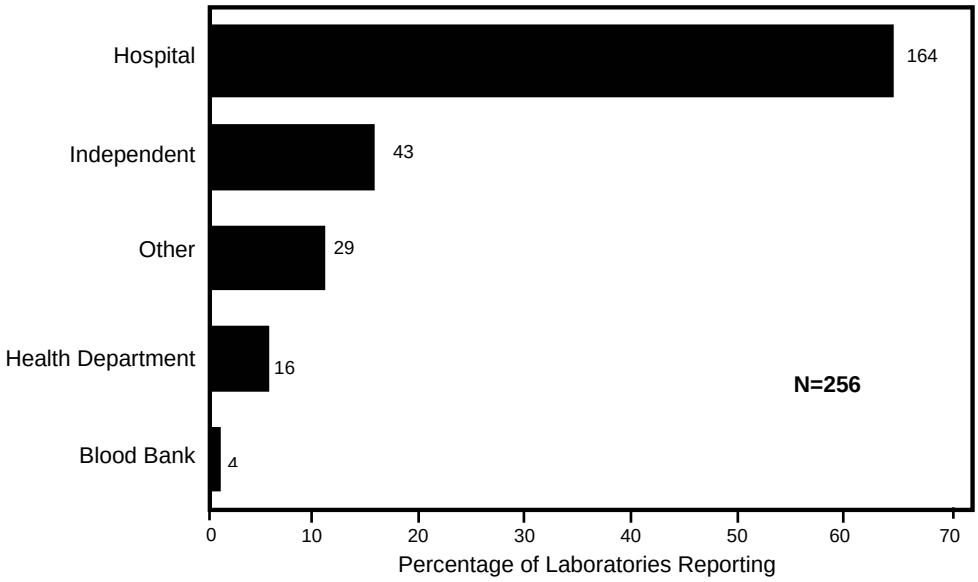
	Cell-Marker Percentage		Absolute Cell Counts			Hematology Results		
Cell Marker	Within 95% Confidence Limits	Outside 95% Confidence Limits	Within 95% Confidence Limits	Outside 95% Confidence Limits			Within 95% Confidence Limits	Outside 95% Confidence Limits
$CD3^+$	94.4%	5.6%				White Blood Cell Count	92.8%	7.2%
$CD4^+$	95.1%	4.9%	92.1%	7.9%		Lymphocyte Percentage	92.1%	7.9%
$CD8^+$	95.3%	4.7%	91.3%	8.7%		Absolute Lymphocyte Count	91.1%	8.9%
$CD14^+$	97.7%	2.3%						
$CD19^+$	95.8%	4.2%						
$CD45^+$	97.0%	3.0%						
$CD3^-/CD56^+$	99.0%	1.0%						
$CD3^-/CD(56+16)^+$	94.2%	5.8%						

Description of Laboratories, Methods, and Instruments

Types of laboratories

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

Figure 1 Types of Participant Laboratories



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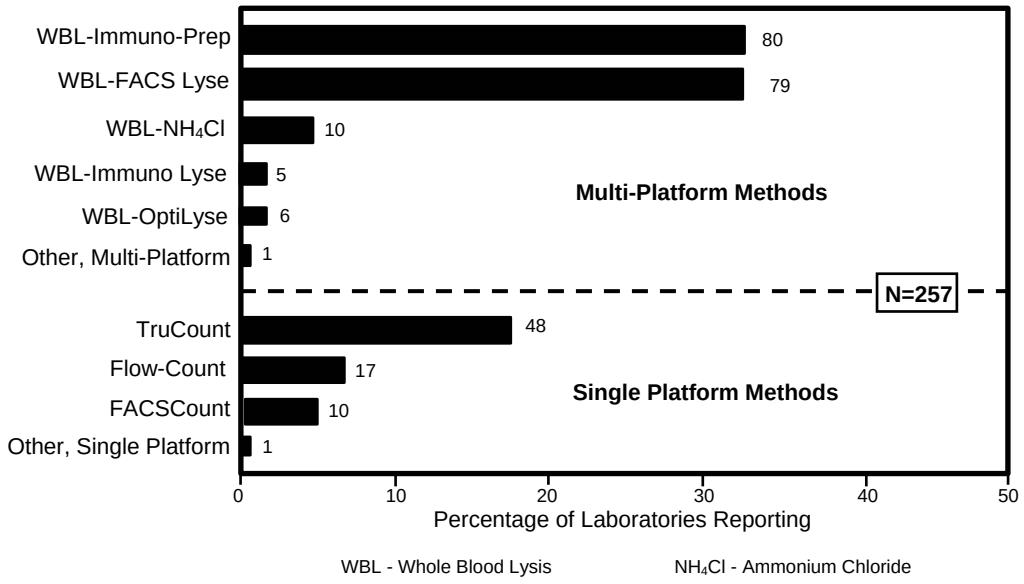
Description of Laboratories, Methods, and Instruments, Continued

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 multi-platform methods reported using a method of whole blood lysis to prepare specimens for $CD4^+$ T-cell (including 2 methods 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" multi-platform method was described as Cal-Lyse (CalTag). The "Other" single-platform method was described as Coulter Tetra-One

