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 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 October 2003

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Table of Contents

Tables

Figures

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

Table 1. Donor Identification for October 2003 Shipment Specimens

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

Analysis of the October 2003 Performance Evaluation Testing Results for $CD4^+$ T-Cell Determinations 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 $CD4^+$ T-cell determination ($CD4^+$ T-cell) performance evaluation specimens sent on October 7 and October 14, 2003. Of the 277 laboratories receiving specimen panels, 259 (93.5%) reported testing results. Of the 18 nonreporting laboratories, two laboratories were no longer performing $CD4^+$ T-cell testing, one laboratory was not performing testing only at the time of the performance evaluation survey, and 15 provided no explanation.

Significant Findings

- As 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.

- 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 six years, and has stabilized at around 30% for the past four survey periods (April 2002, October 2002, April 2003, October 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, 39 (15.1%) of the 259 participant laboratories reported they did not process the specimens on the day they were received.

- Only 15 Health Department laboratories participated in the October 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 October 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 (general linear model). Before calculation, data were analyzed for possible outliers. Only 211 (2.1%) of 10,301 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 October 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
$CD3^+$	95.4%	4.6%				White Blood Cell Count	94.0%	6.0%
$CD4^+$	94.9%	4.1%	92.1%	7.9%		Lymphocyte Percentage	90.8%	9.2%
$CD8^+$	94.0%	6.0%	92.0%	8.0%		Absolute Lymphocyte Count	91.6%	8.4%
$CD14^+$	96.9%	3.1%						
$CD19^+$	95.4%	4.6%						
$CD45^+$	97.3%	2.7%						
$CD56^+$	93.7%	6.3%						
$CD(56+16)^+$	94.3%	5.7%						

