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 2003

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 2003

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Centers for Disease Control and Prevention
Model Performance Evaluation Program for $CD4^+$ T-Cell Determinations

Table 1. Donor Identification for April 2003 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	03	HIV-1 Antibody-Negative
	A3	01	HIV-1 Antibody-Negative
	A5	05	HIV-1 Antibody-Positive
B	B1	01	HIV-1 Antibody-Negative
	B2	03	HIV-1 Antibody-Negative
	B3	05	HIV-1 Antibody-Positive
	B4, B5	04	HIV-1 Antibody-Positive
C	C1, C5	07	HIV-1 Antibody-Positive
	C2	08	HIV-1 Antibody-Negative
	C3	10	HIV-1 Antibody-Positive
	C4	09	HIV-1 Antibody-Negative
D	D1	08	HIV-1 Antibody-Negative
	D2, D4	06	HIV-1 Antibody-Positive
	D3	09	HIV-1 Antibody-Negative
	D5	10	HIV-1 Antibody-Positive

* Human immunodeficiency virus type 1

Analysis of the April 2003 Performance Evaluation Testing Results for $CD4^+$ T-Cell Determinations Program Reported to the Centers for Disease Control and Prevention by Participating Laboratories

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 after they tested the $CD4^+$ T-cell determination ($CD4^+$T-cell) performance evaluation specimens sent on April 8 and April 15, 2003. Of the 280 laboratories receiving specimen panels, 256 (91.4%) reported testing results. Of the 24 nonreporting laboratories, two returned results too late to be included in this data analysis and 22 provided no explanation.

Significant Findings

- 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.

- The percentage of laboratories using single-platform methods, rather than multi-platform methods, to derive absolute $CD4^+$ and $CD8^+$ T-cell counts generally increased over the past five years, and has stabilized at around 30% for the past three survey periods (April 2002, September 2002, April 2003).

- 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. In spite of receiving preshipment letters outlining when to expect receipt of the MPEP $CD4^+$ PE specimens, 34 (13.3%) of the 256 participant laboratories reported they did not process the specimens on the day they were received.

- Only 17 Health Department laboratories participated in the April 2003 shipment. Most of the Nation's capability for performing $CD4^+$ T-cell determinations appears to reside with hospital and independent laboratories. Presumably, most State and local Health Departments rely on hospital and independent laboratories to monitor CD4 T-cell levels in HIV-infected individuals receiving Government supported anti-retroviral therapy.

Materials and Methods

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. Table 1, page 4, contains the specimen numbers and donor information for each performance evaluation specimen.

Laboratories were notified a month in advance of the date they would be receiving specimens. An air-bill tracking number was included in these preshipment letters, enabling the laboratories to locate the specimens in the event the shipment was not received by noon on the scheduled date of their receipt. These notifications also allowed the laboratories to minimize within-

institution delivery delays. Participant laboratories were instructed to process and test the MPEP $CD4^+$ T-cell specimens as they would patient specimens routinely received by their laboratory.

The result reporting booklet used for the April 2003 specimen shipment was designed to be consistent with the CDC guidelines for $CD4^+$ T-cell testing (MMWR, vol. 46, no. RR-2, January 10, 1997). Laboratories were encouraged to use these guidelines in performing $CD4^+$ T-cell determinations on patient specimens. According to these guidelines, specimens should be processed for hematologic testing and flow cytometric immunophenotyping within 30 hours of collection.

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).

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), all results for $CD4^+$ and $CD8^+$ absolute cell counts were grouped. These results were used to calculate 95% confidence limits for each donor and cell marker using the SAS procedure PROC GLM. Before calculation, data were analyzed for possible outliers. Only 235 (2.3%) of 10,357 results were considered to be outliers. These outlier results were removed before the 95% confidence limits shown in Table 3 were calculated. However, 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.

Summary of Results

In general, most laboratories performed well on the donor specimens in the April 2003 shipment. 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 count, lymphocyte percentage, and absolute lymphocyte count are shown in Table 2.