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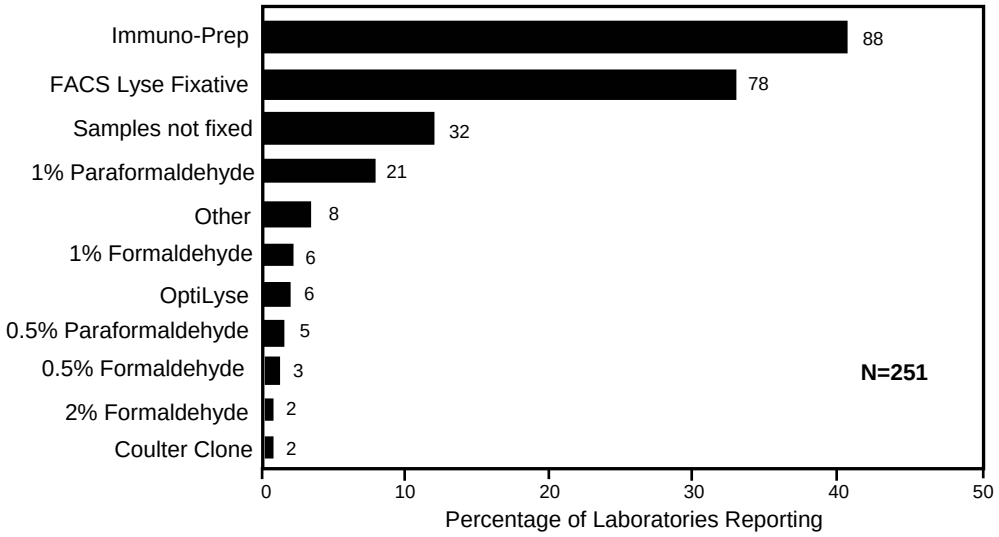
Specimen fixation methods

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, 32 (12.7%) of 251 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



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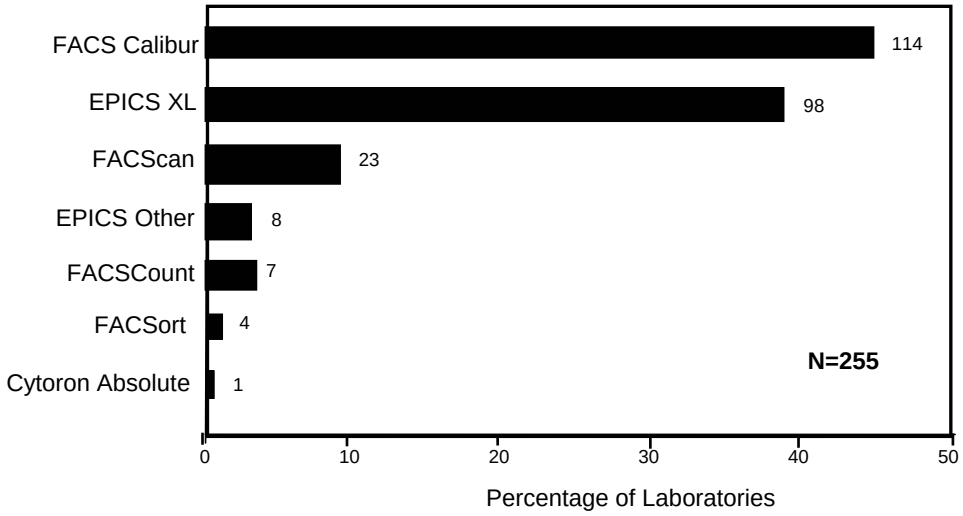
Description of Laboratories, Methods, and Instruments, Continued

Types of flow cytometers used

Figure 4 shows the types of flow cytometers used by the participant laboratories. The eight "Other" EPICS instruments were listed as Cytomics FC-500.

Figure 4 Types of Flow Cytometers Used



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

Among the 256 laboratories reporting results, 219 reported absolute cell counts.

- Of these, 144 (65.8%) of 219 used only a multi-platform method to derive marker-specific absolute cell counts.
- Seventy-four (33.8%) of 219 laboratories used only a single-platform method.
- One laboratory reported results using both single- and multi-platform methods.

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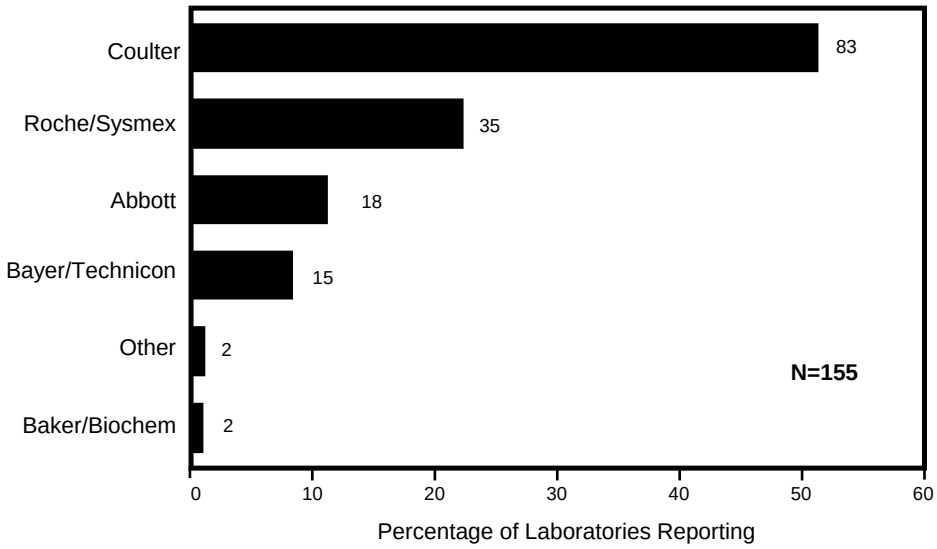
Description of Laboratories, Methods, and Instruments, Continued

Hematology Instruments used

Of the 256 participant laboratories, 155 (60.5%) 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 multi-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 multi-platform results is shown in Figure 7.