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

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

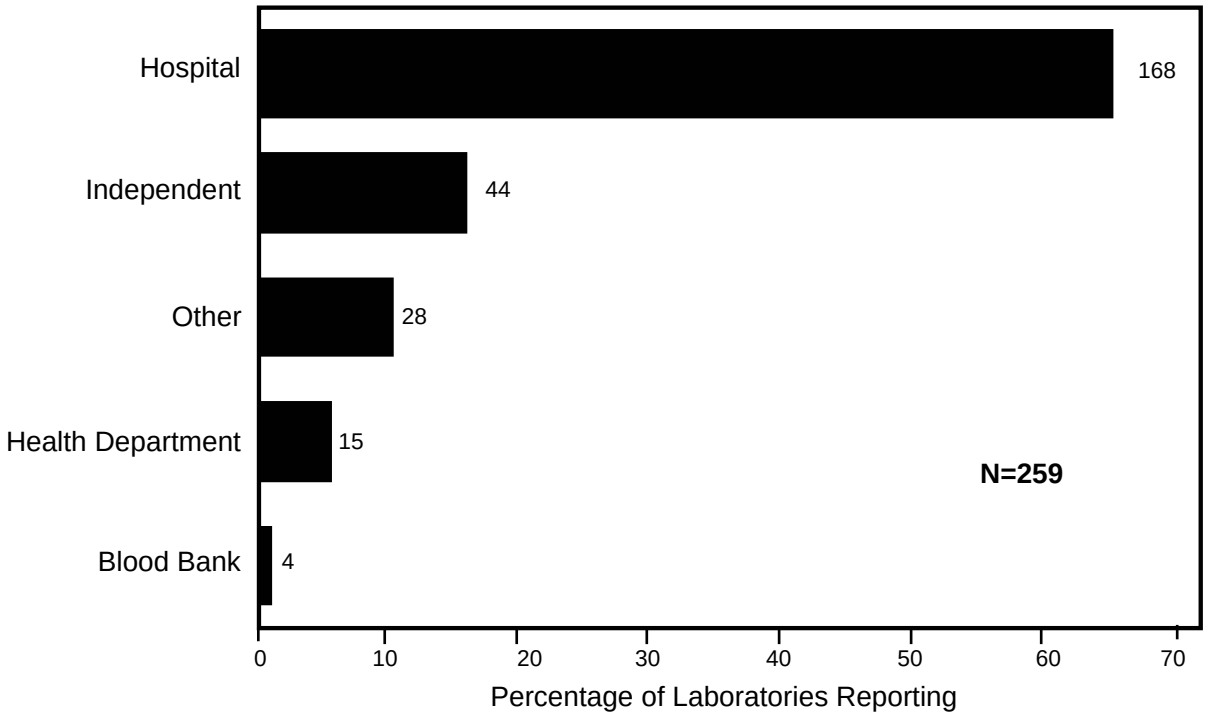
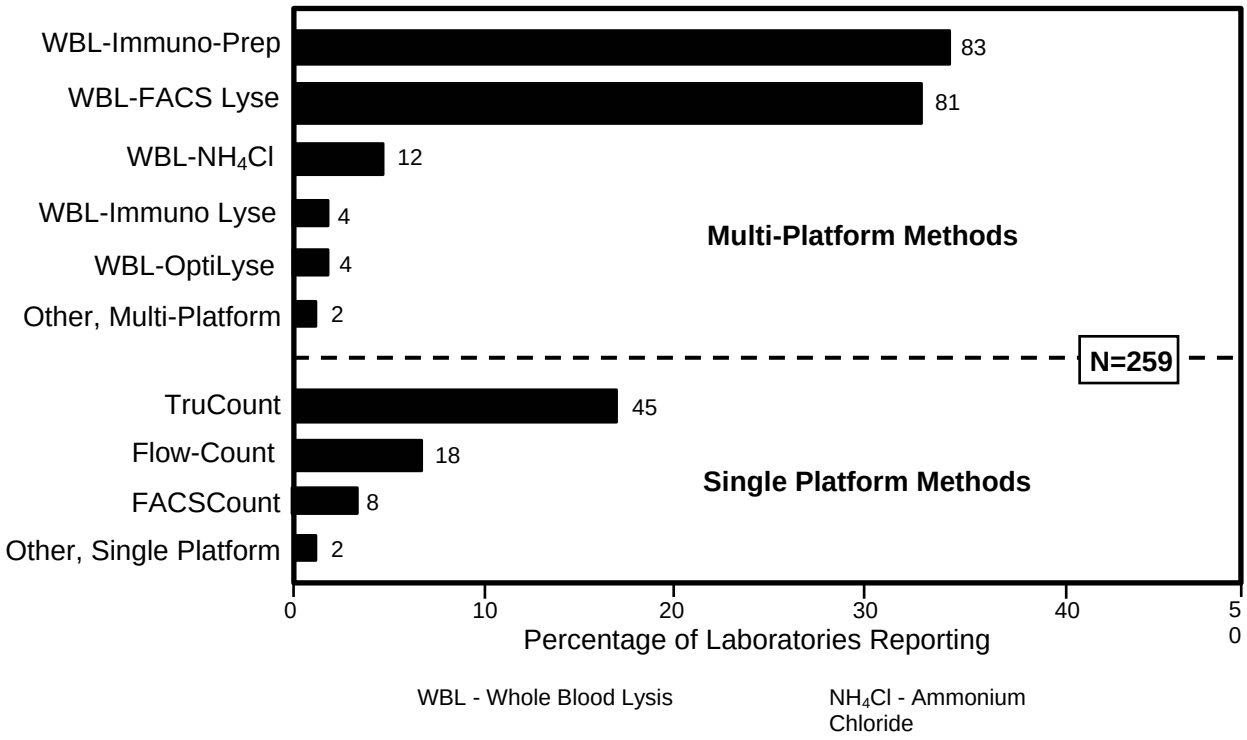


Figure 2 shows the methods used by the laboratories to prepare specimens for $CD4^+$ T-cell determinations. All of the laboratories performing multi-platform methods reported using a method of whole blood lysis to prepare specimens for $CD4^+$ T-cell (including 2 methods described as "Other"). The frequency 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 October 2003 shipment.



"Other" multi-platform methods were described as Cal-Lyse (CalTag) and FACS Count. One "Other" single-platform method was described as Coulter Tetra-One and the second "Other" single-platform method was undefined.

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, 27 (10.7%) of 253 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 used to fix specimens for $CD4^+$ T-cell determinations, reported by participant laboratories to CDC for the October 2003 shipment.