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
$CD3^+$	93.6%	6.4%				White Blood Cell Count	93.6%	6.4%
$CD4^+$	94.6%	5.4%	92.8%	7.2%		Lymphocyte Percentage	92.6%	7.4%
$CD8^+$	95.0%	5.0%	92.2%	7.8%		Absolute Lymphocyte Count	90.6%	9.4%
$CD14^+$	97.4%	2.6%						
$CD19^+$	94.1%	5.9%						
$CD45^+$	95.2%	4.8%						
$CD56^+$	92.8%	7.2%						
$CD(56+16)^+$	93.4%	6.6%						

The types of laboratories participating in the April 2003 $CD4^+$ T-cell determinations shipment are shown in Figure 1.

Figure 1. Primary classification of laboratories participating in the April 2003 shipment.

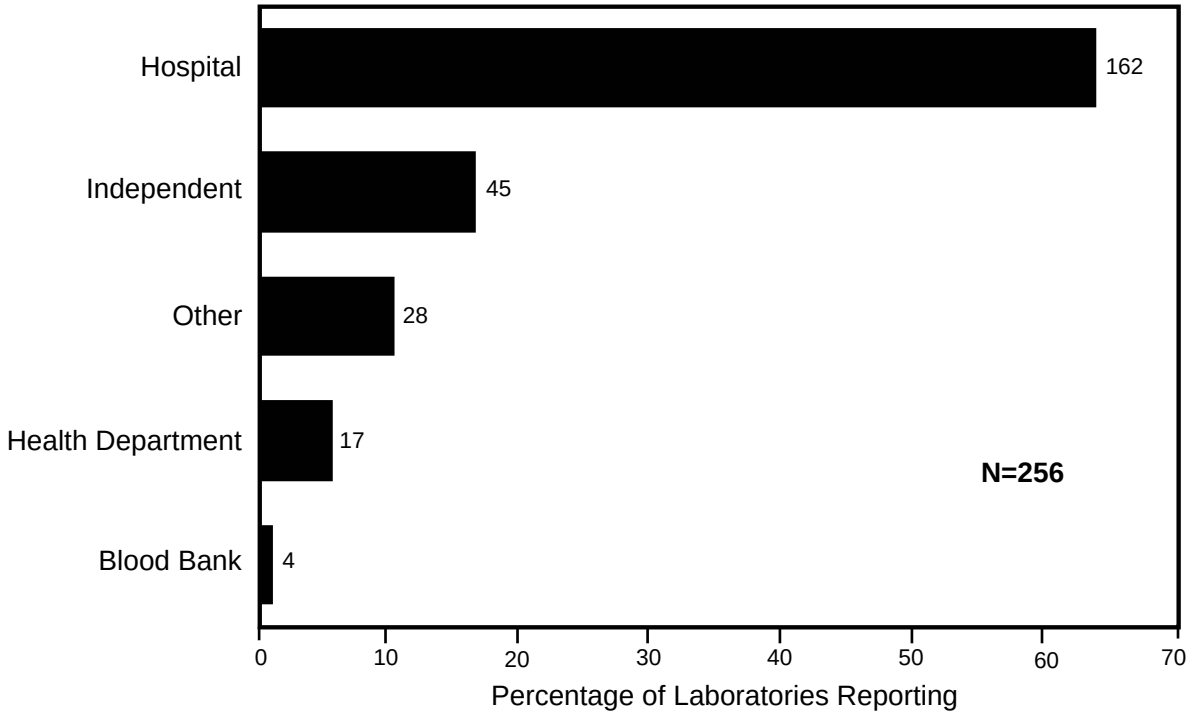
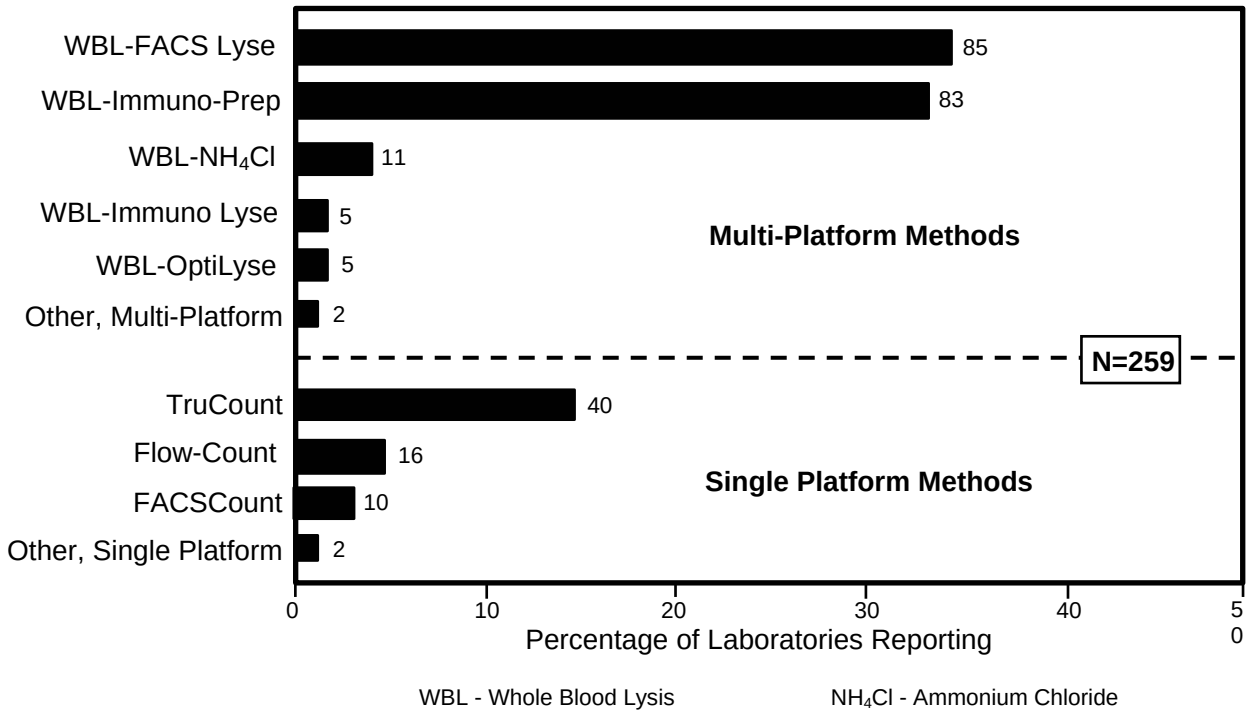