Table 3. Participant Laboratory Results for the April 2004 Shipment

Donor Number 1 (Specimens A2, B4) - Donor Status: HIV-antibody Negative

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	94 - 100	19		
	< 94	1		
CD14	> 1	1		
	0 - 1	19		
	< 0	0		
CD4	> 53	2	> 1,751	6
	46 - 53	114	1,101 - 1,751	104
	< 46	5	< 1,101	1
CD8	> 33	7	> 1,121	7
	28 - 33	112	682 - 1,121	100
	< 28	2	< 682	2
CD19	> 16	1		
	10 - 16	96		
	< 10	3		
$CD3^-/CD56^+$	> 5	1		
	2 - 5	29		
	< 2	0		
$CD3^-/CD56+16^+$	> 7	2		
	2 - 7	63		
	< 2	1		
CD3 Average	> 85	3		
	78 - 85	101		
	< 78	2		
$CD3^-/CD16^+$	5 - 5	1		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 10,051	2
	8,106 - 10,051	75
	< 8,106	2
% Lymphs	> 39	7
	27 - 39	72
	< 27	0
Absolute Lymphs	> 3,737	6
	2,282 - 3,737	73
	< 2,282	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 2 (Specimens A1, A5) - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	90 - 100	16		
	< 90	0		
CD14	> 2	1		
	0 - 2	15		
	< 0	0		
CD4	> 40	3	> 675	7
	31 - 40	111	342 - 675	107
	< 31	0	< 342	0
CD8	> 65	3	> 1,122	7
	52 - 65	106	545 - 1,122	104
	< 52	5	< 545	1
CD19	> 8	0		
	0 - 8	89		
	< 0	0		
$CD3^-/CD56^+$	> 3	0		
	1 - 3	34		
	< 1	0		
$CD3^-/CD56+16^+$	> 9	2		
	2 - 9	50		
	< 2	0		
CD3 Average	> 95	0		
	83 - 95	101		
	< 83	4		
$CD3^-/CD16^+$	5 - 9	2		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 6,588	1
	5,489 - 6,588	69
	< 5,489	4
% Lymphs	> 33	6
	18 - 33	68
	< 18	0
Absolute Lymphs	> 2,003	6
	1,061 - 2,003	70
	< 1,061	0

Table 3. Participant Laboratory Results for the April 2004 Shipment

Donor Number 3 (Specimens A4, B1) - Donor Status: HIV-antibody Negative

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	93 - 100	20		
	< 93	0		
CD14	> 3	0		
	0 - 3	20		
	< 0	0		
CD4	> 58	5	> 1,478	7
	49 - 58	112	928 - 1,478	100
	< 49	4	< 928	4
CD8	> 23	3	> 594	5
	19 - 23	115	357 - 594	101
	< 19	3	< 357	3
CD19	> 16	1		
	12 - 16	96		
	< 12	3		
$CD3^-/CD56^+$	> 11	0		
	4 - 11	30		
	< 4	0		
$CD3^-/CD56+16^+$	> 13	1		
	7 - 13	62		
	< 7	3		
CD3 Average	> 80	6		
	71 - 80	99		
	< 71	1		
$CD3^-/CD16^+$	12 - 12	1		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 8,227	2
	6,377 - 8,227	72
	< 6,377	5
% Lymphs	> 38	5
	25 - 38	74
	< 25	0
Absolute Lymphs	> 2,843	7
	1,771 - 2,843	71
	< 1,771	2

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 (Specimens B2, B3) - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	91 - 100	23		
	< 91	1		
CD14	> 3	2		
	0 - 3	22		
	< 0	0		
CD4	> 48	8	> 1,133	9
	39 - 48	118	471 - 1,133	98
	< 39	2	< 471	1
CD8	> 28	1	> 634	9
	22 - 28	124	291 - 634	96
	< 22	3	< 291	1
CD19	> 21	3		
	14 - 21	103		
	< 14	4		
$CD3^-/CD56^+$	> 12	0		
	7 - 12	26		
	< 7	0		
$CD3^-/CD56+16^+$	> 17	0		
	8 - 17	79		
	< 8	1		
CD3 Average	> 75	6		
	64 - 75	102		
	< 64	0		
$CD3^-/CD16^+$	0 - 0	0		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 7,130	2
	5,347 - 7,130	76
	< 5,347	6
% Lymphs	> 47	7
	15 - 47	76
	< 15	1
Absolute Lymphs	> 2,926	8
	972 - 2,926	75
	< 972	1

Table 3. Participant Laboratory Results for the April 2004 Shipment

Donor Number 5 (Specimens A3, B5) - 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	20		
	< 0	0		
CD4	> 6	2	> 148	4
	3 - 6	118	48 - 148	101
	< 3	0	< 48	5
CD8	> 43	8	> 994	4
	28 - 43	111	539 - 994	98
	< 28	1	< 539	6
CD19	> 14	1		
	6 - 14	97		
	< 6	1		
$CD3^-/CD56^+$	> 50	0		
	18 - 50	28		
	< 18	1		
$CD3^-/CD56+16^+$	> 55	2		
	38 - 55	61		
	< 38	3		
CD3 Average	> 49	9		
	32 - 49	96		
	< 32	1		
$CD3^-/CD16^+$	60 - 60	1		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 4,608	1
	2,421 - 4,608	72
	< 2,421	5
% Lymphs	> 75	3
	48 - 75	71
	< 48	4
Absolute Lymphs	> 2,941	2
	1,391 - 2,941	72
	< 1,391	5

