Analysis of the April 2002 Performance Evaluation Testing Results for T-Lymphocyte Immunophenotyping Reported to the Centers for Disease Control and Prevention by Participating Laboratories

This is an analysis of testing results reported to the Centers for Disease Control and Prevention (CDC) by laboratories participating in the Model Performance Evaluation Program (MPEP) after they tested the T-lymphocyte immunophenotyping (TLI) performance evaluation specimens sent on April 9 and April 16, 2002. Of those laboratories receiving specimen panels, 265 (92.0%) of 288 reported testing results. Two laboratories were unable to report results due to equipment malfunction. One laboratory was unable to report results due to inadvertently storing the specimens in the refrigerator upon arrival. One laboratory was unable to report results due to a delay in sample panel shipment. One laboratory had discontinued testing and, therefore, did not report results. Non-participation by 18 laboratories was unexplained.

The accompanying report entitled "T-Lymphocyte Immunophenotyping: Figures and Table Used for the Analysis of Participant Laboratory Results for the April 2002 shipment" details the responses given by participant laboratories.

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. Page 2 of the accompanying report 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 specimen receipt. These shipment notifications also allowed the laboratories to minimize within institution delivery delays. Participant laboratories were encouraged to process and test the MPEP TLI specimens as they would patient specimens they routinely receive in their laboratory.

The result reporting booklet used for the April 2002 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 by the MPEP to utilize these guidelines in performing TLI on patient specimens. According to these guidelines, specimens should be processed for hematologic testing and flow cytometric immunophenotyping within 30 hours of collection.

Specimen panel receipt was delayed one day for four laboratories due to problems related to the overnight carrier (FedEx). Eight laboratories reported a one day delay, two laboratories reported a two day delay, one laboratory reported a five day delay, and one laboratory reported a 10 day delay in receiving their specimens due to delivery problems within their institution. Additionally, 41 (15.5%) of 265 laboratories reported they did not process the MPEP TLI specimens on the day they were received (37 laboratories, one day delay; three laboratories, two day delay; one laboratory, 10 day delay).

Since the whole blood specimens were collected in K_3EDTA, the laboratories were asked to report absolute lymphocyte counts for $CD4^+$ and $CD8^+$ lymphocytes. Methods used to derive the cell marker specific absolute cell count were classified as either multi-platform or single-platform. Multi-platform methods were those methods which employed the results from the flow cytometer (cell marker percentages) in combination with the results from a hematology analyzer (white blood cell count, percent lymphocytes, absolute lymphocyte count) to calculate the specific absolute cell count. Single-platform methods were defined as those methods whereby the absolute cell count was derived on a single instrument (e.g., FACSCount, TruCount, Coulter GEN-S, or Flow-Count) or in a single procedural assay (e.g., Coulter manual CD4, CD4Trax, or Zymmune).

Summary of Results

The types of laboratories participating in the April 2002 TLI shipment are shown in Figure 1 on page 3 of the accompanying report. The majority of laboratories participating during this shipment period are classified as Hospital, 167 (63.0%) of 265, or Independent, 49 (18.5%) of 265. The remaining laboratory types, i.e., Health Department, Blood Bank and Other, comprised the remaining 18.5%.

Figure 2 on page 3 of the report shows the methods used by the laboratories to prepare specimens for TLI. The majority of laboratories, 197 (74.6%) of 264, reported using a method of whole blood lysis to prepare specimens for TLI (including 2 methods described as "Other"). The frequency of preparation methods specific for single-platform methods is also reflected in this figure: TruCount, 37 (14.0%) of 264; Flow Count, 18 (6.8%) of 264; FACSCount, 9 (3.4%) of 264; and two methods described as "Other".

Figure 3 on page 4 of the report shows the methods used by the laboratories to fix their TLI specimens before flow cytometric analysis. Of laboratories reporting testing results, 26 (10.2%) of 256, specifically stated that they did not fix their TLI specimens before analyzing them even though the panel sent to the laboratories contained known HIV antibody-positive specimens.

The types of flow cytometers used by the laboratories for TLI are shown on in Figure 4 on page 4 of the report. Those reported as used most often were: EPICS XL, 111 (42.9%); FACS Calibur, 98 (37.8%); FACScan, 39 (15.1%); and FACSort, 6 (2.3%). Other types of flow cytometers were used, each with a frequency of three or less.

Among the 265 laboratories reporting results, 215 reported absolute cell counts. Of these, 148 (68.8%) of 215, used only a multi-platform method to derive marker-specific absolute cell counts. Some laboratories, 67 (31.2%) of 215, used only a single-platform method. As can be seen in the Table 1 on the following page, numbers and percentages of laboratories reporting the use of single-platform methods has been increasing across time.