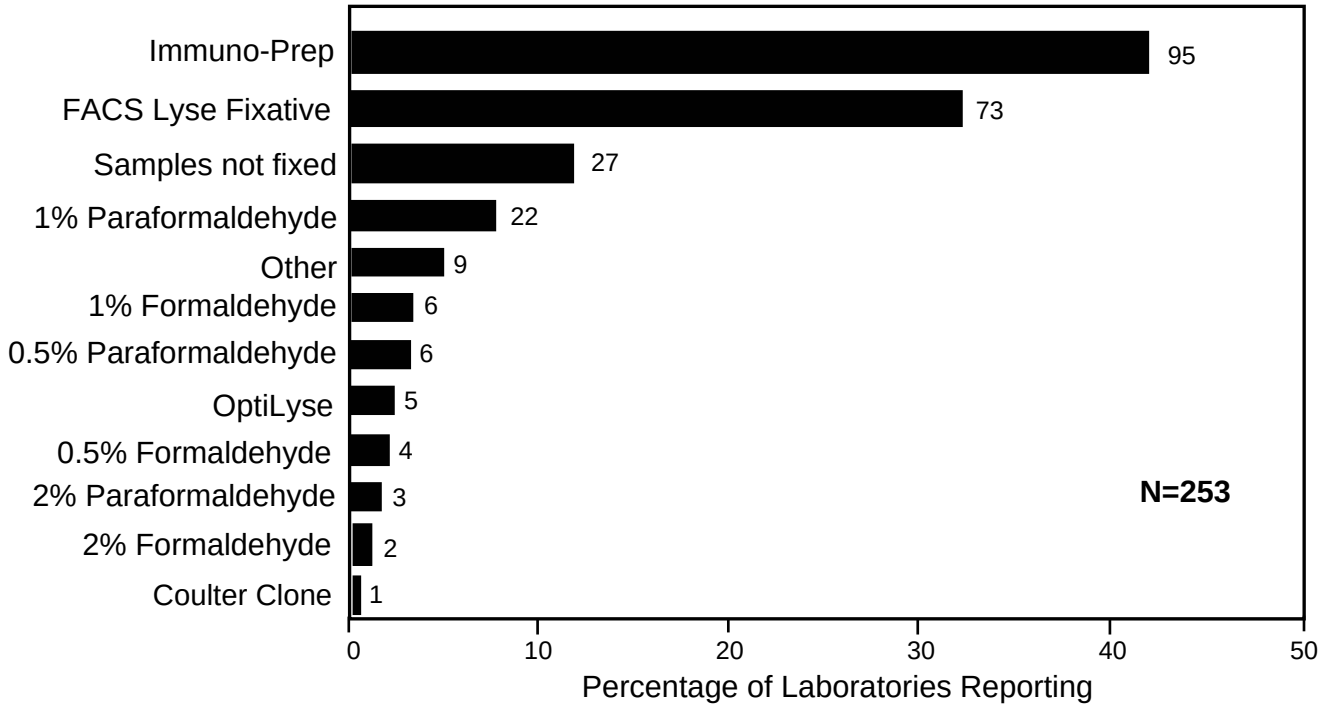
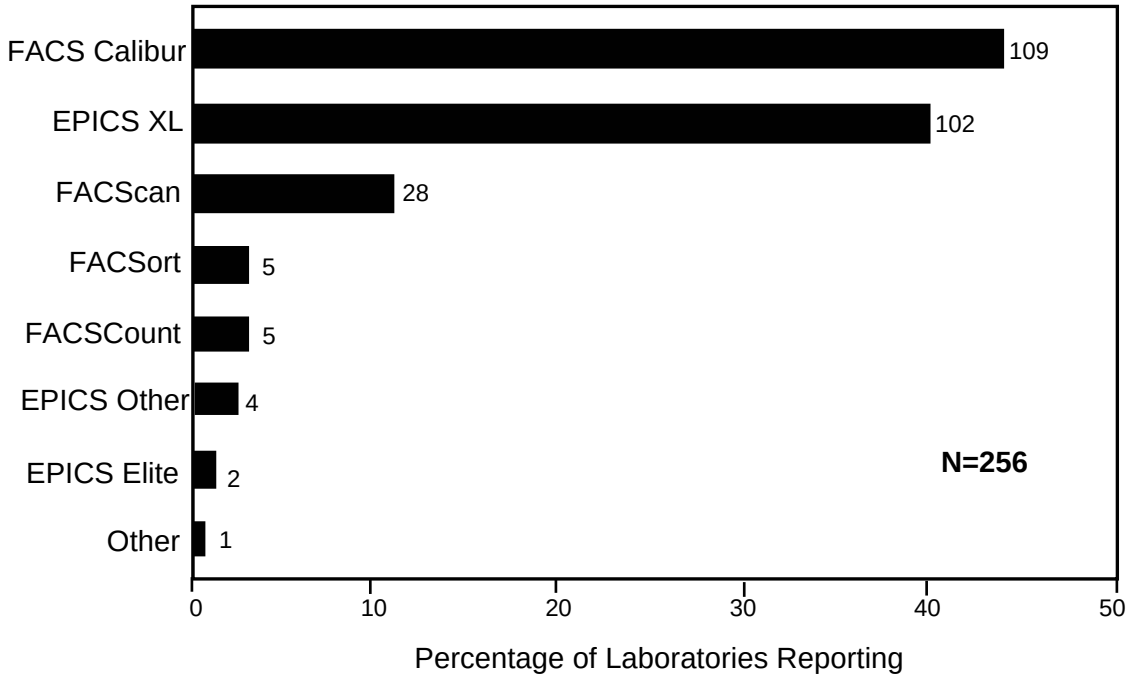