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 (Specimens D1, D4) - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	95 - 100	24		
	< 95	0		
CD14	> 2	0		
	0 - 2	24		
	< 0	0		
CD4	> 50	5	> 1,582	8
	44 - 50	112	864 - 1,582	96
	< 44	1	< 864	2
CD8	> 28	5	> 857	8
	23 - 28	111	459 - 857	94
	< 23	0	< 459	2
CD19	> 30	2		
	16 - 30	96		
	< 16	4		
$CD3^-/CD56^+$	> 3	0		
	0 - 3	24		
	< 0	0		
$CD3^-/CD56+16^+$	> 7	8		
	0 - 7	64		
	< 0	0		
CD3 Average	> 78	7		
	70 - 78	92		
	< 70	1		
$CD3^-/CD16^+$	2 - 23	2		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 8,145	0
	6,955 - 8,145	68
	< 6,955	4
% Lymphs	> 47	4
	25 - 47	66
	< 25	2
Absolute Lymphs	> 3,544	4
	1,889 - 3,544	64
	< 1,889	4

Table 3. Participant Laboratory Results for the April 2004 Shipment

Donor Number 7 (Specimens C1, C3) - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	96 - 100	28		
	< 96	0		
CD14	> 1	0		
	0 - 1	24		
	< 0	0		
CD4	> 32	2	> 576	10
	27 - 32	124	335 - 576	103
	< 27	3	< 335	1
CD8	> 51	2	> 939	10
	45 - 51	125	556 - 939	100
	< 45	1	< 556	4
CD19	> 10	4		
	6 - 10	94		
	< 6	1		
$CD3^-/CD56^+$	> 13	0		
	7 - 13	32		
	< 7	0		
$CD3^-/CD56+16^+$	> 16	0		
	10 - 16	58		
	< 10	5		
CD3 Average	> 82	2		
	74 - 82	108		
	< 74	1		
$CD3^-/CD16^+$	10 - 11	2		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 8,760	2
	7,643 - 8,760	65
	< 7,643	3
% Lymphs	> 25	4
	15 - 25	66
	< 15	0
Absolute Lymphs	> 2,290	4
	1,057 - 2,290	65
	< 1,057	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 (Specimens C5, D3) - Donor Status: HIV-antibody Negative

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	96 - 100	24		
	< 96	2		
CD14	> 1	0		
	0 - 1	24		
	< 0	0		
CD4	> 54	3	> 1,239	5
	47 - 54	120	511 - 1,239	103
	< 47	1	< 511	2
CD8	> 30	2	> 696	7
	26 - 30	120	267 - 696	100
	< 26	1	< 267	2
CD19	> 15	2		
	9 - 15	97		
	< 9	2		
$CD3^-/CD56^+$	> 9	0		
	3 - 9	27		
	< 3	1		
$CD3^-/CD56+16^+$	> 11	1		
	4 - 11	65		
	< 4	2		
CD3 Average	> 83	4		
	76 - 83	101		
	< 76	1		
$CD3^-/CD16^+$	6 - 7	2		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 7,069	3
	5,971 - 7,069	64
	< 5,971	4
% Lymphs	> 40	6
	18 - 40	64
	< 18	1
Absolute Lymphs	> 2,840	4
	864 - 2,840	65
	< 864	2

Table 3. Participant Laboratory Results for the April 2004 Shipment

Donor Number 9 (Specimens C4, D5) - Donor Status: HIV-antibody Negative

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	96 - 100	25		
	< 96	1		
CD14	> 2	1		
	0 - 2	23		
	< 0	0		
CD4	> 58	9	> 2,257	5
	49 - 58	113	1,377 - 2,257	101
	< 49	1	< 1,377	4
CD8	> 24	2	> 915	4
	20 - 24	117	586 - 915	101
	< 20	3	< 586	4
CD19	> 26	1		
	12 - 26	94		
	< 12	5		
$CD3^-/CD56^+$	> 3	0		
	0 - 3	28		
	< 0	0		
$CD3^-/CD56+16^+$	> 8	3		
	0 - 8	64		
	< 0	0		
CD3 Average	> 81	6		
	73 - 81	99		
	< 73	0		
$CD3^-/CD16^+$	1 - 1	2		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 7,601	2
	6,503 - 7,601	66
	< 6,503	3
% Lymphs	> 58	2
	41 - 58	67
	< 41	2
Absolute Lymphs	> 4,183	2
	2,767 - 4,183	66
	< 2,767	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 10 (Specimens C2, D2) - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	94 - 100	24		
	< 94	2		
CD14	> 2	0		
	0 - 2	24		
	< 0	0		
CD4	> 8	4	> 134	4
	5 - 8	119	56 - 134	104
	< 5	0	< 56	2
CD8	> 69	4	> 1,173	4
	61 - 69	117	698 - 1,173	100
	< 61	1	< 698	5
CD19	> 14	2		
	8 - 14	96		
	< 8	2		
$CD3^-/CD56^+$	> 9	0		
	3 - 9	28		
	< 3	0		
$CD3^-/CD56+16^+$	> 13	4		
	4 - 13	62		
	< 4	1		
CD3 Average	> 84	4		
	76 - 84	100		
	< 76	1		
$CD3^-/CD16^+$	5 - 6	2		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 4,296	2
	3,102 - 4,296	68
	< 3,102	1
% Lymphs	> 49	3
	33 - 49	66
	< 33	2
Absolute Lymphs	> 1,885	3
	1,155 - 1,885	66
	< 1,155	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. multi-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 multi-platform ranges were larger than the corresponding single-platform ranges for both $CD4^+$ and $CD8^+$ absolute T-cell counts.