Figure 2 shows the methods used by the laboratories to prepare specimens for $CD4^+$ T-cell determinations. All of the laboratories performing multi-platform methods reported using a method of whole blood lysis to prepare specimens for $CD4^+$ T-cell (including 2 methods described as "Other"). The frequency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

Figure 2. Methods used to prepare specimens for $CD4^+$ T-cell determinations, reported by participant laboratories to CDC for the April 2003 shipment.



"Other" multi-platform methods were described as Coulter Prep Plus 2 and Cal-Lyse (CalTag). "Other" single-platform methods were described as Coulter HMX.

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, 33 (12.4%) of 267, 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.

Figure 3. Methods used to fix specimens for $CD4^+$ T-cell determinations, reported by participant laboratories to CDC for the April 2003 shipment.

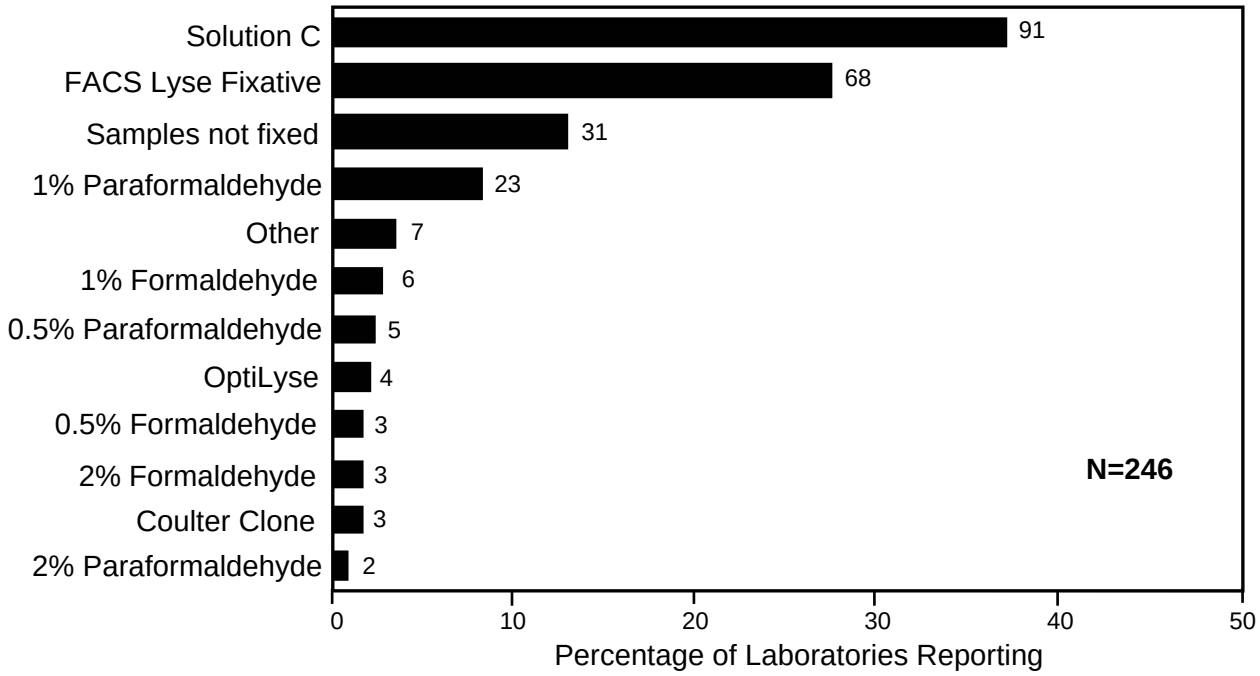
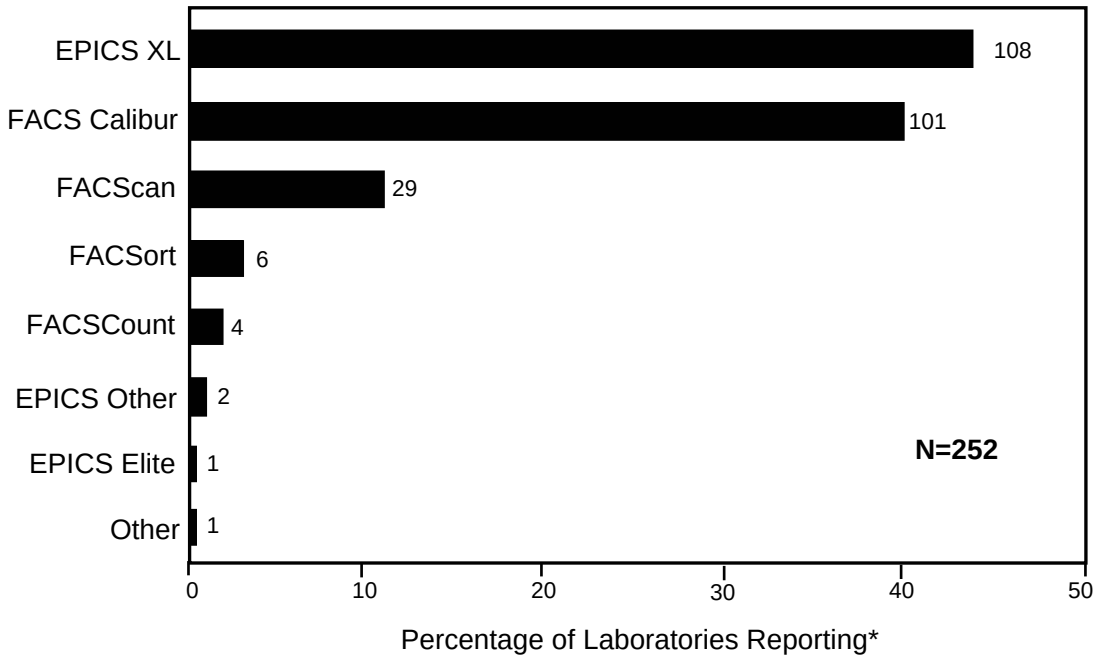


Figure 4. Types of flow cytometers used for $CD4^+$ T-cell determinations, reported by participant laboratories to CDC for the April 2003 shipment.