Table 1. 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
Total # of Labs Reporting	162	188	188	208	205	198	206	205	210	215
# of Labs using Single-Platform	30	36	35	42	42	51	51	57	57	67
% 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

Since not all laboratories provided results for absolute cell counts derived by multi-platform methods, only 169 (63.8%) of 265 laboratories provided information regarding the manufacturer of the hematology instrument in use in their laboratory. The manufacturers of hematology instruments used by the laboratories, shown in Figure 5 of the accompanying report, are as follows: Coulter, 85 (50.3%); Roche/Sysmex, 31 (18.3%); Abbott, 28 (16.6%); Bayer/Technicon, 20 (11.8%); Other, 4 (2.4%); and Baker/Biochem Immunosystems, 1 (0.6%).

Cell Marker Statistical Calculations and Results

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. There were 248 (2.3%) of 10,850 results that were considered to be outliers. These outlier results were removed before calculation of the 95% confidence limits. No data from any laboratory, however, were removed from the aggregate results table comparing values obtained by the laboratories against the 95% confidence limits.

Due to insufficient data, 95% confidence limits could not be calculated for $CD3^-/CD16^+$ or $CD3^-/CD56^+$. The table shows the entire range of laboratory results (maximum and minimum) reported for these two cell markers.

The percentages of participating laboratory results within the 95% confidence limits established for the cell marker percentage results are: CD4, 94.2%; CD8, 94.8%; CD14, 95.2%; CD19, 93.9%; CD45, 96.3%; CD56/16, 94.8%; and CD3 average, 93.4%.

The percentages of participating laboratory results within the 95% confidence limits established for the hematology data are: white blood cell count, 94.1%; lymphocyte percentage, 91.7%; and absolute lymphocyte count, 91.3%.

The percentages of participating laboratory results within the 95% confidence limits established for the absolute cell counts are: CD4, 92.8%; and CD8, 92.6%. As can be seen in Table 2 below, 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 or multi-platform. **Note: These ranges are inclusive ranges (lowest value to highest value) and are <u>not</u> 95% confidence limits as presented in the results in the accompanying report.**

Table 2.
Inclusive* Range of Absolute T-cell Counts Reported, Single-Platform vs. Multi-Platform Derived

Vial Label	Donor Identification	Single-Platform CD4	Multi-Platform CD4	Single-Platform CD8	Multi-Platform CD8	Absolute Lymphocyte Count
A3, B3	1	3 - 42	1 - 300	191 - 443	117 - 708	260 - 2090
A4, B5	2	822 - 1277	759 - 1505	456 - 735	411 – 979	161 - 3202
A2, B1	3	1195 - 1358	1119 - 3266	784 - 1330	365 - 2466	1700 - 6650
A1, A5	4	661 - 870	609 - 1173	494 - 630	476 - 857	900 – 3270
B2, B4	5	585 - 778	537 – 1631	334 - 485	320 – 1099	1068 - 3793
C4, D1	6	709 - 1036	343 – 2290	297 – 595	156 – 983	1080 – 3724
C2, C5	7	522 – 858	569 – 1083	210 – 489	250 – 515	1220 – 2146
C1, D5	8	123 – 215	94 – 533	1082 – 1681	826 – 4643	1631 – 3264
D3, D4	9	390 – 686	401 – 1623	698 – 1119	590 – 2528	1227 – 3432
C3, D2	10	1180 – 1710	984 – 4756	378 – 633	310 – 1383	2100 - 8492

* Inclusive ranges – smallest to largest value, <u>not</u> 95% confidence limits

In all cases, but one (Donor 7, CD8 count), the multi-platform ranges were larger than the corresponding single-platform ranges for both CD4 and CD8 absolute T-cell counts. 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 2, 3, and 10). The magnitude of some of the ranges may be caused by simple reporting errors on the part of the laboratories. For example, one laboratory for one specimen tested reported a lymphocyte count result that was in error by a factor of ten (i.e., the laboratory reported a WBC of 4590 and a lymphocyte percent of 17, which should have yielded a lymphocyte count of 1607, and the laboratory reported a lymphocyte count of 161). There were a total of 12 laboratories that reported lymphocyte counts that were greater than 5% different than the true calculated lymphocyte count (WBC X Lymphocyte percent) on at least one specimen. Of these 12, three laboratories reported inaccurately calculated lymphocyte counts (greater than 5% difference between true and reported) on all 5 specimens tested. The Model

Performance Evaluation Program for TLI is interested in the total testing process, including errors made in reporting due to errors in mathematical calculation.

In summary, most laboratories performed well on the donor specimens in the April 2002 shipment. Not all laboratories used the 2-color and/or 3-color monoclonal antibody combinations recommended in the CDC MMWR $CD4^+$ T-cell testing guidelines. 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 different flow cytometer, hematology instrument, and reagent manufacturer combinations; factors associated with specimen preparation; or reporting errors on the part of the laboratories.