Figure 4 shows the types of flow cytometers used by the laboratories. There were 4 Beckman Coulter model FC500 listed as EPICS "Other" and one Cytomics model listed as an "Other" flow cytometer.

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



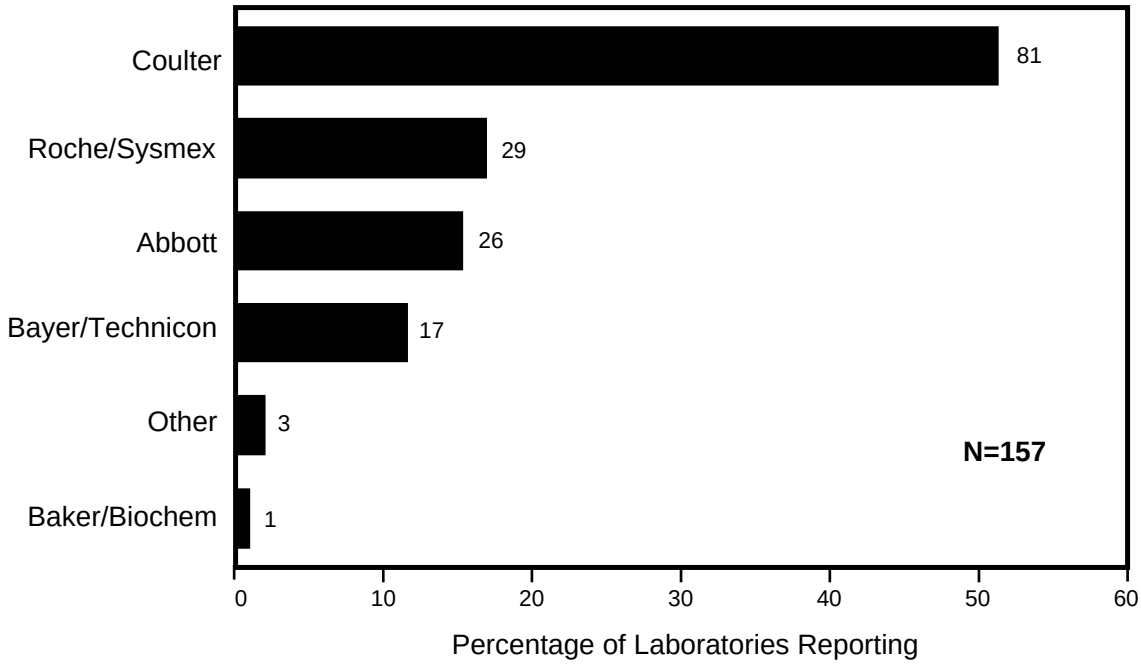
Among the 259 laboratories reporting results, 213 reported absolute cell counts. Of these, 140 (65.7%) of 213 used only a multi-platform method to derive marker-specific absolute cell counts. Seventy-two (33.8%) of 213 laboratories, used only a single-platform method. One laboratory reported results 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

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

Date of Shipment	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	Oct. 2003
Total # of Labs Reporting	162	188	188	208	205	198	206	205	210	215	219	214	213
# of Labs using Single-Platform	30	36	35	42	42	51	51	57	57	67	67	64	72
% 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	33.8

Of the 259 participant laboratories, 157 (60.6%) identified the manufacturer of the hematology instrument being used 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 October 2003 shipment.



Cell Marker Statistical Calculations and Results

Table 4 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. The table 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.