Continued on next page

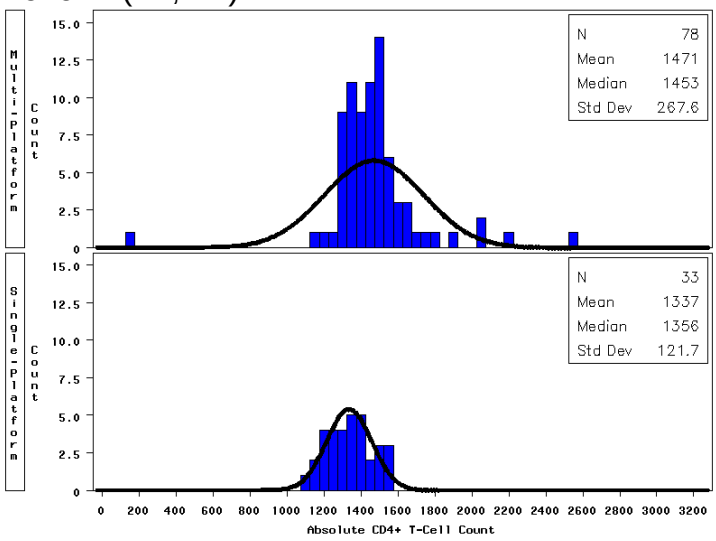
Figure 6. Absolute CD4$^+$ T-cell counts, by donor, by method

Description of graphs depicted below:

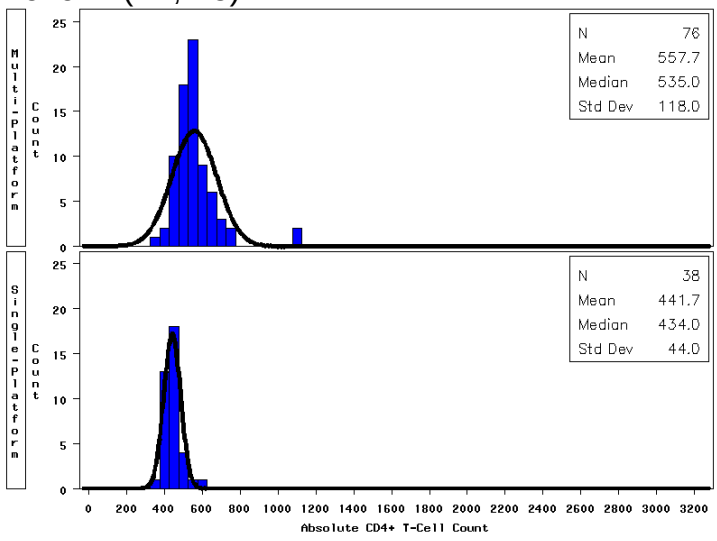
- Upper plot -- absolute CD4$^+$ T-cell count derived using multi-platform methods.
- Lower plot -- absolute CD4$^+$ T-cell count derived using single-platform methods
- X-axis -- range of absolute CD4$^+$ T-cell counts.
- Y-axis --number of laboratories obtaining a particular CD4$^+$ T-cell count.

Absolute CD4$^+$ T-Cell Count

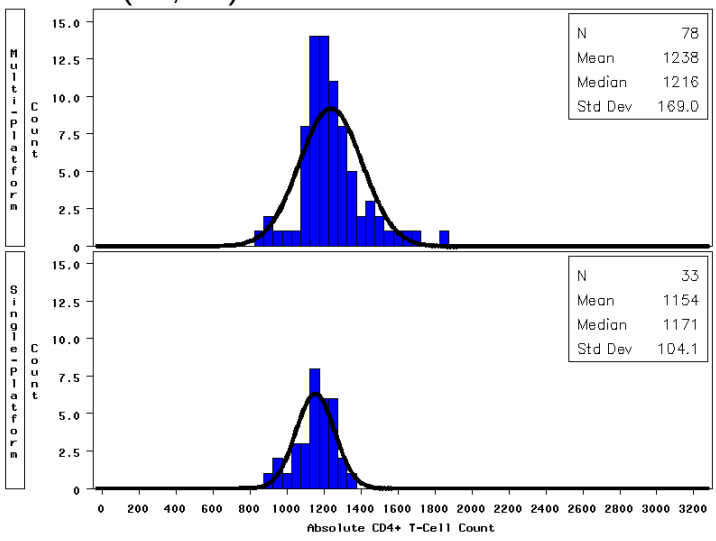
Donor 1 (A2, B4)



Donor 2 (A1, A5)



Donor 3 (A4, B1)



Donor 4 (B2, B3)