* Not all laboratories reported the type of flow cytometer used

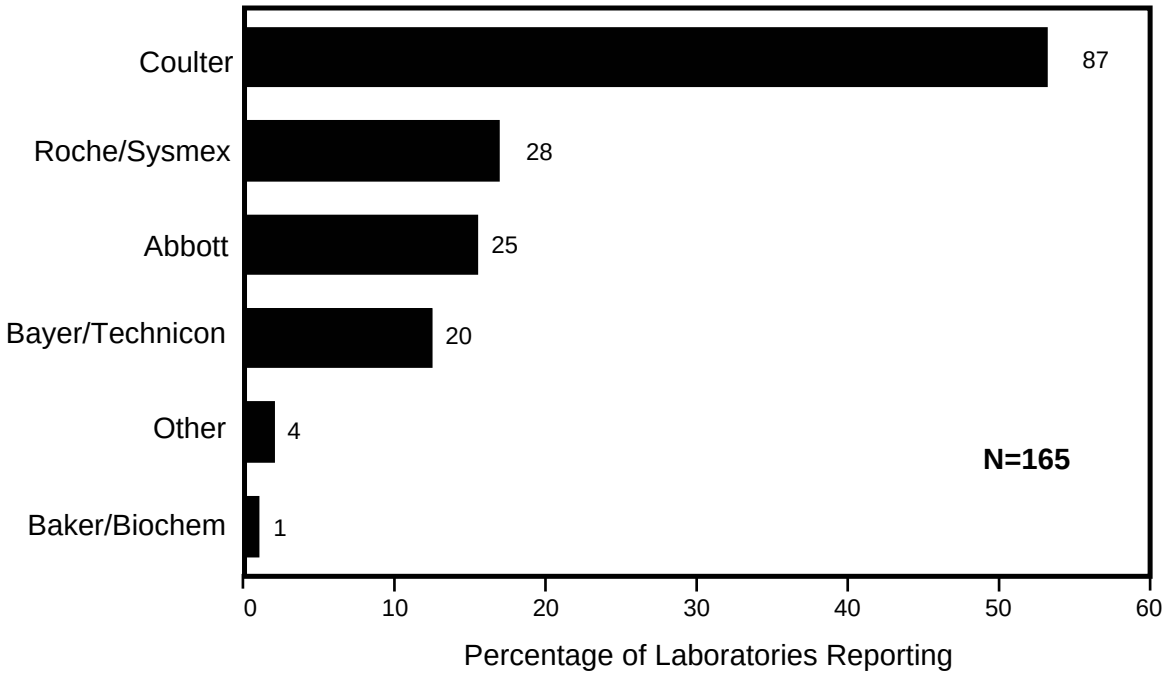
Among the 256 laboratories reporting results, 214 reported absolute cell counts. Of these, 150 (70.1%) of 214 used only a multi-platform method to derive marker-specific absolute cell counts. Sixty-three (29.5%) of 214 laboratories, used only a single-platform method. One laboratory reported results using both single- and multi-platform methods. Table 3 shows the number and percentage of laboratories reporting the use of single-platform methods generally increased during the past six years. However, the use of single-platform methods appears to have stabilized to around 30% during the past 12 months.

Table 3. Laboratories reporting use of single-platform methods for absolute cell counts

Shipments	Sept. 1997	March 1998	Sept./Oct. 1998	April 1999	Oct. 1999	April 2000	Oct. 2000	April 2001	Oct. 2001	April 2002	Oct. 2002	April 2003
Total # of Labs Reporting	162	188	188	208	205	198	206	205	210	215	219	214
# of Labs using Single-Platform	30	36	35	42	42	51	51	57	57	67	67	64
% of Labs using Single-Platform	18.5	19.1	18.6	20.2	20.5	25.8	24.7	27.8	27.1	31.2	30.6	29.9

Of the 256 participant laboratories, 165 (64.5%) identified the manufacturer of the hematology instrument in use in their laboratory. These manufacturers are shown in Figure 5.

Figure 5. Hematology instruments, by manufacturer, used for $CD4^+$ T-cell determinations, reported by participant laboratories to CDC for the April 2003 shipment.



Cell Marker Statistical Calculations and Results

Table 4 contains 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. The table also contains 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.