Table 4. Participant Laboratory Results for the October 2003 Shipment

Donor Number 1 - Donor Status: HIV-antibody Negative

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	96 - 100	26		
	< 96	2		
CD14	> 1	1		
	0 - 1	27		
	< 0	0		
CD4	> 52	3	> 1,888	4
	46 - 52	111	1,276 - 1,888	95
	< 46	2	< 1,276	3
CD8	> 34	1	> 1,231	4
	29 - 34	115	832 - 1,231	95
	< 29	0	< 832	3
CD19	> 16	0		
	10 - 16	85		
	< 10	5		
CD56	> 5	1		
	2 - 5	28		
	< 2	2		
CD56+16	> 6	2		
	1 - 6	56		
	< 1	0		
CD3 Average	> 86	2		
	80 - 86	95		
	< 80	0		
CD16	1 - 3	4		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 9,623	2
	8,167 - 9,623	69
	< 8,167	2
% Lymphs	> 43	6
	32 - 43	67
	< 32	0
Absolute Lymphs	> 3,894	5
	2,774 - 3,894	67
	< 2,774	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 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	92 - 100	25		
	< 92	2		
CD14	> 2	2		
	0 - 2	25		
	< 0	0		
CD4	> 49	3	> 1,247	8
	38 - 49	118	468 - 1,247	97
	< 38	4	< 468	1
CD8	> 26	3	> 627	10
	20 - 26	119	264 - 627	96
	< 20	3	< 264	0
CD19	> 23	1		
	16 - 23	81		
	< 16	6		
CD56	> 14	2		
	6 - 14	30		
	< 6	3		
CD56+16	> 18	3		
	9 - 18	55		
	< 9	2		
CD3 Average	> 75	2		
	60 - 75	95		
	< 60	2		
CD16	8 - 14	5		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 6,996	3
	5,298 - 6,996	73
	< 5,298	2
% Lymphs	> 51	7
	17 - 51	71
	< 17	0
Absolute Lymphs	> 3,158	8
	1,108 - 3,158	70
	< 1,108	0

Table 4. Participant Laboratory Results for the October 2003 Shipment

Donor Number 3 - Donor Status: HIV-antibody Negative

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	95 - 100	27		
	< 95	1		
CD14	> 2	1		
	0 - 2	27		
	< 0	0		
CD4	> 52	2	> 1,646	6
	43 - 52	111	1,009 - 1,646	94
	< 43	3	< 1,009	2
CD8	> 31	3	> 975	7
	24 - 31	108	564 - 975	94
	< 24	5	< 564	1
CD19	> 22	0		
	15 - 22	85		
	< 15	5		
CD56	> 4	2		
	1 - 4	28		
	< 1	1		
CD56+16	> 7	2		
	1 - 7	56		
	< 1	0		
CD3 Average	> 82	3		
	73 - 82	94		
	< 73	0		
CD16	1 - 2	4		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 8,238	3
	6,910 - 8,238	69
	< 6,910	1
% Lymphs	> 46	8
	30 - 46	65
	< 30	0
Absolute Lymphs	> 3,533	6
	2,220 - 3,533	66
	< 2,220	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		
	94 - 100	26		
	< 94	2		
CD14	> 2	1		
	0 - 2	27		
	< 0	0		
CD4	> 40	0	> 1,091	4
	35 - 40	101	728 - 1,091	91
	< 35	4	< 728	4
CD8	> 67	1	> 1,809	2
	51 - 67	94	1,063 - 1,809	91
	< 51	10	< 1,063	6
CD19	> 5	0		
	2 - 5	89		
	< 2	0		
CD56	> 5	1		
	2 - 5	24		
	< 2	1		
CD56+16	> 10	1		
	3 - 10	52		
	< 3	2		
CD3 Average	> 92	0		
	86 - 92	93		
	< 86	0		
CD16	1 - 1	2		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 7,154	2
	5,351 - 7,154	64
	< 5,351	3
% Lymphs	> 51	5
	31 - 51	64
	< 31	0
Absolute Lymphs	> 3,011	5
	2,057 - 3,011	64
	< 2,057	0

