Analysis of the October 1999 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 them on October 12 and October 19, 1999. Of those laboratories receiving specimen panels, 290 (90.6%) of 320 reported testing results.

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. 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 1999 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. These preshipment letters include the airbill tracking number for the use by the laboratories if they do not receive their specimens by noon of the day they were to receive the shipment. These shipment notifications should also allow the laboratories to minimize within institution delivery delays. Twenty-three laboratories reported shipping delays. Seven of these delays were due to late deliveries by the overnight carrier (6 laboratories, 1 day delay; 1 laboratory, 2 day delay). Among sixteen 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 (14 laboratories, 1 day delay; 2 laboratories, 2 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-seven (16.2%) of 290 laboratories reported they did not process the MPEP TLI specimens on the day they were received: 42 laboratories, 1 day delay; 2 laboratories, 2 day delay; 1 laboratory, 5 day delay; 1 laboratory, 6 day delay; and 1 laboratory, 11 day delay.

The types of laboratories participating in the October 1999 TLI shipment are shown in Figure 1. The majority of laboratories participating during this shipment period are classified as Hospital, 186 (64.1%) of 290, or Independent, 56 (19.3%) of 290.

Figure 2 of the report shows the methods used by the laboratories to prepare specimens for TLI. The majority of laboratories, 251 (86.6%) of 290, reported using a method of whole blood lysis to prepare specimens for TLI. The frequency of preparation methods specific for single-platform methods (described below) is also reflected in this figure: TruCount, 25 (8.6%) of 290; FACSCount, 9 (3.1%) of 290; Imagn2000, 5 (1.7%) of 290; and Flow Count, 1 (0.3%) of 290. Forty-two laboratories reported using single-platform methods in the October 1999 shipment compared with 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, 25 (8.9%) of 281, 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, 105 (37.2%); FACScan, 73 (25.9%); FACS Calibur, 68 (24.1%); Ortho CytoronAbsolute, 12 (4.3%); and EPICS Profile II, 10 (3.5%). Other types of flow cytometers were used, each with a frequency of less than 3%.

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 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 absolute 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, 163 (79.5%) of 205, used only a multi-platform method to derive these absolute cell counts. Some laboratories, 40 (19.5%) of 205, used a single-platform method. Two (1.0%) of 205 laboratories provided absolute 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 197 (67.9%) of 290 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, 119 (60.4%); Sysmex, 31 (15.7%); Abbott, 25 (12.7%); Bayer/Technicon, 20 (10.2%); Baker/Biochem Immunosystems, 1 (0.5%) and Other, 1 (0.5%).

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 230 (1.9%) of 12,342 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 percentage of participating laboratory results within the 95% confidence limits established for the cell marker percentage results are: CD3 average, 93.6%; CD4, 95.6%; CD8, 95.7%; CD14, 95.5%; CD19, 94.8%; CD45, 97.2%; and CD56/16, 96.0%.

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, 93.3%; and absolute lymphocyte count, 90.9%.

The percentage of participating laboratory results within the 95% confidence limits established for the absolute counts are: CD4, 91.5%; and CD8, 92.5%. 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 <u>not</u> 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	846 - 1109	648- 1756	671 - 806	422 - 1330	919 - 3630
2	1073 - 1267	804 - 2509	59 - 821	505 - 1599	1128 - 4980
3	64 - 1366	970 - 2255	589 - 799	526 - 1295	1322 - 4777
4	419 - 685	296 - 806	561 - 719	531 - 955	1360 - 2330
5	40 - 80	37 - 122	501 - 711	443 - 1140	58 - 1564
6	588 - 772	529 - 930	512 - 694	88 - 802	1239 - 2189
7	909 - 1169	688 - 1580	776 - 1066	592 - 1371	1966 - 3658
8	701 - 917	665 - 1248	381 - 591	417 - 844	1546 - 2909
9	882- 1338	868 - 4248	328 - 530	241 - 1502	1725 - 6825
10	201 - 346	190 - 360	1685 - 2230	1489 - 2574	1960 - 3795

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

The multi-platform ranges were larger than the corresponding single-platform ranges in 19 (95%) of 20 compared ranges (e.g., single-platform derived CD4, Donor 1 vs. multi-platform derived CD4, Donor1). 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, ~ twenty-six 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 providing the absolute lymphocyte count result of 58 for Donor 5 reported a white blood cell count of 2200 and a percent lymphocyte result of 38% (2200 X .38 = 836 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 October 1999 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 and reagent manufacturer combinations, factors associated with specimen preparation, or reporting errors on the part of the laboratories.