Table 4. Participant Laboratory Results for the April 2003 Shipment

Donor Number 1 - Donor Status: HIV-antibody Negative

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	95 - 100	19		
	< 95	1		
CD14	> 1	1		
	0 - 1	19		
	< 0	0		
CD4	> 69	3	> 1,944	7
	58 - 69	109	489 - 1,944	101
	< 58	4	< 489	1
CD8	> 26	1	> 745	7
	21 - 26	109	167 - 745	99
	< 21	6	< 167	1
CD19	> 7	1		
	3 - 7	93		
	< 3	0		
CD56	> 6	2		
	1 - 6	30		
	< 1	0		
CD56+16	> 8	0		
	3 - 8	56		
	< 3	5		
CD3 Average	> 94	1		
	82 - 94	103		
	< 82	3		
CD16	3 - 5	3		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 9,921	1
	5,282 - 9,921	73
	< 5,282	0
% Lymphs	> 40	4
	17 - 40	67
	< 17	1
Absolute Lymphs	> 3,442	5
	837 - 3,442	65
	< 837	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 2 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	93 - 100	22		
	< 93	2		
CD14	> 2	0		
	0 - 2	24		
	< 0	0		
CD4	> 51	1	> 1,104	10
	42 - 51	110	603 - 1,104	108
	< 42	5	< 603	2
CD8	> 25	2	> 551	10
	20 - 25	111	282 - 551	109
	< 20	3	< 282	1
CD19	> 20	5		
	13 - 20	87		
	< 13	4		
CD56	> 10	3		
	6 - 10	33		
	< 6	0		
CD56+16	> 19	0		
	6 - 19	48		
	< 6	4		
CD3 Average	> 75	6		
	64 - 75	99		
	< 64	1		
CD16	10 - 15	4		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 6,975	1
	5,773 - 6,975	73
	< 5,773	2
% Lymphs	> 41	8
	20 - 41	65
	< 20	1
Absolute Lymphs	> 2,618	5
	1,261 - 2,618	66
	< 1,261	3

Table 4. Participant Laboratory Results for the April 2003 Shipment

Donor Number 3 - Donor Status: HIV-antibody Negative

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	96 - 100	18		
	< 96	2		
CD14	> 1	1		
	0 - 1	19		
	< 0	0		
CD4	> 52	4	> 1,124	4
	45 - 52	106	769 - 1,124	103
	< 45	6	< 769	3
CD8	> 28	3	> 574	3
	22 - 28	110	390 - 574	101
	< 22	3	< 390	4
CD19	> 17	2		
	12 - 17	84		
	< 12	8		
CD56	> 9	2		
	5 - 9	30		
	< 5	0		
CD56+16	> 11	2		
	6 - 11	57		
	< 6	2		
CD3 Average	> 79	5		
	73 - 79	98		
	< 73	4		
CD16	7 - 8	3		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 7,036	2
	6,059 - 7,036	68
	< 6,059	4
% Lymphs	> 35	4
	26 - 35	69
	< 26	0
Absolute Lymphs	> 2,364	3
	1,634 - 2,364	67
	< 1,634	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 4 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	97 - 100	16		
	< 97	0		
CD14	> 0	2		
	0 - 0	14		
	< 0	0		
CD4	> 33	4	> 713	5
	27 - 33	110	531 - 713	93
	< 27	2	< 531	2
CD8	> 55	6	> 1,208	5
	49 - 55	110	927 - 1,208	89
	< 49	0	< 927	2
CD19	> 11	1		
	7 - 11	88		
	< 7	3		
CD56	> 4	1		
	2 - 4	27		
	< 2	0		
CD56+16	> 6	2		
	2 - 6	68		
	< 2	0		
CD3 Average	> 89	3		
	82 - 89	101		
	< 82	4		
CD16	3 - 3	2		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 4,567	5
	4,024 - 4,567	66
	< 4,024	1
% Lymphs	> 51	3
	45 - 51	69
	< 45	0
Absolute Lymphs	> 2,340	2
	1,795 - 2,340	66
	< 1,795	4

Table 4. Participant Laboratory Results for the April 2003 Shipment

Donor Number 5 - Donor Status: HIV-antibody Positive

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	> 10	4	> 171	7
	6 - 10	109	76 - 171	100
	< 6	1	< 76	1
CD8	> 68	2	> 1,238	6
	56 - 68	107	617 - 1,238	99
	< 56	5	< 617	1
CD19	> 5	5		
	1 - 5	88		
	< 1	0		
CD56	> 19	1		
	11 - 19	30		
	< 11	1		
CD56+16	> 28	1		
	13 - 28	56		
	< 13	3		
CD3 Average	> 82	2		
	68 - 82	100		
	< 68	4		
CD16	14 - 22	3		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 5,628	1
	4,759 - 5,628	68
	< 4,759	5
% Lymphs	> 37	4
	23 - 37	68
	< 23	1
Absolute Lymphs	> 1,979	3
	1,103 - 1,979	66
	< 1,103	4