Table 4. Participant Laboratory Results for the October 2003 Shipment

Donor Number 5 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	95 - 100	27		
	< 95	0		
CD14	> 1	0		
	0 - 1	27		
	< 0	0		
CD4	> 6	2	> 150	5
	2 - 6	111	45 - 150	96
	< 2	0	< 45	1
CD8	> 44	11	> 1,146	2
	27 - 44	100	554 - 1,146	96
	< 27	2	< 554	4
CD19	> 9	2		
	4 - 9	85		
	< 4	1		
CD56	> 57	0		
	12 - 57	28		
	< 12	1		
CD56+16	> 65	0		
	38 - 65	53		
	< 38	5		
CD3 Average	> 50	6		
	30 - 50	87		
	< 30	1		
CD16	1 - 58	3		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 4,738	1
	2,362 - 4,738	67
	< 2,362	5
% Lymphs	> 80	1
	59 - 80	65
	< 59	7
Absolute Lymphs	> 3,419	0
	1,519 - 3,419	68
	< 1,519	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 - 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	33		
	< 0	0		
CD4	> 51	7	> 1,591	4
	44 - 51	125	971 - 1,591	101
	< 44	0	< 971	5
CD8	> 32	4	> 954	3
	26 - 32	124	589 - 954	100
	< 26	4	< 589	5
CD19	> 24	3		
	14 - 24	97		
	< 14	6		
CD56	> 5	4		
	0 - 5	38		
	< 0	0		
CD56+16	> 5	2		
	1 - 5	56		
	< 1	0		
CD3 Average	> 82	5		
	74 - 82	107		
	< 74	1		
CD16	0 - 0	0		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 7,290	1
	5,880 - 7,290	74
	< 5,880	5
% Lymphs	> 50	6
	34 - 50	73
	< 34	1
Absolute Lymphs	> 3,329	4
	2,120 - 3,329	74
	< 2,120	2

Table 4. Participant Laboratory Results for the October 2003 Shipment

Donor Number 7 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	97 - 100	14		
	< 97	0		
CD14	> 2	0		
	0 - 2	14		
	< 0	0		
CD4	> 44	5	> 1,291	7
	37 - 44	119	812 - 1,291	106
	< 37	4	< 812	3
CD8	> 39	3	> 1,105	3
	32 - 39	119	731 - 1,105	104
	< 32	6	< 731	7
CD19	> 10	0		
	7 - 10	105		
	< 7	3		
CD56	> 7	0		
	2 - 7	30		
	< 2	0		
CD56+16	> 8	2		
	3 - 8	67		
	< 3	3		
CD3 Average	> 89	3		
	83 - 89	111		
	< 83	2		
CD16	3 - 5	4		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 5,896	1
	4,711 - 5,896	62
	< 4,711	3
% Lymphs	> 56	6
	44 - 56	59
	< 44	1
Absolute Lymphs	> 3,156	2
	2,181 - 3,156	60
	< 2,181	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 8 - Donor Status: HIV-antibody Negative

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	92 - 100	24		
	< 92	0		
CD14	> 1	1		
	0 - 1	22		
	< 0	0		
CD4	> 58	5	> 1,321	7
	50 - 58	122	738 - 1,321	104
	< 50	3	< 738	2
CD8	> 20	2	> 450	8
	15 - 20	126	216 - 450	100
	< 15	2	< 216	3
CD19	> 18	1		
	13 - 18	103		
	< 13	3		
CD56	> 11	0		
	4 - 11	34		
	< 4	2		
CD56+16	> 13	0		
	8 - 13	61		
	< 8	4		
CD3 Average	> 78	4		
	69 - 78	108		
	< 69	3		
CD16	8 - 8	2		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 8,566	0
	7,030 - 8,566	70
	< 7,030	3
% Lymphs	> 34	6
	17 - 34	66
	< 17	1
Absolute Lymphs	> 2,743	5
	1,240 - 2,743	65
	< 1,240	2

Table 4. Participant Laboratory Results for the October 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	24		
	< 95	0		
CD14	> 1	1		
	0 - 1	23		
	< 0	0		
CD4	> 53	5	> 1,245	7
	46 - 53	121	830 - 1,245	101
	< 46	4	< 830	5
CD8	> 30	2	> 697	5
	25 - 30	125	437 - 697	102
	< 25	3	< 437	4
CD19	> 15	2		
	9 - 15	103		
	< 9	2		
CD56	> 10	0		
	5 - 10	35		
	< 5	1		
CD56+16	> 11	0		
	8 - 11	62		
	< 8	3		
CD3 Average	> 81	6		
	74 - 81	107		
	< 74	2		
CD16	7 - 8	2		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 6,119	1
	5,029 - 6,119	70
	< 5,029	2
% Lymphs	> 46	6
	31 - 46	66
	< 31	1
Absolute Lymphs	> 2,607	4
	1,666 - 2,607	65
	< 1,666	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 - Donor Status: HIV-antibody Positive

