Analysis of the April 2001 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 10 and April 17, 2001. Of those laboratories receiving specimen panels, 272 (90.4%) of 301 reported testing results. Two laboratories were unable to report results due to equipment malfunction. One laboratory was unable to report results because the specimen panel was inadvertently placed in the refrigerator upon arrival.

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 April 2001 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 air bill 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.

Participant laboratories are encouraged to process and test the MPEP TLI specimens as they would patient specimens they normally receive in their laboratory. Specimen panel receipt was delayed 1 day for six laboratories due to overnight carrier (FedEx) problems. Nine laboratories reported a 1 day delay in receiving their specimens due to delivery problems within their institution. Additionally, 41 (15.1%) of 272 laboratories reported they did not process the MPEP TLI specimens on the day they were received (39 laboratories, 1 day delay and 2 laboratories, 2 day delay).

The types of laboratories participating in the April 2001 TLI shipment are shown in Figure 1. The majority of laboratories participating during this shipment period are classified as Hospital, 177 (65.1%) of 272, or Independent, 49 (18.0%) of 272.

Figure 2 of the report shows the methods used by the laboratories to prepare specimens for TLI. The majority of laboratories, 212 (77.9%) of 272, 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 (described below) is also reflected in this figure: TruCount, 32 (11.8%) of 272; Flow Count, 12 (4.4%) of 272; FACSCount, 12 (4.4%) of 272; and GEN-S, 1 (0.4%) of 272. Of those laboratories reporting absolute cell counts, 57 of 205 (27.8%) laboratories reported using single-platform methods in the April 2001 shipment compared with 51 of 206 (24.7%) laboratories in the October 2000 shipment, 51 of 198 (25.8%) laboratories in the April 2000 shipment, 42 of 205 (20.5%) laboratories in the October 1999 shipment, 42 of 208 (20.2%) laboratories in the April 1999 shipment, 35 of 188 (18.6%)

laboratories in the September/October 1998 shipment, 36 of 188 (19.1%) laboratories in the March 1998 shipment, and 30 of 162 (18.5%) 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, 28 (10.6%) of 264, 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, 114 (42.5%); FACS Calibur, 91 (34.0%); FACScan, 45 (16.8%); and FACSort, 7 (2.6%). Other types of flow cytometers were used, each with a frequency of five or less.

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 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, 148 (72.2%) of 205, used only a multi-platform method to derive these absolute cell counts. Some laboratories, 55 (26.8%) of 205, used a single-platform method. Two laboratories (1.0%) of 205 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 April 2001 shipment because of the 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 170 (62.5%) of 272 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, 89 (52.4%); Abbott, 30 (17.6%); Roche/Sysmex, 27 (15.9%); Bayer/Technicon, 21 (12.4%); 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 219 (2.0%) of 11,180 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, 95.2%; CD4, 94.2%; CD8, 94.6%; CD14, 96.7%; CD19, 95.1%; CD45, 97.1%; and CD56/16, 95.8%.

The percentages of participating laboratory results within the 95% confidence limits established for the hematology data are: white blood cell count, 93.1%; lymphocyte percentage, 92.9%; 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, 91.9%; and CD8, 91.7%. 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 to highest value).**

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
A5, B4	1	901 - 1378	946 - 2817	573 - 1030	662 - 1834	1859 - 6552
B2, B3	2	550 - 990	606 - 1225	1014 - 1714	1237 - 2147	1902 - 3927
A3, B5	3	473 - 1189	784 - 3674	208 - 447	265 - 1249	1290 - 7348
A2, B1	4	4 - 95	5 - 262	656 - 1092	475 - 1334	759 - 1627
A1, A4	5	679 - 993	743 - 1516	1067 - 1459	1038 - 2274	2145 - 4459
D3, D4	6	1095 - 1407	943 - 2456	1284 - 1865	1310 - 2735	2864 - 6201
C2, C5	7	294 - 600	330 - 982	256 - 413	250 - 1824	806 - 2130
C4, D1	8	688 - 1419	211 - 2938	334 - 600	177 - 1386	396 - 3527
C3, D2	9	250 - 1039	686 - 2124	320 - 644	406 - 1630	1460 - 2812
C1, D5	10	14 - 42	11 - 100	403 - 743	361 - 1663	420 - 2279

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

In all cases 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 1 and 3). 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 1386 for Donor 8 reported an absolute lymphocyte count of 3276 and a CD8 percentage result of 22% (3276 X .22 = 721 for the correct absolute CD8 count). Similarly, this same laboratory reported a multi-platform derived absolute CD8 count result of 1792 for Donor 7, yet reported an absolute lymphocyte count of 1064 and a CD8 percentage result of 32% (1064 X .32 = 340 for the correct absolute CD8 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 2001 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.