

## **Analysis of the October 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 October 10 and October 17, 2000. Of those laboratories receiving specimen panels, 275 (89.6%) of 307 reported testing results.

Each laboratory received a total of five whole blood specimens collected in K<sub>2</sub>EDTA, 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 October 2000 specimen shipment was designed to be consistent with the CDC guidelines for CD4<sup>+</sup> 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 the scheduled date of specimen receipt. These shipment notifications should also allow the laboratories to minimize within institution delivery delays. For this performance survey, fourteen laboratories reported shipping delays. Two of these delays were due to late deliveries by the overnight carrier (1 day delay). Among the twelve 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 until the next day.

Participant laboratories are encouraged to process and test the MPEP TLI specimens as they would patient specimens they normally receive in their laboratory. Thirty-five (12.7%) of 275 laboratories reported they did not process the MPEP TLI specimens on the day they were received; 34 laboratories, 1 day delay and 1 laboratory, 2 day delay.

The types of laboratories participating in the October 2000 TLI shipment are shown in Figure 1. The majority of laboratories participating during this shipment period are classified as Hospital, 186 (67.6%) of 275, or Independent, 50 (18.2%) of 275.

Figure 2 of the report shows the methods used by the laboratories to prepare specimens for TLI. The majority of laboratories, 221 (80.4%) of 275, reported using a method of whole blood lysis to prepare specimens for TLI (including 3 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%) of 275; Flow Count, 11 (4%) of 275; FACSCount, 8 (2.9%) of 275; and GEN-S, 1 (0.4%) of 275. Fifty-one laboratories reported using single-platform methods in the October 2000 shipment compared with 50 laboratories 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, 30 (11.2%) of 267, 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, 117 (43.0%); FACS Calibur, 86 (31.6%); FACScan, 48 (17.6%); and FACSort, 10 (3.7%). Other types of flow cytometers were used, each with a frequency of three or less.

Since the whole blood specimens were collected in K<sub>2</sub>EDTA, the laboratories were asked to report absolute lymphocyte counts for CD4<sup>+</sup> and CD8<sup>+</sup> 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 Zymune). The majority of laboratories, 155 (75.2%) of 206, used only a multi-platform method to derive these absolute cell counts. Some laboratories, 49 (23.7%) of 206, used a single-platform method. Two laboratories (1.0%) of 206 provided absolute cell counts derived from both multi-platform and single-platform methods. Several laboratories which normally use the Becton Dickinson Biosciences Imagn2000 system were unable to participate in the October 2000 shipment because of the sudden manufacturer recall of testing reagents in late September 2000.

Since not all laboratories provided results for absolute cell counts derived by multi-platform methods, only 182 (66.2%) of 275 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, 96 (52.8%); Abbott, 33 (18.1%); Roche/Sysmex, 29 (15.9%); Bayer/Technicon, 22 (12.1%); and Baker/Biochem Immunosystems, 2 (1.1%).

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<sup>+</sup> 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 216 (1.9%) of 11,472 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<sup>-</sup>/CD16<sup>+</sup> or CD3<sup>-</sup>/CD56<sup>+</sup>. 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, 95.3%; CD4, 95.5%; CD8, 94.3%; CD14, 94.1%; CD19, 95.6%; CD45, 96.8%; and CD56/16, 95.7%.

The percentage of participating laboratory results within the 95% confidence limits established for the hematology data are: white blood cell count, 92.8%; lymphocyte percentage, 92.4%; and absolute lymphocyte count, 92.1%.

The percentage of participating laboratory results within the 95% confidence limits established for the absolute cell counts are: CD4, 92.2%; and CD8, 92.6%. 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).**

<b>Inclusive* Range of Absolute T-cell Counts Reported, Single-Platform vs. Multi-Platform Derived</b>					
Donor Identification	Single-Platform CD4	Multi-Platform CD4	Single-Platform CD8	Multi-Platform CD8	Absolute Lymphocyte Count
1	733 - 1274	458 - 1398	404 - 669	192 - 977	27 - 2625
2	551 - 910	559 - 1373	814 - 1271	809 - 2169	33 - 3498
3	827 - 2001	420 - 2030	307 - 670	244 - 1024	26 - 4161
4	6 - 35	0 - 69	501 - 967	304 - 1580	49 - 2000
5	725 - 1391	605 - 1600	1182 - 1775	956 - 1946	1820 - 3577
6	332 - 591	364 - 644	780 - 1486	940 - 1492	1530 - 2564
7	336 - 690	342 - 916	958 - 1737	950 - 2240	1485 - 4312
8	1436 - 1989	1494 - 2863	587 - 1289	462 - 8944	2070 - 4190
9	931 - 1246	860 - 1440	597 - 836	1 - 970	1829 - 3130
10	31 - 70	39 - 103	618 - 1665	909 - 1772	1148 - 2568

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

The multi-platform ranges were larger than the corresponding single-platform ranges in 18 (90.0%) of 20 compared ranges (e.g., single-platform derived CD4, Donor 1 vs. multi-platform derived CD4, Donor 1). 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., Donors 1-4). 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 one for

Donor 9 reported an absolute lymphocyte count of 2496 and a CD8 percentage result of 28% ( $2496 \times .28 = 699$  for the correct absolute CD8 count). The extremely low values reported for the absolute lymphocyte counts for Donors 1-4 may also reflect reporting errors since all of these low values were provided by a single laboratory. 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 October 2000 shipment. Not all laboratories used the 2-color and/or 3-color monoclonal antibody combinations recommended in the CDC MMWR CD4<sup>+</sup> T-cell testing guidelines. Differences in laboratory performance of cell marker analysis may be related to: the use of the CDC CD4<sup>+</sup> 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.