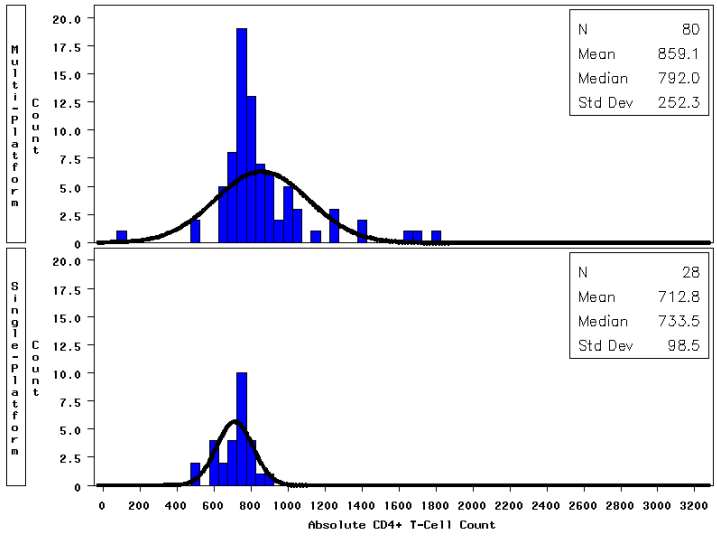
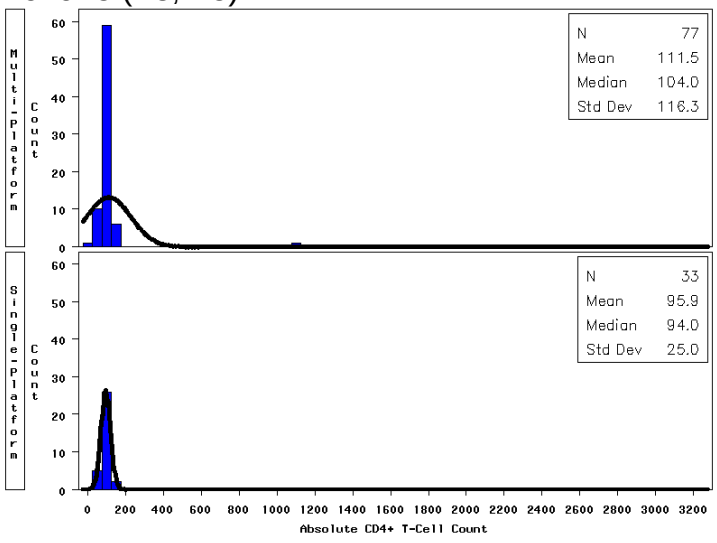
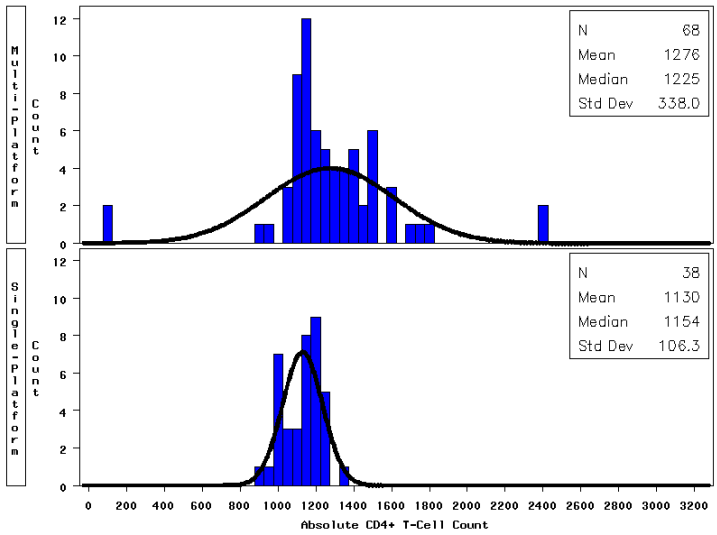


Figure 6 continued. **Absolute CD4$^+$ T-cell counts, by donor, by method**

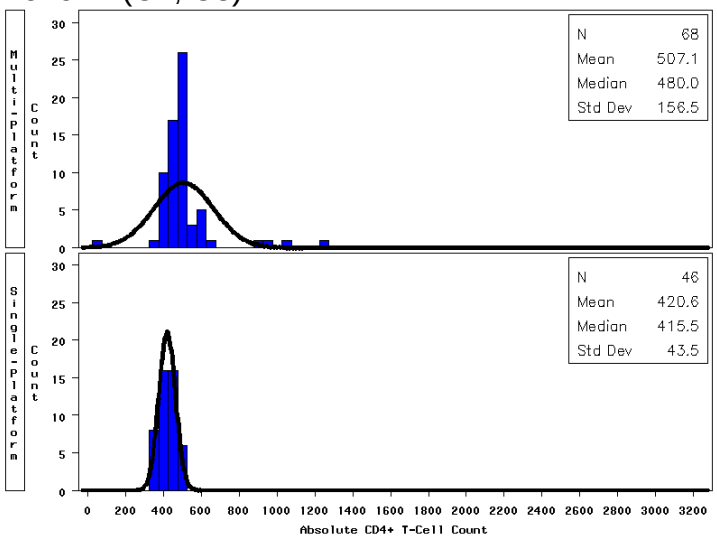
Donor 5 (A3, B5)



Donor 6 (D1, D4)



Donor 7 (C1, C3)



Donor 8 (C5, D3)

