

**Analysis of the April 2000 Performance Evaluation Testing Results for
T-Lymphocyte Immunophenotyping Reported to the
Centers for Disease Control and Prevention by Participating Laboratories**

This report is an analysis of results furnished 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 11 and April 18, 2000. Of those laboratories receiving specimen panels, 264 (86.6%) of 305 reported testing results.

Each laboratory received a total of five whole blood specimens collected in K₂EDTA, 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. The page immediately following the acknowledgment page contains the specimen numbers and donor information for each performance evaluation specimen.

The result reporting booklet used for the April 2000 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 have been 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.

Laboratories are notified a month in advance of the date they will be receiving specimens. An airbill tracking number is included in these preshipment letters which enables the laboratories to locate the specimens in the event the shipment is not received by noon on scheduled date of specimen receipt. These shipment notifications should also allow the laboratories to minimize within institution delivery delays. Nine laboratories reported shipping delays. Two of these delays were due to late deliveries by the overnight carrier (1 day delay). Among the seven other reported delays, the performance panels were delivered to the institution's specimen receiving area within 24 hours, but were not delivered to the testing laboratories within the institution in a timely manner (4 laboratories, 1 day delay; 2 laboratories, 2 day delay; 1 laboratory, 8 day delay).

Participant laboratories are encouraged to process and test the MPEP TLI specimens as they would patient specimens they normally receive in their laboratory. Forty-four (16.7%) of 264 laboratories reported they did not process the MPEP TLI specimens on the day they were received: 37 laboratories, 1 day delay; 2 laboratories, 2 day delay; 1 laboratory, 3 day delay; 2 laboratories, 5 day delay; and 2 laboratories, 6 day delay.

The types of laboratories participating in the April 2000 TLI shipment are shown in Figure 1. The majority of laboratories participating during this shipment period are classified as Hospital, 167 (63.3%) of 264, or Independent, 49 (18.6%) of 264.

Figure 2 of the report shows the methods used by the laboratories to prepare specimens for TLI. The majority of laboratories, 214 (81.4%) of 263, reported using a method of whole blood lysis to prepare specimens for TLI (including 3 of 4 methods described as "Other"). The frequency of preparation methods specific for single-platform methods (described below) is also reflected in this figure: TruCount, 33 (12.5%) of 263; FACSCount, 6 (2.3%) of 263; Imagn2000, 6 (2.3%) of 263; and Flow Count, 3 (1.1%) of 263. Fifty laboratories (including two using the CytoronAbsolute) reported using single-platform methods in the April 2000 shipment compared with 42 laboratories in the October 1999 shipment, 42 laboratories in the April 1999

shipment, 35 laboratories in the September/October 1998 shipment, 24 laboratories in the March 1998 shipment, and 15 laboratories in the September 1997 shipment.

Figure 3 shows the methods used by the laboratories to fix their TLI specimens before flow cytometric analysis. Of laboratories reporting testing results, 26 (10.3%) of 252, 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 in Figure 4. Those reported as used most often were: EPICS XL, 96 (37.6%); FACS Calibur, 70 (27.5%); FACScan, 65 (25.5%); and FACSort, 10 (3.9%). Other types of flow cytometers were used, each with a frequency of five or less.

Since the whole blood specimens were collected in K₂EDTA, 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 cytometry instrument (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, Flow-Count, or Imagn2000) or in a single procedural assay (e.g., Coulter manual CD4, CD4Trax, or Zymmune). The majority of laboratories, 147 (74.2%) of 198, used only a multi-platform method to derive these absolute cell counts. Some laboratories, 50 (25.3%) of 198, used a single-platform method. One laboratory (0.5%) of 198 provided absolute cell counts derived from both multi-platform and single-platform methods.

Since not all laboratories provided results for absolute cell counts derived by multi-platform methods, only 173 (65.5%) of 264 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, are as follows: Coulter, 94 (54.3%); Sysmex, 27 (15.6%); Abbott, 26 (15.0%); Bayer/Technicon, 23 (13.3%); Baker/Biochem Immunosystems, 2 (1.2%) and Other, 1 (0.6%).

All cell marker percentage results reported by the laboratories were grouped according to the cell marker of interest, regardless of the flow cytometry instrument or monoclonal antibody combination used to derive the specific result, e.g., CD4⁺ results were grouped from laboratories using CD3/CD4, CD3/CD4/CD8, or CD45/CD3/CD4. 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 240 (2.2%) of 11,125 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: CD3 average, 95.4%; CD4, 94.9%; CD8, 95.4%; CD14, 95.5%; CD19, 97.2%; CD45, 95.2%; and CD56/16, 95.4%.

The percentage of participating laboratory results within the 95% confidence limits established for the hematology data are: white blood cell count, 91.4%; lymphocyte percentage, 92.8%; and absolute lymphocyte count, 91.0%.

The percentage of participating laboratory results within the 95% confidence limits established for the absolute cell counts are: CD4, 91.9%; and CD8, 90.5%. As can be seen in the following table, 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 not the same ranges presented in the Results table (95% confidence limits) but rather are inclusive ranges (lowest value - highest value).**

Inclusive* Range of Absolute T-cell Counts Reported, Single-Platform vs. Multi-Platform Derived					
Donor Identification	Single-Platform CD4	Multi-Platform CD4	Single-Platform CD8	Multi-Platform CD8	Absolute Lymphocyte Count
1	801 - 1055	658 - 1332	429 - 670	386 - 725	958 - 2833
2	222 - 444	284 - 1113	441 - 858	356 - 1262	400 - 2700
3	784 - 1011	515 - 2676	300 - 423	199 - 988	994 - 5611
4	417 - 598	455 - 1060	958 - 1414	1076 - 2694	1690 - 4240
5	59 - 110	32 - 1513	989 - 1379	76 - 1749	68 - 2100
6	790 - 1210	902 - 2212	1062 - 1788	190 - 3188	2440 - 6507
7	536 - 859	213 - 1189	1310 - 2164	473 - 2871	1700 - 4976
8	866 - 1390	321 - 2568	641 - 1055	235 - 1932	1723 - 5972
9	958 - 1446	307 - 3033	500 - 730	165 - 1548	2133 - 6191
10	25 - 48	7 - 64	548 - 922	198 - 1144	248 - 1400

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

The multi-platform ranges were larger than the corresponding single-platform ranges in all 20 (100%) of the compared ranges (e.g., single-platform derived CD4, Donor 1 vs. multi-platform derived CD4, Donor 1). Obviously, the ranges of multi-platform results were affected by the magnitude of the ranges of the absolute lymphocyte count results (last column), which in some cases were quite large (e.g., Donor 5, ~ thirty-one fold difference between smallest and largest absolute lymphocyte count determinations). The magnitude of some of the ranges may be caused by simple reporting errors on the part of the laboratories.

For example, the laboratory that provided the multi-platform derived absolute CD8 count result of 76 for Donor 5 reported an absolute lymphocyte count of 1650 and a CD8 percentage result of 82% ($1650 \times .82 = 1353$ for the correct absolute CD8 count). Another example is the laboratory that provided the absolute lymphocyte count of 68 for Donor 5 reported a white blood cell count of 4400 and a lymphocyte percentage of 31% ($4400 \times .31 = 1364$ for the correct absolute lymphocyte count). The Model Performance Evaluation Program for TLI is interested in the total testing process, including errors made in reporting.

In summary, most laboratories performed well on the donor specimens in the April 2000 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.