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		
	96 - 100	34		
	< 96	0		
CD14	> 1	0		
	0 - 1	34		
	< 0	0		
CD4	> 54	4	> 1,958	6
	47 - 54	122	1,366 - 1,958	112
	< 47	0	< 1,366	2
CD8	> 38	4	> 1,358	6
	33 - 38	120	962 - 1,358	108
	< 33	0	< 962	6
CD19	> 11	1		
	6 - 11	90		
	< 6	5		
CD56	> 2	2		
	0 - 2	36		
	< 0	0		
CD56+16	> 3	2		
	0 - 3	52		
	< 0	0		
CD3 Average	> 93	6		
	87 - 93	93		
	< 87	1		
CD16	0 - 1	4		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 6,578	3
	5,624 - 6,578	76
	< 5,624	3
% Lymphs	> 59	2
	51 - 59	77
	< 51	3
Absolute Lymphs	> 3,933	4
	2,828 - 3,933	75
	< 2,828	3

Table 4. Participant Laboratory Results for the April 2003 Shipment

Donor Number 7 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	94 - 100	31		
	< 94	3		
CD14	> 1	0		
	0 - 1	33		
	< 0	0		
CD4	> 28	4	> 1,137	1
	22 - 28	114	536 - 1,137	83
	< 22	2	< 536	6
CD8	> 58	9	> 2,319	1
	51 - 58	110	1,325 - 2,319	83
	< 51	1	< 1,325	6
CD19	> 16	2		
	7 - 16	99		
	< 7	3		
CD56	> 3	2		
	1 - 3	32		
	< 1	2		
CD56+16	> 8	2		
	2 - 8	58		
	< 2	2		
CD3 Average	> 85	4		
	78 - 85	91		
	< 78	1		
CD16	5 - 7	4		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 9,210	0
	5,337 - 9,210	76
	< 5,337	8
% Lymphs	> 52	2
	41 - 52	80
	< 41	2
Absolute Lymphs	> 4,245	0
	2,487 - 4,245	75
	< 2,487	7

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		
	93 - 100	32		
	< 93	2		
CD14	> 2	0		
	0 - 2	34		
	< 0	0		
CD4	> 57	3	> 1,499	8
	50 - 57	116	745 - 1,499	97
	< 50	4	< 745	0
CD8	> 20	3	> 533	7
	16 - 20	119	246 - 533	96
	< 16	0	< 246	2
CD19	> 20	2		
	11 - 20	95		
	< 11	3		
CD56	> 10	0		
	4 - 10	35		
	< 4	2		
CD56+16	> 13	1		
	7 - 13	55		
	< 7	2		
CD3 Average	> 77	4		
	69 - 77	92		
	< 69	2		
CD16	7 - 11	4		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 7,389	3
	6,088 - 7,389	78
	< 6,088	2
% Lymphs	> 44	8
	20 - 44	75
	< 20	0
Absolute Lymphs	> 3,256	8
	1,210 - 3,256	73
	< 1,210	1