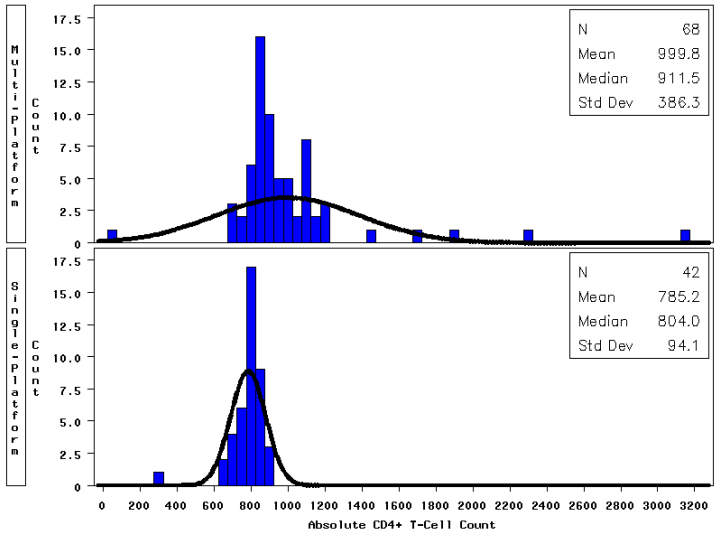
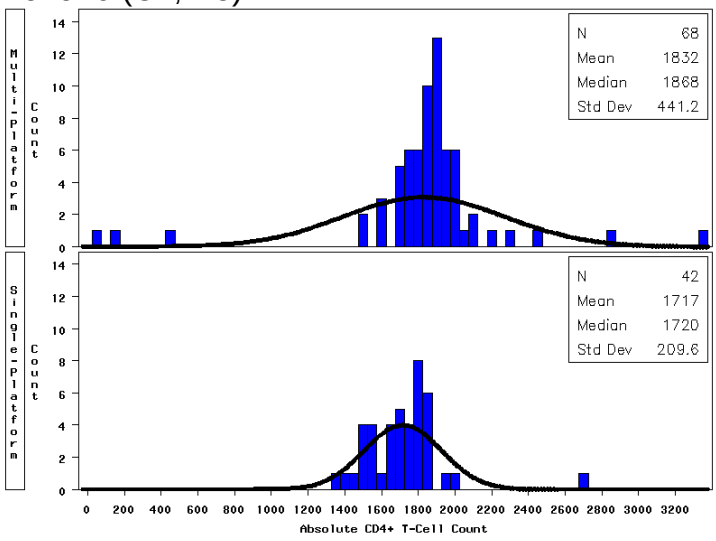
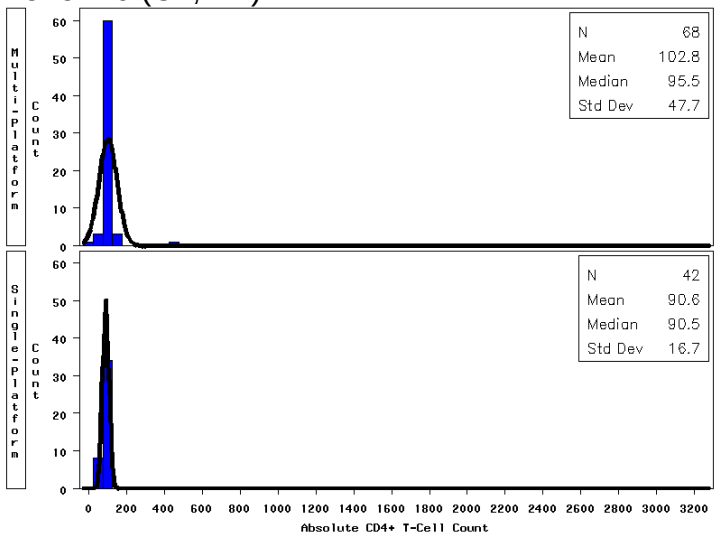


Figure 6 continued. **Absolute CD4$^+$ T-cell counts, by donor, by method**

Donor 9 (C4, D5)



Donor 10 (C2, D2)



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 all five 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 7020 and a lymphocyte percent of 47, which should have yielded a lymphocyte count of 3299; however, the laboratory reported a lymphocyte count of 333).
- Nine 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. Of the nine, three laboratories inaccurately calculated lymphocyte counts (greater than 5% difference between true and reported) on all 5 specimens tested.

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 multi-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 significant.

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

Donor	Multi-platform mean CD4 value	Single-platform mean CD4 value	p value	Significance
1	1471	1337	p=0.0004	Significant*
2	557	441	p=<0.0001	Significant
3	1238	1154	p=0.0020	Significant
4	859	713	p=<0.0001	Significant
5	112	96	p=0.2669	Not Significant
6	1276	1130	p=0.0015	Significant
7	507	421	p=<0.0001	Significant
8	1000	785	p=<0.0001	Significant
9	1832	1717	p=0.0688	Not Significant
10	103	91	p=0.0586	Not Significant
* Significant if p-value is <0.05				

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

The ranges of multi-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

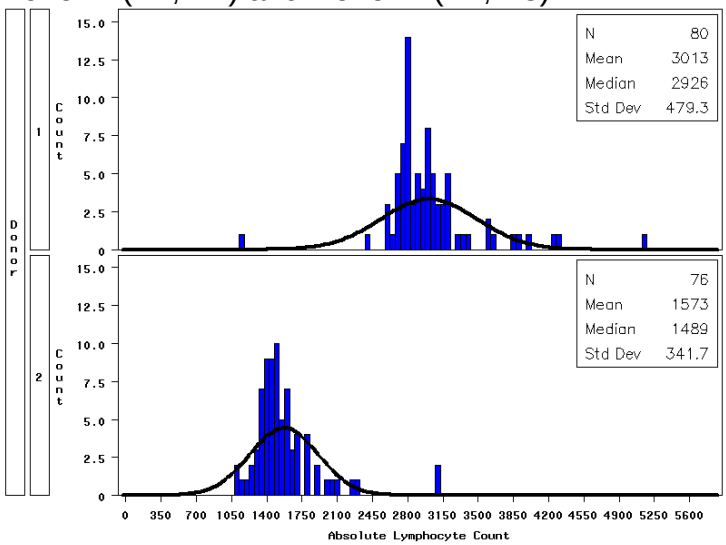
Figure 7 Absolute Lymphocyte Counts, by Donor

Description of graphs depicted below:

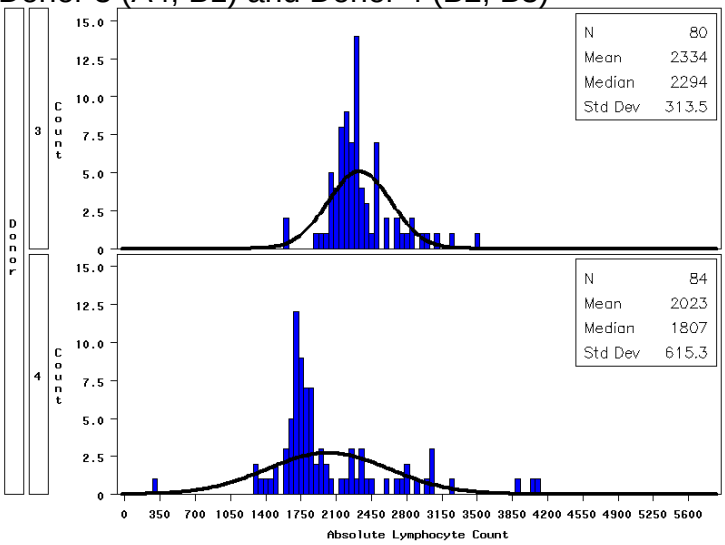
- Upper plot -- absolute lymphocyte count for one donor.
- Lower plot -- absolute lymphocyte count for another donor.
- The identity of the donors can be read in the bars on the left hand side of the plot.
- X-axis -- range of absolute lymphocyte counts.
- Y-axis -- number of laboratories obtaining a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

Absolute Lymphocyte Count (Hematology Instrument)

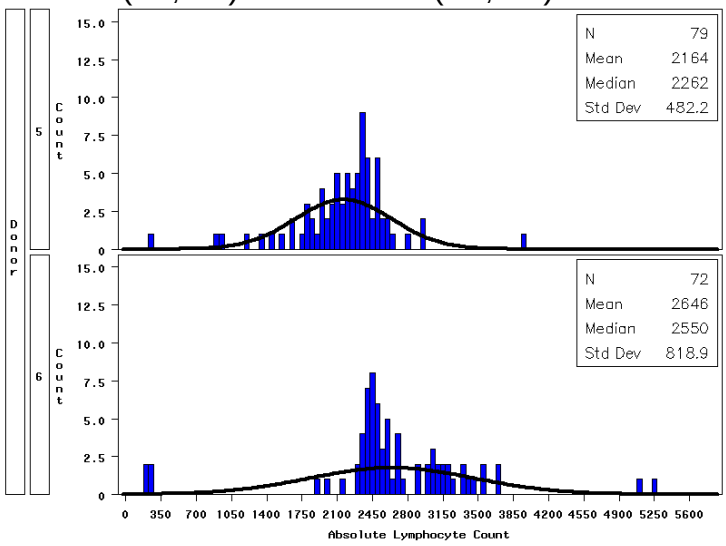
Donor 1 (A2, B4) and Donor 2 (A1, A5)



Donor 3 (A4, B1) and Donor 4 (B2, B3)



Donor 5 (A3, B5) and Donor 6 (D1, D4)



Donor 7 (C1, C3) and Donor 8 (C5, D3)

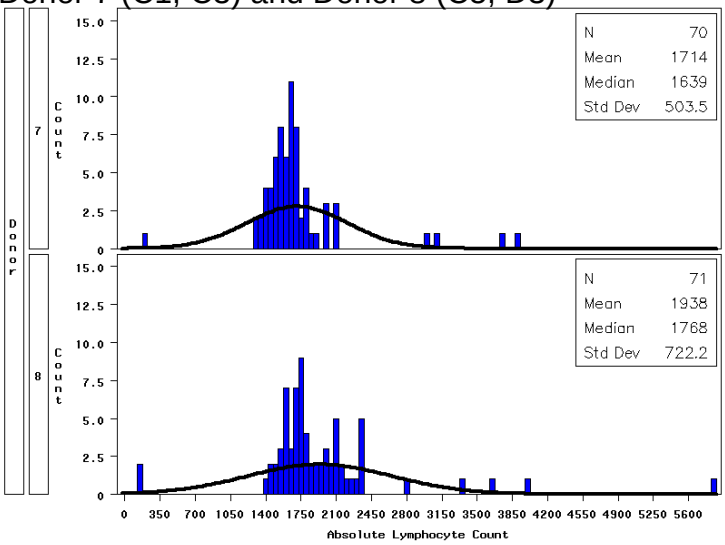


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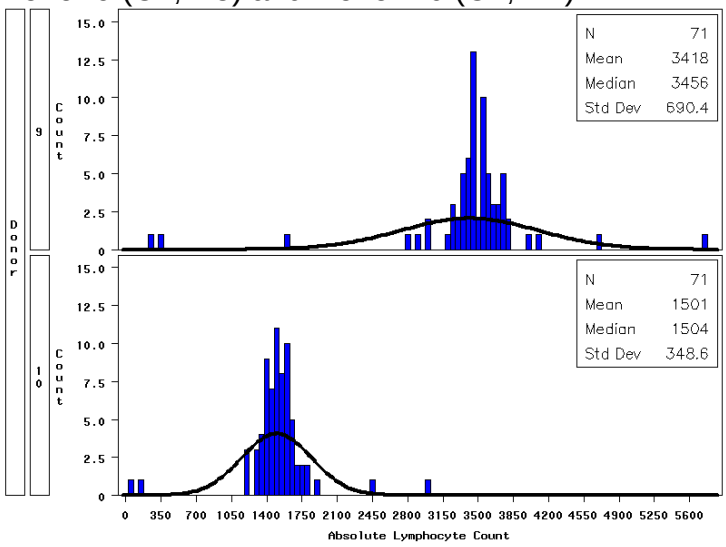
Figure 7 continued**. Absolute Lymphocyte Counts, by Donor**

Donor 9 (C4, D5) and Donor 10 (C2, D2)



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 58 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 58 laboratories reporting specimen preparation delays, 34 laboratories reported one or more results outside the established 95% confidence ranges.
- One laboratory reported 29 results 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 multi-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 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 a 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].