Cell Marker	Percentage Results Range	No.	Absolute Counts Range	No.
CD45	> 100	0		
	90 - 100	24		
	< 90	0		
CD14	> 2	1		
	0 - 2	22		
	< 0	0		
CD4	> 14	6	> 196	5
	8 - 14	123	96 - 196	106
	< 8	1	< 96	2
CD8	> 62	5	> 911	4
	49 - 62	122	537 - 911	103
	< 49	3	< 537	4
CD19	> 17	3		
	9 - 17	102		
	< 9	2		
CD56	> 16	0		
	5 - 16	36		
	< 5	0		
CD56+16	> 23	0		
	11 - 23	61		
	< 11	4		
CD3 Average	> 77	5		
	62 - 77	108		
	< 62	2		
CD16	12 - 15	2		

Hematology Results

Hematology Parameter	Range	No.
WBC	> 3,728	1
	2,974 - 3,728	69
	< 2,974	3
% Lymphs	> 47	4
	32 - 47	68
	< 32	1
Absolute Lymphs	> 1,625	2
	1,027 - 1,625	66
	< 1,027	4

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	
A1, B3	1	1097 - 1848	1358 - 2570	677 - 1231	873 - 1667	2772 - 5410
A3, A5	2	559 – 852	61 – 1756	304 – 473	336 – 904	1620 – 4076
A2, B4	3	827 – 1426	1081 – 2371	494 – 834	564 – 1392	2181 - 5474
B2, B5	4	665 – 996	77 - 1385	960 – 1594	659 - 2287	2100 – 3688
A4, B1	5	35 – 171	49 – 1050	511 – 1016	496 – 1762	1280 - 3100
D2, D5	6	966 – 1463	749 – 2506	601 – 887	470 – 1514	1650 - 5220
C1, C4	7	770 – 1362	789 – 1534	432 – 1321	675 – 1270	1775 - 3510
C5, D4	8	770 – 1232	655 – 3040	19 – 372	193 – 1013	181 - 5630
C2, D1	9	799 – 1189	588 – 1515	373 – 679	348 – 831	1480 - 3030
C3, D3	10	106 – 208	82 – 261	387 – 896	401 – 1079	680 - 1850

* 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 2.7 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 3, 6, and 8).

The magnitude of the ranges shown in Table 5 may be due to simple reporting errors on the part of the laboratories. For example, one laboratory for one of the 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 7500 and a lymphocyte percent of 24, which should have yielded a lymphocyte count of 1800; however, the laboratory reported a lymphocyte count of 181). Five 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 five, one laboratory inaccurately calculated lymphocyte counts (greater than 5% difference between true and reported) on all 5 specimens tested. One laboratory reported the exact same lymphocyte count (2304) for two different samples. The correctly calculated

lymphocyte count (WBC X Lymphocyte percent) for one of these samples was 4736. This discrepancy in reporting may be due to an error in transcription.

The MPEP for $CD4^+$ T-cell determinations focuses on the total testing process, including errors resulting from incorrect calculations and result transcription.

Laboratories are reminded that the regulations implementing the Clinical Laboratory Improvement Amendments (CLIA) include a requirement for ensuring that manual or electronic results calculations are accurate and reliable [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."

Discussion

Several laboratories reported delays in preparing specimens for analysis. These delays were related to: 1) specimen panel receipt delay due to problems with the overnight courier, 2) specimen panel receipt delay due to delivery problems within the receiving institution, and 3) delay in processing the specimens after receipt in the laboratory. Specimen panel receipt was delayed one day for nine laboratories due to problems related to the overnight carrier. Ten laboratories reported a one-day delay and one laboratory reported a two-day delay in receiving their specimens due to delivery problems within their institution. Thirty-nine laboratories reported they did not process the MPEP $CD4^+$ T-cell specimens on the day they were received (34 laboratories, one-day delay; three laboratories, two-day delay; one laboratory, four day-delay; one laboratory, seven day-delay). A total of 56 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 56 laboratories reporting specimen preparation delays, 36 laboratories reported one or more results outside the established 95% confidence ranges, with one laboratory reporting 24 results outside the 95% confidence ranges.

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 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].