Table 4. Participant Laboratory Results for the April 2003 Shipment

Donor Number 9 - Donor Status: HIV-antibody Negative

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	95 - 100	32		
	< 95	2		
CD14	> 2	2		
	0 - 2	32		
	< 0	0		
CD4	> 53	5	> 1,966	8
	47 - 53	116	1,151 - 1,966	97
	< 47	2	< 1,151	0
CD8	> 27	2	> 962	8
	22 - 27	119	554 - 962	97
	< 22	1	< 554	0
CD19	> 23	1		
	15 - 23	93		
	< 15	6		
CD56	> 4	3		
	0 - 4	34		
	< 0	0		
CD56+16	> 5	4		
	2 - 5	53		
	< 2	1		
CD3 Average	> 80	6		
	73 - 80	91		
	< 73	1		
CD16	2 - 3	4		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 8,145	2
	6,992 - 8,145	79
	< 6,992	2
% Lymphs	> 52	7
	31 - 52	76
	< 31	0
Absolute Lymphs	> 4,060	6
	2,349 - 4,060	74
	< 2,349	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 10 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	91 - 100	32		
	< 91	1		
CD14	> 1	1		
	0 - 1	32		
	< 0	0		
CD4	> 9	6	> 242	4
	6 - 9	116	39 - 242	101
	< 6	1	< 39	0
CD8	> 50	9	> 1,049	7
	38 - 50	113	567 - 1,049	98
	< 38	0	< 567	0
CD19	> 19	1		
	9 - 19	95		
	< 9	4		
CD56	> 33	2		
	10 - 33	33		
	< 10	2		
CD56+16	> 40	1		
	28 - 40	52		
	< 28	5		
CD3 Average	> 59	8		
	45 - 59	90		
	< 45	0		
CD16	28 - 34	4		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 5,372	1
	4,072 - 5,372	78
	< 4,072	4
% Lymphs	> 48	7
	30 - 48	75
	< 30	1
Absolute Lymphs	> 2,353	6
	1,381 - 2,353	74
	< 1,381	2

As can be seen in Table 5, 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. **Note: These are inclusive ranges (lowest value to highest value) and are not 95% confidence limits as presented in the results in the previous tables.**

Table 5. Inclusive* Range of Absolute T-cell Counts Reported, Single-Platform vs. Multi-Platform Derived

		$CD4^+$ T-cell Count		$CD8^+$ T-cell Count		Absolute Lymphocyte Count (Hematology Instrument)
Vial Label	Donor Identification	Single-Platform	Multi-Platform	Single-Platform	Multi-Platform	
A3, B1	1	841 - 1124	64 - 3244	305 - 428	20 - 1097	156 – 4770
A1, A4	2	662 - 1771	42 - 1690	290 - 947	24 - 810	1144 – 3520
A2, B2	3	737 - 1322	42 - 1287	342 - 719	22 - 630	185 – 2600
B4, B5	4	523 - 762	487 - 985	922 - 1255	937 - 1684	196 – 3178
A5, B3	5	76 - 145	6 – 232	634 - 988	61 – 1738	140 – 2650
D2, D4	6	1304 - 1844	274 – 2788	874 – 1285	192 – 1989	326 - 5510
C1, C5	7	471 – 955	216 – 1287	1235 – 2086	1132 – 2334	2000 – 4244
C2, D1	8	869 - 1107	746 - 3047	312 – 409	234 - 1045	199 – 5500
C4, D3	9	1249 - 1639	1159 - 3605	599 - 843	566 - 1730	320 – 5310
C3, D5	10	94 - 163	97 - 1520	631 - 840	574 - 2360	182 - 5076

* Inclusive ranges – smallest to largest value, not 95% confidence limits

The multi-platform ranges were larger than the corresponding single-platform ranges for both $CD4^+$ and $CD8^+$ absolute T-cell counts (on average, more than 3.5 times larger). The ranges of multi-platform results were affected by the magnitude of the ranges of the absolute lymphocyte count results (last column), which were often quite large (e.g., Donors 1, 6, 9, and 10). For some, the magnitude of the ranges may be due to simple reporting errors on the part of the laboratories. For example, one laboratory for all five specimens tested reported a lymphocyte count result that was in error by nearly a factor of 10 (e.g., the laboratory reported a WBC of 6190 and a lymphocyte percent of 25, which should have yielded a lymphocyte count of 1548; however, the laboratory reported a lymphocyte count of 156). Eleven 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 11, three laboratories inaccurately calculated lymphocyte counts (greater than 5% difference between true and reported) on all 5 specimens tested. The MPEP for $CD4^+$ T-cell determinations focuses on the total testing process, including errors resulting from incorrect calculations.

Discussion

Specimen panel receipt was delayed one day for seven laboratories due to problems related to the overnight carrier. Nine laboratories reported a one-day delay in receiving their specimens due to delivery problems within their institution. Additionally, 34 (13.3%) of 256 laboratories reported they did not process the MPEP $CD4^+$ T-cell specimens on the day they were received (32 laboratories, one-day delay; two laboratories, two-day delay). These delays may have affected the testing results from these laboratories.

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.

Those laboratories performing $CD4^+$ T-cell determinations using a single-platform method should 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].